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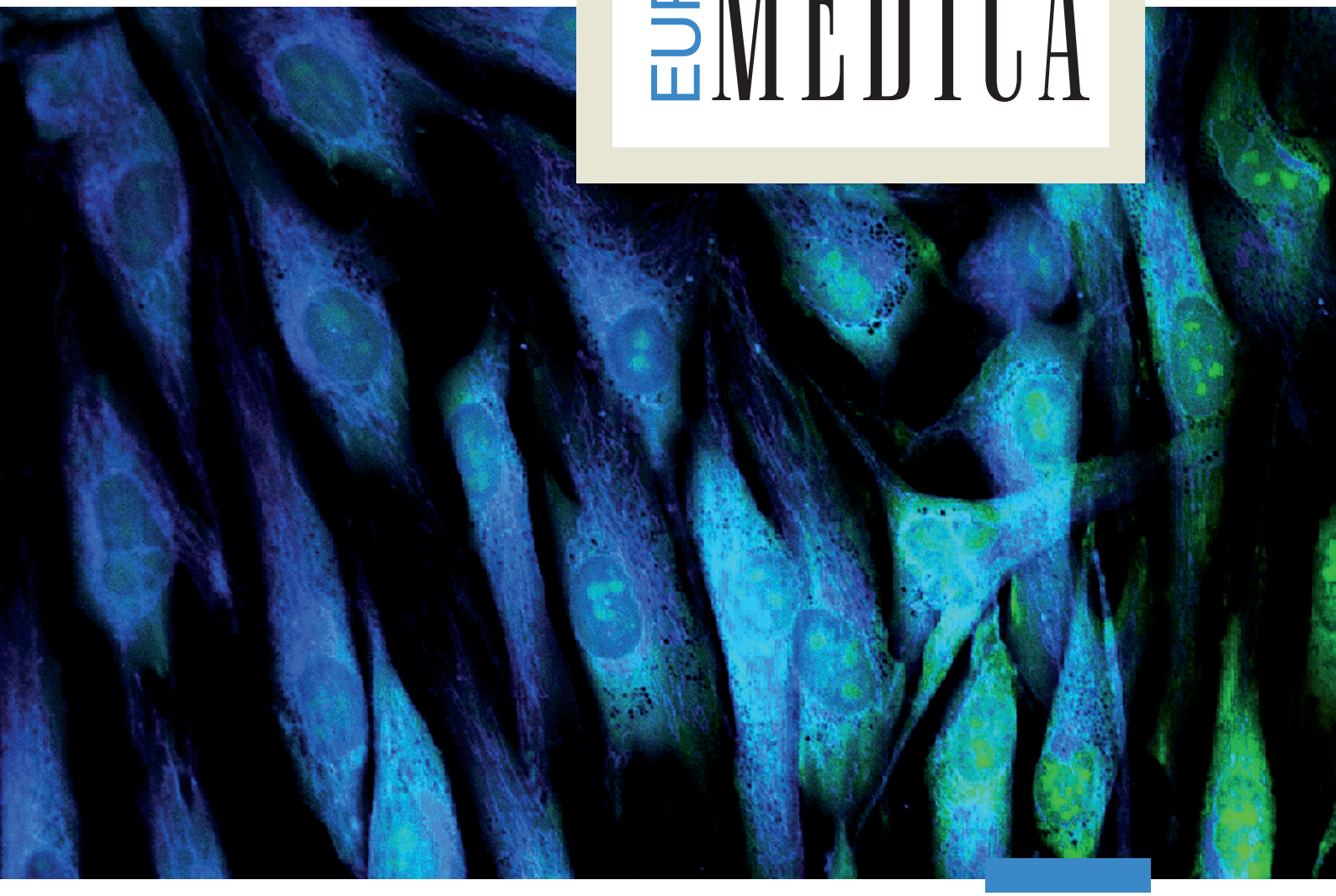

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CONTENTS

CONTENTS

STOMATOLOGY
EXPERIMENTAL RESEARCH

STOMATOLOGY
CLINICAL RESEARCH

EDITORIAL

Dear colleagues,

Our current issue has been largely dedicated to problems of the oral cavity. The dental and oral diseases are most common; they influence the rate of total human morbidity and their clinical manifestations are not only associated with a decay of dental health but substantially affect the health-related quality of life of millions of people.

The level of dental morbidity and the quality of dental care has been attributed to the professionalism and commitment of dentists and the medical staff. State-of-the-art modern dentistry has emerged due to innovative high quality technologies for conservative, orthopedic, orthodontic and surgical treatment of different dental and craniofacial diseases as well as their diagnostics and prevention.

The present issue of the journal "Archiv EuroMedica" is aimed at providing scientists and clinicians with robust solutions for major challenges to modern medicine. The authors made their best to present a compact but nonetheless comprehensive set of articles on diagnostics and treatment of most important dental problems. In their opinion, the obtained research data should contribute in finding solutions to actual problems.

The authors of our dental section have accumulated a great deal of clinical experience, personal and literary information; they performed rigorous scientific studies in different dental disciplines. It is feasible to utilize this valuable data in planning and selecting the methods and volumes for adequate treatment, foreseeing and evaluation of its outcomes as well as improving the quality of dental services.

Executive Editor
Prof. Dmitry Domenyuk

MORPHOLOGICAL SPECIFICS OF CRANIOFACIAL COMPLEX IN PEOPLE WITH VARIOUS TYPES OF FACIAL SKELETON GROWTH IN CASE OF TRANSVERSAL OCCLUSION ANOMALIES

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ABSTRACT — Harmonious structure of the craniofacial complex is the major component for physical, mental and social well-being. Therefore, delayed diagnostics and treatment of dentoalveolar pathology may lead not to social maladjustment only, yet also to an increased risk of periodontal diseases, carious lesions, and temporomandibular joint functional issues. Following the outcomes of studies involving head anthropometric measurements, computed tomograms and lateral teleradiography of 68 people with physiological occlusion and various types of the facial skeleton growth, a method has been proposed, which can be employed to evaluate the facial structure balance, based on the match between the anteroposterior dimensions of the maxilla alveolar process and the mandible alveolar part. Only 76.47±5.14% of patients have been identified as having balanced ratios; 14.71±4.29% of the patients were found to have maxillary sagittal dimensions prevailing, with another 8.82±3.44% featuring the prevalence of the mandible anterior-posterior dimensions. Besides, the dominance of the mandible body dimensions in relation to the branch has been found in all types of growth of the head facial area. The obtained data have been compared with the anthropometric and radiological data of 12 patients with cross occlusion. It has been proven that in patients with cross occlusion, a change in the face configuration and the dental system morphological status depends on the anomaly shape as well as on the degree of the mandible transversal shift.

KEYWORDS — physiological occlusion; transversal anomalies of occlusion; type of head facial area growth; lateral teleradiography; cone-beam computed tomography.

INTRODUCTION

The prevalence of dental anomalies and deformities in Russia, which are leading among the maxillofacial issues, varies considerably in the range of 30.9–82.8%. The progressing growth of dentition malformations, leading to social maladjustment, increased risk of developing carious lesions, periodontal diseases, and temporomandibular joint (TMJ) functional disorders, demands the development and implementation of advanced and highly effective methods for dental pathology diagnostics and treatment [1–9]. Diagnostics of the dental system anomalies and deformations is based on the clinical examinations results and on additional (laboratory) data, while the treatment tactics for this group of patients depends on a whole set of factors, the leading ones being the type of pathology, the severity of changes, the stage of occlusion development (the patient's age) [10–21].

Orthodontic care in childhood (adolescence) implies creating favorable conditions for balanced growth, the development of the child's dental system and facial skeleton, which is achieved through eliminating bad habits and improving respective functions. In case of older age groups, this set of measures is expanded with orthodontic equipment, which is often combined with surgical interventions. Experts note that it is a combination of conservative and surgical treatments based on comprehensive diagnostics involving radiological and functional methods that allows achieving a morphological, functional, and aesthetic balance in the dentofacial system structure and functioning along with stable remote clinical outcomes [22–38].

Cross occlusion, related to the anomalies of dentition closure in the transverse direction, is one of the most complex issues entailing maxillofacial aesthetic, morphological and functional changes. The occurrence of cross occlusion among all dentofacial anomalies is not the same for different age categories — in children (adolescents) it is up to 1.9%, whereas in people of mature age it goes up to 3%. The following factors lay the ground for cross occlusion: jaw bones impaired growth due to inflammation issues; reduced chewing function or one-sided chewing with

early tooth extraction and multiple caries; changed sequence and timing of teething; uneven occlusal contacts and unworn milk teeth tubercles; improper swallowing; obstructed nasal breathing; congenital cleft palate; somatic pathologies caused by impaired phosphorus-calcium metabolism; traumatic injuries; disturbed myodynamic balance between the masticatory, buccal, temporal musculature and suprahyoid muscles; musculoskeletal system issues with organic or peripheral type genesis (scoliosis); dysplastic bone disease (osteopathy); osteochondral tissue systemic lesions [39–41].

A mismatch in size, shape and position of the jaw bones or dentition, which cause anomalies in the transversal direction, underlie the classification of cross occlusion types depending on the type of morphological changes leading to the anomaly: articular, dentoalveolar, gnathic. The prevalence of the articular type in case of cross occlusion goes up to 77% of all transversal anomalies. Each type of cross occlusion can be symmetrical or asymmetric, unilateral or bilateral. Depending on the morphological changes in the dental system, L.S. Persin (2004) identified three types of cross occlusion: palatal occlusion, lingual occlusion and vestibular occlusion. Clinically, cross occlusion is determined by the anomaly type (severity), the nature of the disturbances (uni- or bilateral), the degree and extent of the dentition junction disturbance, as well as the shape (skeletal or dentoalveolar) of the anomaly, and is manifested by the following facial signs: face asymmetry; disturbed face configuration; chin shift towards the lips; chin slope [42].

In the maxillofacial area, the following functional disorders are typical of all types of cross occlusion: TMJ dysfunction; disturbed chewing efficiency; bruxism; functional failure of the chewing muscles; impaired lateral movements and blockage of the mandible. The most serious changes in the TMJ structures are to be observed in case of occlusion anomalies in the transverse direction. Clinical experts consider articular crossbite as an independent nosological type, where the etiology is determined not only by the TMJ issues, yet also by the asymmetry of the jaws or their parts — abnormal position; misproportionate branches; impaired growth of the body and/or the mandible alveolar part [43–45].

Employing cone-beam computed tomography as an advanced, high-tech, precision method allows not only determining structural and functional disorders of the dentomaxillary system elements in a linear, three-dimensional format, yet also conducting precise and accurate morphometric examinations to diagnose and select proper treatment tactics for patients suffering from maxillofacial area pathologies [46–57].

Knowing the morphological and functional features of dental system alterations through cross occlusion will help improve the methods for differential diagnostics of occlusal disorders in the transverse direction; reduce the time spent on diagnostics; offer explanation for the choice of the plan and method of treatment for dental anomalies; prevent potential complications (relapse); ensure stable long-term results.

Aim of study:
to carry out a morphometric evaluation of the dentition status in people featuring different various facial area growth types with physiological and cross occlusion.

MATERIALS AND METHODS

The survey involved 68 people in their first mature age, with a full set of permanent teeth, physiological occlusion, as well as 12 patients with cross occlusion, comparable in terms of age and gender. Subject to the age-periodization scheme recommended by the VII All-Union Conference on Age Morphology, Physiology and Biochemistry (Moscow, 1965), the first mature age for males is 22–35; for females — 21–35. Patients with physiological occlusion were divided into three groups depending on the jaw growth type. Following clinical experts' recommendations, the jaw growth type was identified by the mandible angle size and Bjork total angle. Group 1 included 41 people (60.3%) with a neutral growth type; Group 2 was 8 people (11.8%) with a vertical growth type, while Group 3 included 19 persons (27.9%) with a horizontal jaw growth type. Clinical, X-ray studies were conducted in strict compliance with the ethical principles of biomedical research and obtaining voluntary informed consent of all patients. The developed and approved provisions were fully consistent with the basic ethical legal and regulatory documents required for conducting research with human participation (Nürnberg Code, 1947; World Medical Association Declaration of Helsinki, 1964).

X-ray research methods for the maxillofacial area involved cone-beam computed tomography and lateral projection X-ray diffraction. Computed tomography was performed on a PaX-i3DSC conical tomograph with FOV cephalostat (17×15 cm) (VATECH Global, South Korea). Processing, storage and export of the X-ray images involved the Ez Dent-i™ software, a multiplanar reconstruction and a three-dimensional (3D) reconstruction — using the Ez 3D-i™ tomograph software for 3D diagnostics; viewing the saved data with an importing option was performed using the Viewer™ software. The thickness of the tomographic

section was 1 mm, the reconstruction step was 1 mm, the rotation step — 1 mm. Of the entire variety of research methods, points and reference lines, the major guidelines were selected for identifying the size of the apical jawbone: the ***A*** point is the upper jaw apical basis or the projection of the medial upper incisor root apex on the alveolar process vestibular surface; the ***B*** point is the mandible apical basis or projection of the medial lower incisor root apex on the vestibular surface of the mandible alveolar part. The occlusal plane was identified following the generally accepted methods. The distal point was the apex of the chewing surface vestibular distal tubercle on the mandible second molar. The anterior (interincisal) point was located in the middle of the distance between the medial incisors cutting edges on both jaws.

To identify the jaws size in the sagittal direction, the occlusal plane was used as the main guideline. The front point for calculating the upper jaw sagittal size was taken in a constructive point at the intersection of the perpendicular to the occlusal plane, drawn from the subspinal ***A*** point. Similar action was performed for the lower jaw, from the supramental ***B*** point. From the distal side, a perpendicular was drawn for the upper jaw, running to the occlusal plane from the ***TM*** point located on the convexity of the upper jaw tubercle distal surface (tuber maxillae) of the infratemporal surface (facies infratemporalis). The distance from the anterior point (***A′***) to the posterior point (***TM′***) matched the sagittal size of the maxilla alveolar process. On the lower jaw, the distal point of the alveolar part was set at the intersection of the mandible angle and the occlusal plane bisector. The distance from the anterior point (***B′***) to the distal point (***Go′***) matched the sagittal size of the mandible alveolar part. Besides, on the computed tomograms there had been the supramental ***B*** point marked in the projection of the antagonist root top apex. The mandible plane ran through the most convex points of the mandible body lower surface. Then a tangent line was drawn to the lower jaw branch. The intersection of the lines shaped the angle of the lower jaw at the ***Go*** point (gonion). The top point of the mandible articular head was defined as ***Cond*** (condylion). Then, a perpendicular from the B point was drawn descending onto the mandible line, while the point obtained through crossing the lines was set as the ***B′′*** point (Fig. 1).

Teleroradiograpy in the lateral projection was performed on an X-ray machine Rayscan Symphony Alpha 3D (South Korea). The results were processed using the RayScanver. 2.0.0.0 software offering the options of receiving, processing and storing data in a DICOM 3.0 compatible format. Shooting features: sensor type — CMOS; resolution detector — 630×1024 pixels; focal spot — 0.5 mm; voxel size — 140–230 microns; magnification — 1.3; time — 2–14 s; panoramic image size — 148 mm. The type of the facial area growth was identified by the mandible angle size, which was shaped by tangent lines to the lower edge of the mandible body and branch. The angle ranging within 119° to 123° corresponded to the neutral type of jaw growth. A decrease or an increase in the mandible angle pointed at the horizontal and vertical growth types of the facial area, respectively (Fig. 2).

Computer software SimPlant, Viewer™, Ez 3Dent-i™, Ez 3D-i™ allowed obtaining precision digital indicators without additional measurements, which significantly reduced the study time and increased the data gained through the study. The photostatic face photographs of the patients with cross occlusion, made in frontal and lateral projections, were used to identify the jaws location in the craniofacial structure. The key reference points were the pupillary line, as well as the perpendicular that was drawn to the pupillary line from the ***N*** point (*nasion*) — the aesthetic center line. A shift of the mental ***Gn*** point (*gnation*) was measured in degrees, between the vertical lines of the aesthetic center and the ***N–Gn*** line. Besides, the horizontal lines position was evaluated, in particular the line passing through the mouth corners in relation to the pupillary line. In case of non-parallelism of these lines, the angle of the labial line was identified. Photostatic side-projection images were used to perform a face analysis following Schwarz A.M. with identifying the profile angles, the location of the jaws in relation to the lines proposed by Kantorowicz A., Ricketts R.M., Simon P.W. and other professionals that are generally recognized in orthodontic practice. An orbital line and the aesthetic center line were also drawn on a computed tomogram in the frontal projection, which was required to evaluate the deviation degree for the chin point and the mandible angles symmetry. An analysis of the teleradiographies was performed subject to generally accepted techniques in front and side projections using a package of applied computer software (Fig. 3).

For statistical analysis of the results, the software products STATISTICA 8.0 and SPSS 22.0 (StatSoft, USA) were used. For each feature, the following were determined: the arithmetic mean value and the arithmetic mean error. To identify the significance of the difference between the averages from the counterlateral sides, Student's t-criterion was identified. To examine the significance of the differences between the mean values, the dispersions analysis (ANOVA) was used.

RESULTS AND DISCUSSION

When identifying the balance of the antero-posterior size of the maxilla alveolar process and the

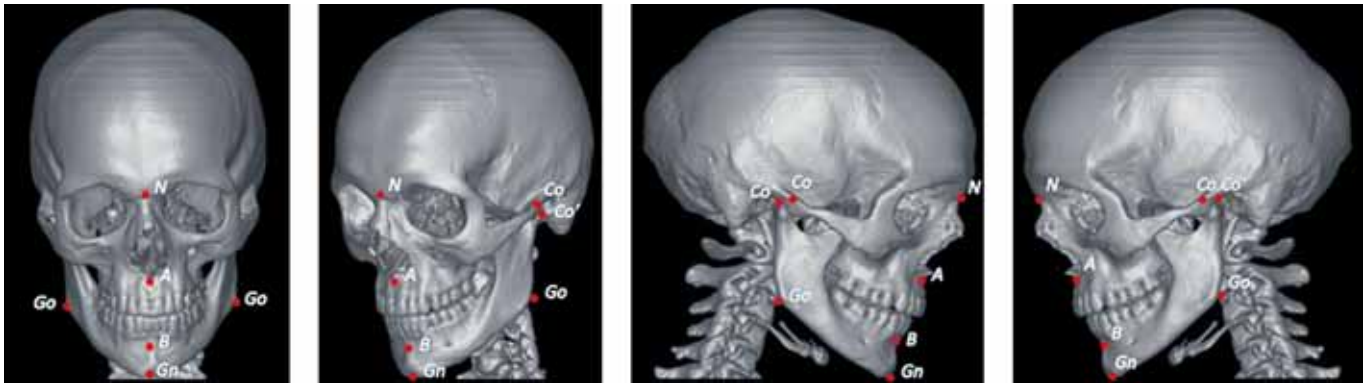


Fig. 1. *Localization of points on a computed tomogram used to study the balance evaluation for the facial area structure: a — frontal; b — turning the head to the right by 3/4; c — lateral left projection; d — lateral right projection*

mandible alveolar part, the lateral teleradiographies revealed that the A′–TM′ distance corresponded to the B′–Go′ length for 52 people of the studied group, which was 76.47±5.14 % of the total number of the participants involved. In 10 people (14.71±4.29%), the sagittal sizes of the maxilla alveolar process exceeded those of the mandible alveolar part by an average of 2.87±1.02 mm. At the same time, the antagonist teeth occlusive relations corresponded to the age and physiological norm and, as a rule, were observed in people with upper medial incisors physiological retrusion, at which the subspinal point A had an anterior shift. In 6 patients (8.82±3.44%), the dimension of the mandible alveolar part was 1.95±1.08 mm larger than the maxillary alveolar bone sagittal size, which, in our opinion, is due to the upper medial incisors physiological protrusion and the posterior shift of the upper apical basis (A point). As a result of the study, we proposed a method for identifying the balance of the mandible branch and body on the lateral teleradiographs.

Method description.

The mandible body sagittal dimension was identified from the Go point to the anterior point (B″), which was located at the intersection of the perpendicular built from the B point to the mandible plane. We suggested the vertical size of the branch be measured from the Go point to the Cond (condylion) point projection on the tangent line to the mandible branch. Taking into account the different scaling of teleradiographs, we used not absolute values of body length and branch height, yet relative indicators, defined as the percentage of the branch height to the mandible body.

In Group 1, the percentage ratio between the mandible branch and the body was 81.72±1.06%, which means the predominance of the jaw body to the branch. In case of the vertical growth type, this ratio was 80.91±1.12%; with the horizontal growth type — 82.19±0.97%. An analysis of lateral projection teleradiographies revealed that the patients with the neutral growth type of the facial area had the mandible angle 120.73±1.18°. During that, the maxillofacial angle shaped by the intersection of the craniofacial and mandible planes was 43.51±2.87°. In people with the horizontal type of the face growth, the mandible angle was significantly smaller ($p \leq 0.05$), 108.93±3.62° in the group as a whole. A significant decrease in the maxillofacial angle down to 36.61±2.17° was also observed. The vertical type of face growth contributed to an increase in the angles up to 126.11±2.19° and 51.24±1.22°, respectively, which can be seen from Fig. 4.

Table 1 offers a view on the results of examining the main angular parameters in the lateral teleradiographies in people with various types of the facial area growth.

Lateral teleradiographies angular measurements in patients with physiological bite revealed that regardless of the facial area growth type, the position of the jaw (upper, lower) in relation to the main anatomical and topographical craniofacial reference marks fell within the physiological norm.

There were no statistically significant differences detected in people with different facial growth types in terms of the ANS angle showing the location of the upper jaw in the facial skull, and the size of the BNS angle, which establishes the mandible orientation regarding the skull. The ANS angle parameters in people with physiological occlusion varied within 84–89°. The BNS angle was slightly smaller than the ANS angle,

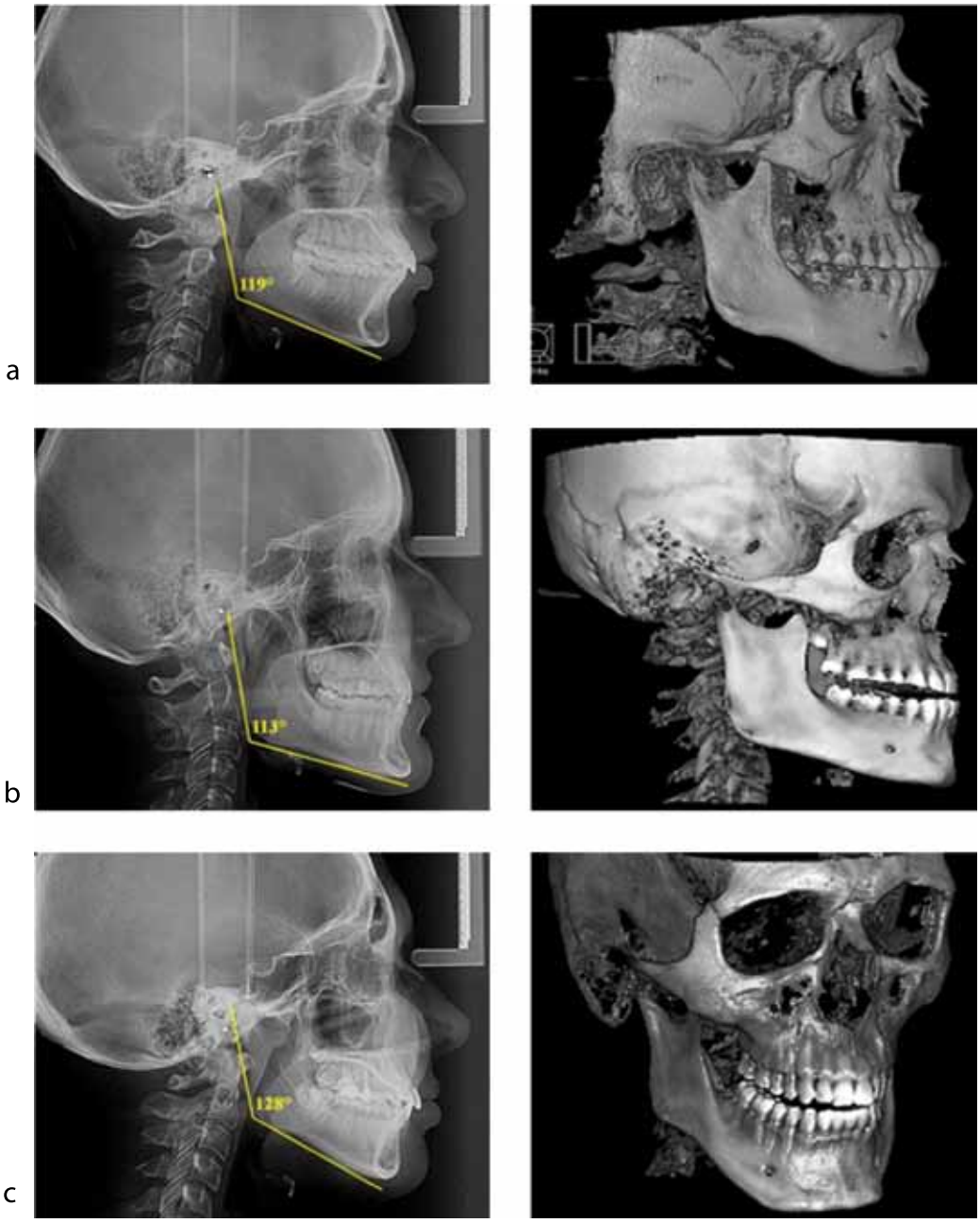
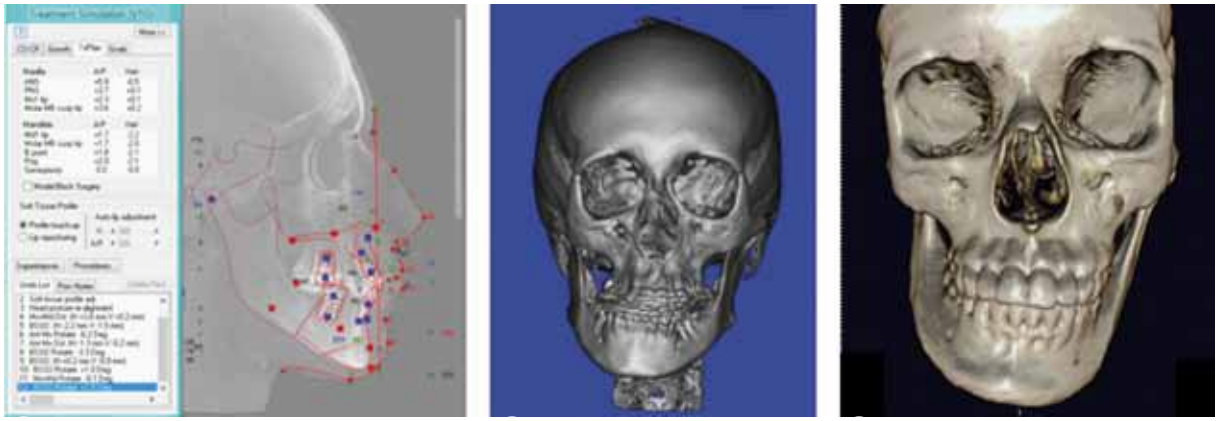


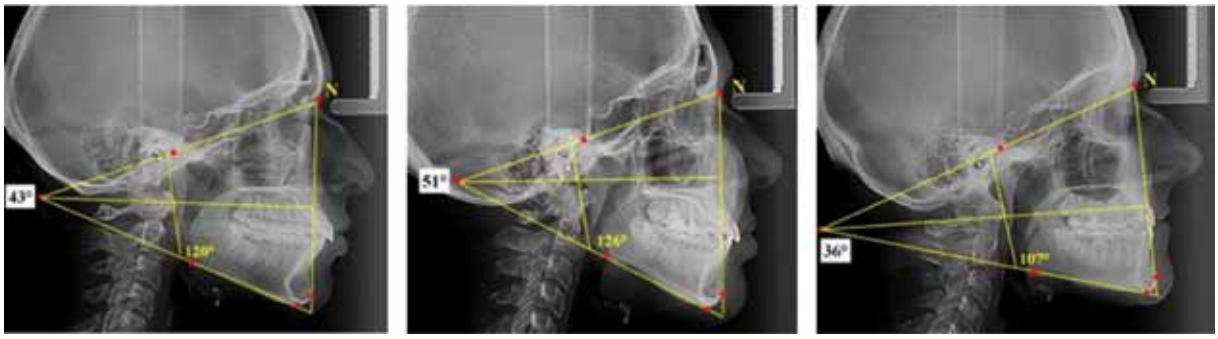
Fig. 2. *The prominence of the mandible angle on computed tomograms and lateral teleradiographies with the neutral (a), horizontal (b) and vertical (c) types of the facial area growth*

yet also did not depend on the facial growth type. The study outcomes showed that in people with different facial growth types, the fluctuation of the ANB angle indicators is 2–3°, while a slight increase in the angle is typical for people with the vertical growth type, and a decrease in the angle — for those with the horizontal type of facial growth. Of interest is the inconsistency of Bjork total angle with the tailored prescriptions since for all the growth types its value was lower than what data from respective literature suggest. However, significant differences within the groups involved in the study, were identified. In people with the neutral growth type, the angle varied from 380° to 384°, with a decrease in the angle typical of people with the horizontal growth type, and an increase — for the vertical facial growth type.



a b c

Fig. 3.Radiography image of a patient with cross occlusion in the lateral projection (a) and computed tomogram in the frontal projection (b, c)



a b c

Fig. 4. Specific features of maxillofacial angle and mandible angle with neutral (a), vertical (b) and horizontal (c) growth types

Table 1. *Lateral teleradiographies major angular parameters in people with various types of facial area growth (degrees), (M ± m)*

The parameters of radiographs	X-ray indices for growth type:		
	horizontal	neutral	upright
ANS angle	87,33±1,15	87,11±1,21	86,52±1,34
BNS angle	85,67±1,46	84,17±1,35	82,38±1,96
ANB angle	1,67±0,96	2,94±0,81	4,12±1,83
Ricketts angle	98,89±1,39	90,44±1,14	86,68±1,38
Bjork angle	375,33±1,27	382,06±2,06	388,53±1,67
N-Go-Me angle	64,33±1,26	71,61±1,98	79,15±1,82
Mandibular angle	113,22±0,84	120,07±1,24	127,65±1,68
OL-ML angle	9,02±0,88	15,04±0,48	17,52±0,51
NL-ML angle	19,78±1,92	29,39±1,75	36,48±2,87

Images of the oral cavity in the anterior projection in patients with buccal (a), lingual (b), buccal-lingual types of cross bite before and after orthodontic treatment (Fig. 5).

Clinical and laboratory examinations revealed that almost all patients featured a shifted mental ***Gn*** point (*gnation*) deviating from the aesthetic center line by 8–12°, depending on the pathology severity. The mouth angle on the shift side, as a rule, was elevated, while drawn down on the opposite side, whereas the line connecting the mouth corners converged with the pupillary line at an angle of 6–10° (Fig. 6).

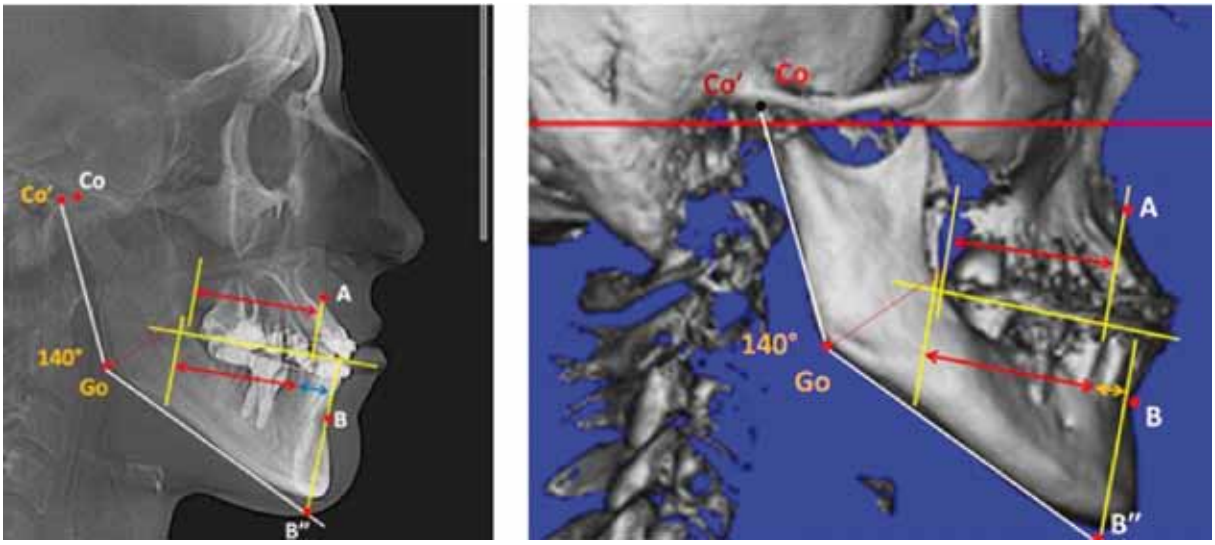
Patients with gnathic cross occlusions typically featured a mandible angle different from that of people with physiological occlusion; the branch vs. body ratio did not meet the age norm, nor did it meet the gnathic and dental facial types (Fig. 7).

Virtually all the patients with cross occlusion featured a shift of the Gn mental point from the vertical line. Notable were the unequal mandible angles, while the tooth-containing parts, both in the maxilla and mandible, often matched each other (Fig. 8).

Further tactics and scope of complex orthodontic and surgical treatment, aimed at improving the jaws

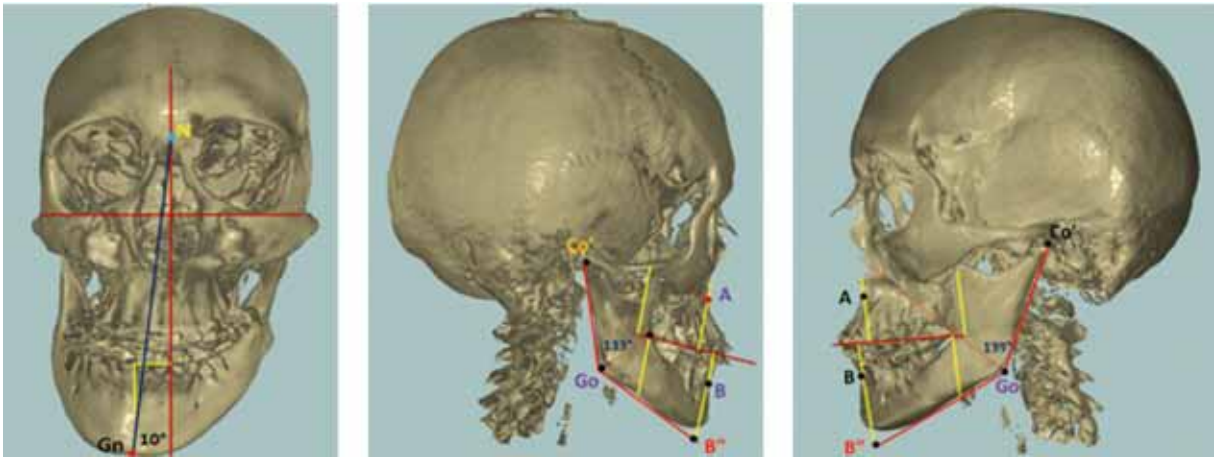
Fig. 5. *Images of the oral cavity in anterior projection in patients with buccal (a), lingual (b), buccal-lingual types of cross bite before and after orthodontic treatment*

Fig. 6. *Facial features in patient with cross occlusion in direct (a,b) and lateral (c) projections*



a b

Fig. 7. Maxillofacial angle and mandible angle features in the lateral teleradiography and computed tomogram in sagittal projection in patient with cross occlusion



a b c

Fig. 8. The position of the mental Gn point regarding the vertical line (a); unequal angles of the mandible on computed tomogram in sagittal projection in patient with cross occlusion, right (b) and left (c)

size and position was developed via computer simulation in view of the maxillofacial pathology severity (Fig. 9).

Comprehensive treatment, as a rule, allows achieving a functional and aesthetic optimum for the maxillofacial area (Fig. 10).

After surgical treatment, orthodontic and prosthetic treatments were continued, aimed at improving the occlusal relations and certain morphometric parameters.

CONCLUSIONS

1. The most reliable and diagnostically significant indicator of all linear and angular parameters that determine the direction and type of the facial area growth is Bjork total angle, which, along with the lower gonial angle (<NGoMe), the front angle according to Ricketts (<BaNPtGn), the angel determining the position of the plane of the mandible base in relation to the anterior skull base (<ML–NSL), the maxillary angle (<B) and the ratio of the face posterior

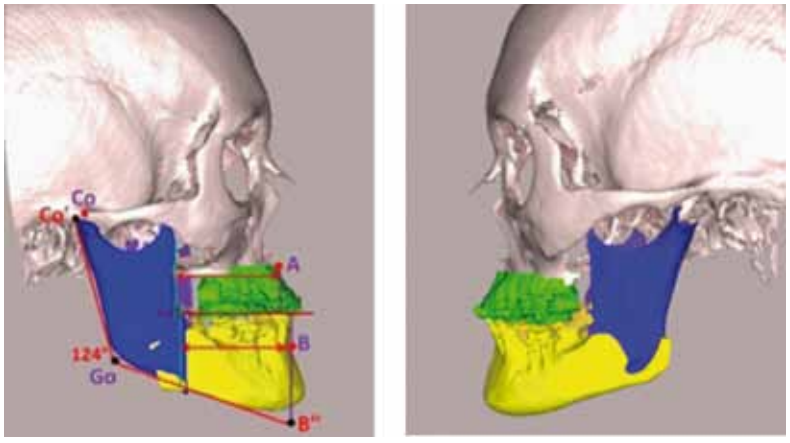


Fig. 9. Planning of surgical treatment for patient with cross occlusion, computed tomograms in sagittal projection, right (a) and left (b)

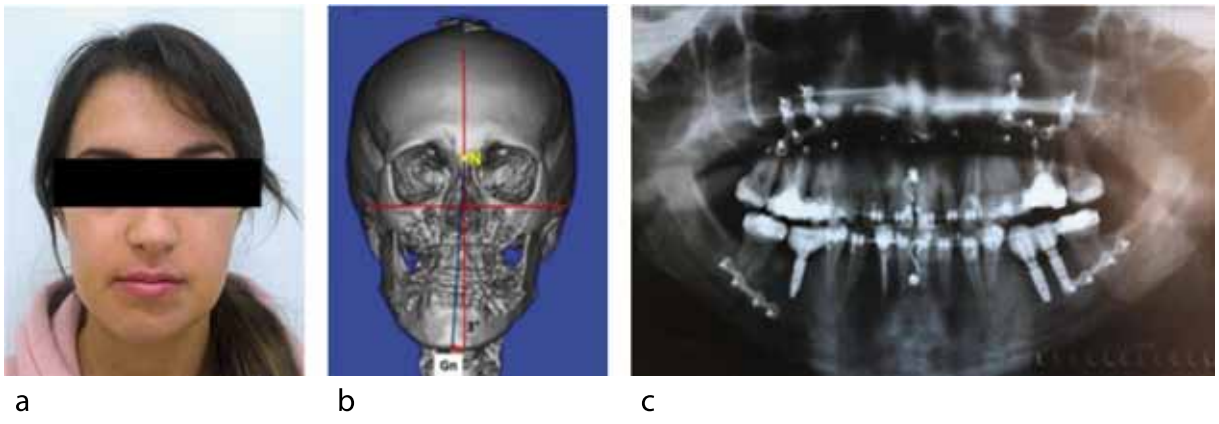


Fig. 10. Maxillofacial area after comprehensive treatment performed on patient with cross occlusion: a — improved facial features; b — reduced mental Gn point shift from the vertical line according to computed tomography (b) and x-ray outcomes (c) in direct projection

height to the face anterior height (SGo:NMe) allows an objective assessment of the facial growth types.

2. A method has been proposed for assessing the balance of the facial area structure, based on the match between the maxilla alveolar process anteroposterior size and the mandible alveolar part, calculated using the head lateral projection teleradiography patterns. We have identified that 76.47±5.14% of the total number of the examined patients with physiological occlusion feature proportionate balance; 14.71±4.29% of the patients had the maxillary sagittal dimensions prevailing, with another 8.82±3.44% patients featuring the prevalence of the mandible anterior-posterior dimensions.

3. The data obtained through the analysis of the clinical & radiology method for detecting the mandible branch vs. body balance indicate that in case of the neutral facial growth type, the size of the mandible body prevails (the mandible vs. body ratio is 81.72±1.06%). The dominance of the mandible body dimensions in relation to the branch was observed in case of the vertical (80.91±1.12% ratio), as well as at the horizontal (82.19±0.97% ratio) facial growth types.

4. Changed face configuration in patients with cross occlusion depends on the mandible transversal shift degree, and include the following set of disturbed facial features: chin shift away from the aesthetic center line (8–12°); raised angle of the mouth on the shifted side, and lowered – on the opposite side; retracted upper lip on the shifted side; lower face flattening side opposite to the shift; the angle shaped by the line connecting the mouth corners and the pupillary line equals 6–10°.

5. The data from the head lateral teleradiographies and computed tomograms in patients with cross

occlusion indicate that the size of the mandible branch and body (shortening) on the shift side does not correspond to the age norm, as well as to the gnathic and dental face types. The mandible angle on the shift side approaches 90° (right angle), while on the opposite side it is 136–140° (developed angle).

6. Offering an accurate and reliable diagnosis, as well as planning a correct and rational orthodontic treatment for patients with cross occlusion, should take studying the head lateral teleradiographic patterns and evaluating the face skeleton growth type.

7. The inclusion of angular parameters that identify the direction and facial growth type in the «Clinical protocols for diagnostics and outpatient orthodontic treatment of dental and maxillary anomalies» will reduce significantly the time that orthodontists spend at the clinical examination and diagnostics stages; improve the efficiency of early diagnostics for transversal occlusion anomalies; prevent temporomandibular joint pathology development; ensure a stable prolonged effect of treatment while reducing the risk of long-term negative effects.

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MODERN METHODS OF NON-INVASIVE CORRECTION FOR DISTURBED REGIONAL BLOOD CIRCULATION THROUGH PHYSIOTHERAPEUTIC MEASURES (LITERATURE REVIEW)

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ABSTRACT — Microcirculatory disorders resulting from disturbed mechanisms for blood flow regulation, or developing as secondary disturbances, are the focal point within the pathogenesis of most pathological conditions in cardiology, surgery, dentistry and other areas of medicine. Aim of study: to analyze and summarize the literature data focusing on the contemporary opportunities for non-invasive correction of regional blood circulation disorders using physiotherapeutic methods.

KEYWORDS — terahertz therapy, disturbed microcirculation.

INTRODUCTION

The microcirculatory bed provides transportation needed for plastic and energy metabolism directly into the body cells, as well as for the removal of their metabolism products, thereby creating the conditions for due functioning in tissues and organs. Coherent interaction of all the links in this system is possible due to regulatory influences from the nervous, humoral mechanisms, as well as the involvement of factors released by endotheliocytes [1, 2].

Considering the important role that microcirculation system plays in most pathological processes, potential correction of microcirculatory disorders and improving trophism in tissues appears a very attractive option in treating a variety of diseases and pathological conditions in dental practice, as well as in offering rehabilitation for patients who underwent reconstructive interventions on maxillofacial areas.

RESULTS AND DISCUSSION

Microcirculation disorders developing as a result of regulatory mechanisms disturbances are known to be the cause behind a number of pathological conditions; in some cases they may be of secondary nature, anyway they are present in most diseases in cardiology, surgery, dentistry, etc. [3, 4].

A powerful factor that aggravates the state of the endothelium in the vascular wall is tobacco smoking. Tobacco intoxication doubles the number of endothelium cells circulating in the peripheral blood, which is a sign of endothelium desquamation. This effect is caused not only by the capacity of nicotine to inhibit NO-synthase, which is involved in the production of endothelial nitric oxide, yet also by the direct toxic effect that cigarette smoke works on endothelial cells [5].

The deterioration of regional blood flow through the development of hypoxia and trophic disorders, changing permeability of the vascular walls, play an important role in the pathogenesis of inflammatory and dystrophic periodontal diseases. It has been also found that in case of insufficient oxygen supply to the tissue, free radical oxidation is activated, which, under progressive inflammatory reaction, will only aggravate the course of the pathological process [6, 7].

Of great interest are the changes in microcirculation occurring in the tissues after a dental implant installation. Numerous studies focusing on this issue have revealed that the status of the vascular bed and the level of blood flow play a key role in reaching osseointegration. Therefore, under ischemization of the surrounding tissues, there is a tendency towards the development of fibrous and cartilage tissues rather than bone structures, which has a negative impact on orthopedic treatment prognosis [8, 9].

For non-invasive correction of microcirculation disorders, the pharmaceutical industry offers antihypoxants or antioxidants — drugs that increase tissues resistance to hypoxia or inhibit the development of free radicals, respectively. Drugs belonging to these groups can be used systemically or locally as part of comprehensive therapy for treating periodontal dis-

eases, in case of face and jaws traumas [10, 11].

However, in case of using pharmacological agents, it is important to consider the risk of side effects and in increase in the cost of treatment. These drawbacks of pharmacotherapy point at the need to search for new non-medication methods for correcting microcirculation issues. Such methods include low-intensity electromagnetic radiation (EMI) of the millimeter-wave range with a wavelength (λ) of 1–10 mm and an extremely high frequency of 30–300 GHz (EHF-therapy), as well as laser therapy, where only the wavelength range is specified (100 to 30,000 nm). It should be stressed that when the requirements for these methods are observed, no side effects will occur, be that mutagenic or carcinogenic. At the same time, the processes of reparation are stimulated, microhemodynamics is activated, while nutrients are improved in the tissues; besides, immunomodulation and analgesic effects are triggered [12].

The advantages of EHF and low-intensity laser therapy include their good compatibility with other physiotherapy methods as well as with each other. Parfenova S.V., et al. (2018), for instance, in her respective work showed possible correction of the platelets aggregation and adhesive activity in chronic inflammatory periodontal diseases, employing a combination of EHF and laser irradiation using a Matrix device [13].

Recently, a large number of works have been focused on the therapeutic application of terahertz frequency range electromagnetic radiation (EMR THF; 100 GHz – 10 THz), which stands in between extremely high frequencies and the optical infrared range. This wavelength range is interesting in terms of its containing the molecular emission and absorption spectra (MSEA) of such inevitably important cellular metabolites as molecular oxygen, nitrogen oxide, carbon dioxide and a number of others. The potential for controlling the reactive capacity of the mentioned metabolites in order to mediate metabolic regulation in cells and tissues explains the huge interest that researchers take in this method [14].

A number of papers have focused on studying the effect of terahertz radiation at the nitric oxide MSEA frequencies of 150, 176–150, 664 GHz. Nitric oxide (NO) is well known as one of the most important vascular wall tone regulator. Being a powerful vasodilator and antiplatelet agent, nitric oxide is important in regulating blood circulation and proper blood supply to organs and tissues [15].

The effectiveness of THF therapy in this frequency range has been shown in a series of experiments on animals where hemodynamic disturbances were triggered by acute or chronic immobilization stress [16].

The normalizing effect of EMR THF at frequencies 150, 176–150, 664 GHz on the antioxidant blood system [17], on the state of the gas and electrolyte composition of blood [18] has been shown.

Clinical studies of THF-radiation of this frequency range showed their high efficiency in cardiology, traumatology, and dentistry. Techniques have been developed for exposure to EMR THF at nitric oxide frequencies, which increase the effectiveness of treatment for patients with angina pectoris [19].

The capacity of terahertz radiation at frequencies of 150, 176–150, 664 GHz to improve blood rheological properties and microcirculation allow using it for prevention of venous thrombosis in patients with lower limb fractures [20], as well as a factor stimulating regeneration and shortening the consolidation period for fractured bones [21].

THF electromagnetic radiation at the frequency of nitric oxide MSEA has found its application in dentistry. A.V. Zelenova, N.V. Bulkina, et al. (2015) compared the results obtained through using a standard mode for treating patients with rapidly progressing periodontitis and the outcomes of applying the same mode yet combined with a course of THF therapy at NO frequencies. A comparative analysis involving the two groups showed that there was better dynamics of inflammation relief, as well as a more complete restoration of blood supply in periodontal tissues observed in the group that featured THF therapy as part of the treatment [22].

Equally interesting for experimental and clinical medicine is the radiation of the terahertz range at the frequencies of the atmospheric oxygen radiation and absorption molecular spectrum of 129 ± 0.75 GHz. In biological systems, oxygen acts as the main oxidant, an essential link in energy-intensive metabolic processes. It is part of plastic and energy metabolism reactions, the synthesis of biologically active substances required to maintain the vital functions of the body [23].

The group of authors conducted a series of experimental studies demonstrating possible correcting effect of terahertz range electromagnetic waves with a frequency of 129.0 ± 0.75 GHz on blood coagulation and fibrinolytic properties, nitrite concentration, and systemic hemodynamic parameters [24, 25, 26].

The antianginal effect of terahertz therapy on the atmospheric oxygen molecular spectrum frequency has been studied, as well as its effectiveness in treating unstable angina has been demonstrated [27].

The study by L.A. Zyulkina (2018) proved, both experimentally and clinically, the feasibility of using a specially developed course of irradiation with electromagnetic waves in the terahertz range at a frequency of 129.0 GHz through various stages of treating inflammatory and dystrophic periodontal lesions [28].

According to N.V. Bulkina, L.A. Zyulkina, et al. (2017), planning of reconstructive dental interventions should be carried out in view of the oral microcirculation system functional status. Dystrophic and inflammatory changes in the oral cavity are accompanied by disturbed microcirculation and functions of the vascular wall endothelium, which affects the course of the regeneration & reparation. Improving microcirculation through THF-therapy at the frequency of atmospheric oxygen of 129.0 GHz in the intervention zone takes place as a result of restoring the vascular wall balance, i.e. due to the endothelium factors (both vasoconstricting (endothelin (1-38, big), asymmetric dimethylarginine (ADMA) and vasodilating (endothelial nitric oxide synthase (eNOS), nitrite level) [29] expressed by the vascular wall.

Non-invasive correction of regional blood flow disturbances through physiotherapy can be used for treating various pathologies in the maxillofacial region [30-41].

CONCLUSION

In view of the above, the data available from respective literature shows that the modern physiotherapy method, terahertz range electromagnetic radiation, has a powerful potential in regulating various physiological processes, including systemic and regional hemodynamics and blood rheological properties. Considering the fundamental role that blood supply plays in most pathological processes affecting organs and tissues, search for new ways of its non-invasive correction through THF-therapy appears quite deserving due effort and requires further investigation.

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KETOGENIC DIETS: OPPORTUNITIES AND LIMITATIONS IN PRACTICAL APPLICATION (LITERATURE REVIEW)

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ABSTRACT — During almost 100 years ketogenic diets (KD) were used for treatment of epilepsy and certain metabolic deficiencies (GLUT1 deficiency and Pyruvate dehydrogenase (PDHD). It has been widely suggested that KD and their preparations might be used to treat obesity, cancer and to prevent neurodegenerative diseases (dementia, Alzheimer's, etc.), diabetes and cardiovascular diseases and even aging.
The literature review provides evidence for KD efficiency in reduction of epileptic seizures in children and adults. However, the majority of research and the professional communities of dietologists, such as German Nutrition Society and German Cancer Soceity warn of adverse health effects of KD when used for other conditions. Additional studies are therefore required.

KEYWORDS — classic ketogenic diet, ketogenic diet, modified Atkins diet, Low Glycemic Index-Treatment, intermittent fasting, Alternate - day fasting, High Fat Low Carbohydrate, time-restricted feeding, overnight-fasting, medium chain fatty acids, long chain triglycerides, medium chain triglycerides, fatty acids, carbohydrates, proteins, amino acid, ketones, β-hydroxybutyrate, adenosine triphosphate, ketogenic metabolic therapy, obesity, epilepsy, glucose transporter type 1 deficiency syndrome, Pyruvatdehydrogenase Deficiency, Multiple Acyl-CoA-Dehydrogenase Deficiency, hyperinsulinism, type 2 diabetes.

Abbreviations:

FA: fatty acids
CH: carbohydrates
AA: amino acid
βHB: β-hydroxybutyrate
AcAc: acetoacetate
MCT: medium chain triglycerides
MCFA: medium chain fatty acids
LCT: long chain triglycerides
LCFA: long chain fatty acids
ATP: adenosine triphosphate
Glut1-DS: glucose transporter type 1
MADD: Multiple Acyl-CoA-Dehydrogenase Deficiency
PDHM: Pyruvatdehydrogenase-Mangel
KD: ketogenic diet
CKD: classic ketogenic diet
HFLC: High Fat Low Carbohydrate
MAD modifed Atkins diet
LGIT: Low Glycemic Index-Treatment:
IFD: intermittent fasting
TRF: time-restricted feeding
OF: overnight-fasting
ADF: Alternate - day fasting
T2D: type 2 diabetes
DGE: German Nutrition Society

KETOGENIC DIETS

The popularity of the classic ketogenic diet (CKD), developed for the treatment of childhood epilepsy in the 1920s and widely used in the decade to follow, has declined with the confirmation of the effectiveness of anti-convulsants.

In the mid-1990s, the Hollywood producer Jim Abraham, whose son had successfully dealt with his seizures by means of the classic ketogenic diet, established a foundation to attract attention and revive scientific interest in the diet.

Experts believe that a ketogenic diet works in half of the cases, and in one third of cases it produces very good results (1). In 2008, a randomised clinical trial found an advantage in children with difficult cases of epilepsy who adhere to a classic ketogenic diet (CKD). An improvement in treatment over a period of 6–24 months was observed in over 90% of cases (1, 57).

A "classic" ketogenic diet (CKD) is a strictly balanced diet consisting of a fixed weight ratio (content) between fats, proteins and carbohydrates (standard 4:1, i.e. 4 grams of fat to 1 gram of protein and carbohydrates). For children under 2 years of age, this ratio is 3:1, otherwise the age requirement for protein is not guaranteed (1).

The fats used in the classic ketogenic diet (CKD) are mainly triglycerides with long-chain fatty acids (LCT). There are also options for the use of medium chain triglycerides (MCTs).

The protein content of the ketogenic diet is calculated using DGE recommendations for each age group (2).

In general, the amount of fat, protein and carbohydrates is calculated proportionally according to a fixed weight ratio. This fixed ratio should be maintained for each meal [3].

The KD is based on the formation of ketone bodies from acetyl-CoA, which is formed by the breakdown of fatty acids. In this context, ketone bodies are the water-soluble molecules (acetoacetate(AcAc), beta-hydroxybutyrate (βHB), and the spontaneous breakdown product of acetoacetate, acetone) (4, 5).

The path to the formation of ketone bodies (ketogenesis) occurs in the mitochondria of liver cells.

In this catabolic pathway, fatty acids are converted into acetoacetate at the end of β-oxidation through acetyl-KoA.

There are three ways to trigger ketosis – meaning an increase in the level of ketone bodies in the blood. These are fasting (hunger), exercises, or a very high-fat diet with simultaneous reduction of carbohydrate intake (6, 7, 8). In all three cases, the glucose deficit and the stimulation of lipolysis trigger the production of an alternative source of energy, which is particularly important for the brain. This alternative source of energy is the ketone bodies, and especially βHB, which is the most abundant among them (9).

In order to significantly increase the level of ketone bodies in the blood (> 2 mmol/L), 60–90% of daily energy intake (E%; Low Glycemic Index Treatment (LGIT) vs. classic kD 4:1) need to come from fat (10). Generally, it is assumed that in adults, a carbohydrate (CH) intake of over 50 g per day will not produce ketosis or will only do so to a very limited extent (11). The fact that this diet is so different from a normal balanced diet leads to problems with compliance, which in turn leads to treatment discontinuation (12, 13).

Therefore, ketone bodies are the norm and are present in human blood at all times, albeit at very low concentrations.

Recent discoveries have revealed that ketones, such as acetoacetate (AcAc) and its precursor β-hydroxybutyrate (β-HB), are not only viable fuel sources for all cells with mitochondria, including the brain (14, 15) but are also legitimate signalling molecules, eliciting advantageous changes in inflammation (16), cognition (17,18), oxidative stress (19) and more. Beyond pathology, ketones may also be a relevant metabolic fuel in the context of physical activity, improving athletic performance (20) and myocardial adenosine triphosphate (ATP) generation (21, 22).

It is important to note that the ketotic metabolic state for the most part of the history of humans was a physiological state. People had to move a lot, and the availability of food was not sufficient and guaranteed.

However, over the past 200 years, by increasing the consumption of simple carbohydrates, increasing energy consumption and changing lifestyles, ketosis has been an exceptional state of the body.

The first signs of ketone bodies in the body were found in the urine of diabetic patients in the middle of the 19th century. For a long time, it was believed that these were undesirable, even non-physiological by-products of incomplete fat burning. Based on this assumption, the well-known expression "fats burn in the carbohydrate furnace" was invented, which according to the current state and knowledge is considered to be obsolete.

Once considered "metabolic garbage," ketones have become the focus of significant efforts within the realm of cardiometabolic research.

Initially, a ketogenic diet was used only for the treatment of pharmacoresistant epilepsy in children. Back in the 1980s, exogenous intake of ketone bodies had already caught the attention of the field of sports medicine (23, 24). Later on, possible medical applications started to be discussed and compassionate use treatments were carried out using sodium βHB in various rare metabolic diseases, such as Multiple Acyl-CoA-Dehydrogenase Deficiency (MADD), PDHD, and hyperinsulinism (25–27).

Therefore, earlier clinical studies have focused on the use of "extreme" versions of ketogenic diets for the treatment of seizures in epilepsy and other conditions.

However, recent studies suggest that metabolic changes associated with the treatment of epilepsy, weight loss, metabolic syndrome and type 2 diabetes can be achieved with more gentle approaches. They are less restrictive of carbohydrates and proteins, and are, therefore, safer and easier to implement, also in other groups of patients (14).

A "well-formulated" ketogenic diet is generally characterised by a total carbohydrate intake of less than 50 g/d and a moderate protein intake of approximately 1.5 g/d per kg of reference weight (28). This typically increases circulating ketone bodies (β-hydroxybutyrate (BHB) and acetoacetate (ACA) from concentrations that are typically less than 0.3 mM into the range of nutritional ketosis, which for BHB, we define as 0.5–3 mM (29). This range is below the typical 5–10 mM range for BHB that occurs during prolonged fasting, and well below concentrations characteristic of ketoacidosis (28, 29, 30). From the perspective of meeting energy demands, the reduced carbohydrate and moderate protein intakes necessarily make ketogenic diets high in fat.

Thus, ketogenic diets have been studied sporadically for over 100 years, however, over the past 15 years an increasing number of researchers have concluded that the process of keto-adaptation may be associated with a wider range of health benefits than just the treatment of epilepsy (30-54).

Increasing general and scientific interest in the KD has led to new areas of application being opened up. Despite contradiction with mainstream dietary guidelines, ketogenic diets may be beneficial for many health conditions, particularly the previously mentioned conditions related to mitochondrial impairment, which includes obesity (31, 32), diabetes (33–34), cardiovascular disease (35–37), cancer (31, 39–47), neurodegenerative diseases (dementia, Alzheimer's, etc.) (40, 41, 48–51), and even aging (52–54, 55, 56).

In terms of trend diets using ketone bodies or the KD, the appetite-reducing effect is one of the most commonly cited advantages (30).

The possible benefits of using a ketogenic diet for a wide range of different diseases are still being actively discussed.

Thus, a ketogenic diet simulates metabolic hunger, creating a glucose deficit.

However, in the case of a ketogenic diet (KD), ketones are formed from the fatty acids in the diet, not only from the body's own fat reserves.

The brain usually prefers to use glucose as the main source of energy. Glucose accumulates in the body, primarily in the liver, in the form of a special substance, glycogen. Glycogen forms an energy reserve, which can be quickly mobilised to compensate for sudden glucose deficiency, if necessary.

Some tissues, such as brain and red blood cells, depend on a constant supply of glucose. If the amount of carbohydrates obtained from the food is insufficient, the necessary concentration of glucose in the blood may be maintained for some time by the breakdown of glycogen by the liver. With a glucose deficiency in the body, glycogen under the influence of enzymes is broken down to glucose, which enters the bloodstream. If these reserves are also depleted, the liver starts the de novo synthesis of glucose, gluconeogenesis.

Glucose can also be obtained from proteins and some other sources (gluconeogenesis). However, after only about two weeks of food deprivation, protein reserves will be depleted solely because of the necessary supply of glucose to the brain, and death will inevitably occur (58).

During periods of starvation, however, when glucose is not sufficient, ketone bodies can replace glucose as the main source of energy for the human brain.This is a prerequisite for the body to survive long periods of starvation using fatty deposits, without carbohydrates during periods of hunger (57, 59).

In addition to supplying the brain, ketone bodies also play an important role in the metabolism of hunger to provide energy to the heart muscle, skeletal muscles and kidneys.

The heart and kidneys can also use ketone bodies as an energy supplement under normal physiological conditions. In adults, the liver can produce up to 185 g of ketone per day. The proportion of free ketone bodies is individually considered very different (60).

Based on β-hydroxybutyrate (βHB), the normal concentration in the blood serum after meals is approximately 0.05 mmol/l and 0.4 mmol/l after an overnight fasting. After 2–3 days of fasting, the levels of 2–3 mmol/l can be reached. In case of prolonged fasting it can reach 7 mmol/l, and in case of a strict ketogenic diet — it is possible to reach a concentration of 4 mmol/l (61).

If too many ketone bodies are formed, they accumulate in the blood and penetrate the urine. In medicine, this condition is called ketonuria, or acetonuria, and its common name is "acetone in the urine". It is very dangerous and can cause serious poisoning, and in the case of people with diabetes or small children it may lead to an acetonaemic coma.

Healthy people have acetone in their urine when they are overworked and hypothermic, and the energy is used more quickly than it is replenished.

A ketogenic diet forces the body to use fats as the main source of energy. Usually, carbohydrates from food are processed into glucose, which is essential for the nourishment and functioning of the brain. However, if the diet contains little carbohydrates, the liver converts fat into fatty acids and ketone bodies. Ketone bodies enter the brain and are used as an energy source instead of the glucose.

KETOSIS AS A RESULT OF A DIET WITH HIGH FAT CONTENT AND LOW CARBOHYDRATE CONTENT

In addition to the already mentioned method of increasing the concentration of ketone bodies in the blood serum due to starvation, this can also be achieved by intensive physical activity.

However, there is a possibility of a targeted diet with a very high fat content and very low carbohydrate content (High Fat Low Carbohydrate, HFLC) (66).

Typically, if the consumption of carbohydrates exceeds 100 g, no significant increase in ketone bodies in the blood (ketosis) should be expected. An increase is only possible with an intake of up to 40 g, however, there are strong personal differences in this regard (60).

Frequently, a maximum carbohydrate level of 50 g per day also causes ketosis (65).

Physiological ketosis, which also occurs during a ketogenic diet (KD), must be clearly differentiated from pathological ketoacidosis, such as diabetes mellitus.

In case of a dietary ketoacidosis, maximum ketone body concentrations of up to 7–8 mmol/l are achieved, whereas in diabetic ketoacidosis concentrations can rise above 25 mmol/l.

The target ketosis (the desired concentration of the ketone body in the blood) in the context of such a diet varies from 2 to 7 mmol/l and can only be achieved if strict dietary requirements are met (5).

Depending on the references, there are also values in the range of 3–5 mmol/l or 2–5 mmol/l (according to S1's KD recommendations) (64, 67).

In addition, in the case of physiological ketosis, there is no glucosuria (urinary glucose) associated with hyperglycemia and concomitant reduction of plasma pH (59, 62).

When using ketogenic diets, an important consideration is also given to the concept of the ketogenic ratio.

The ketogenic ratio is the ratio between fat and carbohydrate/protein content in the diet. In a classic ketogenic diet, the ratio is 4:1, perhaps even 3:1.

Thus, per 1 g of carbohydrates + proteins (in total), it is necessary to obtain 4 g (3 g) of fat with food.

The common assumption that any of the low-carbohydrate diets leads to a significant increase in ketones in the blood is not true. Regardless of the level of carbohydrates in low-carbohydrate diets, protein intake is usually too high and the amount of fat is too low (57).

Some proteins, or, more precisely, certain amino acids, can be metabolised to glucose and thus prevent ketogenesis. These "glucogenic" amino acids make up to 58% of the dietary protein (58).

The main area of application and recognised indications for ketogenic diets.

Pharmacoresistant epilepsy in childhood and adolescence. Epilepsy is a complex condition that makes a child susceptible to seizures. Seizures result from abnormal electrical activity in the brain: Some parts of the brain get over-excited and fire off too many electrical signals. Treatments for epilepsy have expanded greatly in recent years to include many new medications, specialized diets and a wide range of surgical strategies. (70). If there is no effect from the treatment of two or more professionally used anticonvulsants, ketogenic diet can be assigned. Diet therapy can sometimes be a good option for childhood epilepsy when medications don't control seizures or cause intolerable side effects. It can be especially helpful for certain types of epilepsy, such as myoclonic astatic epilepsy (Doose syndrome). The mid-term effects of a classic ketogenic diet in children are comparable with modern anticonvulsants (Levy et al., 2012). Some cases (about 10%) show pharmacoresistant (also refractory) epilepsy, which cannot be adequately controlled by drugs (68).

In these cases, KD (the ketogenic diet) is a good option (67, 68), and its short- and medium-term effects in children, according to Cochrane reviews, are comparable to those of modern anticonvulsants (73).

A modern approach to epilepsy provides diet therapy despite a good pharmaceutical therapy success. A number of different diets can be used for epilepsy. These include the Classic Ketogenic Diet (CKD), the Modified Atkins Diet (MAD), the Medium Chain Triglyceride Diet (MCTD) and the Low Glycemic Index Treatment Diet (LGITD). Which diet is best for child will depend on the epilepsy diagnosis, child's age and eating habits, and family needs and preferences. It is possible and sometimes helpful to transition between the various diets.

Diet therapy takes a strong commitment, but it may offer children a better chance of seizure control than trying a new medication. Because there isn't yet a good way to tell exactly how a child will respond to diet therapy, a trial of three to four months is usually recommended.

The ketogenic diet has been shown to be effective for many children with epilepsy when drugs fail. It can provide control of seizures for about 30 percent of children with epilepsy. The mechanism of action of KD in epilepsy is still unclear. There are many suggestions as to why this dietary treatment is showing good results (67). In addition to the assumption that ketone bodies are responsible for anticonvulsant properties, there is evidence of the anticonvulsant effects of some fatty acids (capric acid, C10) (70, 71, 72).

In its strictest form, the ketogenic diet (CKT) provides more than 90 percent of its calories through fat (compared to the 25 to 40% usually recommended for children). When the body burns fat for energy, rather than glucose from carbohydrates, it produces compounds known as ketone bodies. The increase in ketones is called ketosis. Experts believe ketosis reduces seizure activity in the brain, although exactly how this works is still unknown.

Though the diet is challenging, it's easier than it used to be. As interest in the diet has increased, "keto-friendly" products have become more widely available. These include coconut oil, low-carbohydrate noodles, sugar-free syrups and flavorings, and nut flours.

Nowadays, the use (CKT) in epilepsy is mainly limited to the childhood period. However, good therapeutic results are also reported for adults (74). Similar side-effects (hypoglycemia, excessive ketosis, etc.) have been described in children and adults during (CKT), but adolescents and adults have a higher tendency to not follow this complex diet therapy (74).

Because of easier use, adolescents and adults prefer MAD (modified Atkins' diet) to the CKT (69). Overall, the adult refusal rate is more than 50% for the classic diet (CKT) and an average of 28–42% for MAD (69, 74).

Ketogenic diets have also been used to treat rare brain energy metabolic defects, such as Glut1 and Pyruvate dehydrogenase deficiency (PDHD) deficiencies.

The first description of the GLUT1 deficiency syndrome was made in 1991 (75). The glucose transporter type 1 (Glut1) is the most important energy

carrier of the brain across the blood–brain barrier. In the early nineties, the first genetic defect of Glut1 was described and known as the Glut1 deficiency syndrome (Glut1-DS). It is characterised by early infantile seizures, developmental delay, microcephaly, and ataxia. Recently, milder variants have also been described. The clinical picture of Glut1 defects and the understanding of the pathophysiology of this disease have significantly grown. A special form of transient movement disorders, the paroxysmal exertion-induced dyskinesia, absence epilepsies particularly with an early onset absence epilepsy and childhood absence epilepsy, myoclonic astatic epilepsy, episodic choreoathetosis and spasticity, and focal epilepsy can be based on a Glut1 defect.

The use of KD is based on the fact that ketone bodies pass through the hemato-encephalic barrier through another receptor (MCT1) and thus serve the brain as a substrate for alternative energy. The treatment is usually done using classic KD with a ratio of 3:1 or 4:1. Over the last few years, there has been growing evidence that MAD is also suitable for the treatment of GLUT1 deficiencies (76, 77). MAD can also be used for adult patients or in case of non-compliance. There is not yet enough data for such statements to be made about the LGIT or MCT diet (77).

Pyruvate dehydrogenase deficiency (PDHD) is a rare neurometabolic disorder characterised by a wide range of clinical signs with metabolic and neurological components of varying severity. Manifestations range from often fatal, severe, neonatal lactic acidosis to later-onset neurological disorders.

Pyruvate, the final product of glycolysis, can only be metabolised to acetyl-CoA for a limited energy use in the citric acid cycle (76, 78). The consequences of this disorder include, but are not limited to, increased lactate (hyperlactatemia) and lack of energy (78). The neurological type is characterised by lesions of the brain and nervous system. However, it is worth noting that the isolated form of the disease is extremely rare. As a rule, patients are found to have a transient type of the disease, which is manifested by neurological and metabolic disorders.

The KD bypasses the pyruvate dehydrogenase reaction. Acetyl-CoA, which is essential for the citric acid cycle, is formed from fats rather than the glucose (76). In general, a KD is a safe and effective treatment for most PDG patients (Pyruvate dehydrogenase deficiency (PDHD) (78).

Ketones may be an alternative energy supply for the reduced glucose transport through the hemato-encephalic barrier (Glut1 defeciency) or impaired glucose degradation (PDH deficiency) and thus effectively treat emerging epileptic encephalopathy (79).

New ketogenic diets are becoming increasingly popular (57):

- in older children, adolescents, in cases of problems with compliance with treatment requirements;
- therapy for neurodegenerative diseases and craniocerebral injury (Masino and Rho, 2012);
- weight loss in adults.

Increased central nervous system energy reserves lead to higher neuronal resistance, a reduction in free radicals and a decrease in inflammatory activity, which makes it possible to use ketogenic diets more widely. Ketogenic diets are performed under medical supervision and are achieved only in the team of the patient, parent, pediatrician and specialist nutritionist.

It is necessary to calculate the ratio of LPG individually and to add carbohydrate-free additives (vitamins, minerals, trace elements) in all ketogenic diets.

The ketogenic diets are not currently recommended for adults (57).

DIFFERENT TYPES OF KD DIETS

In clinical use, the general term "ketogenic diet" includes four diets (80, 81):

- the modified Atkins Diet (MAD),
- MCT (medium-chain triglyceride diet), triglycerides with a medium chain length,
- low glycemic index treatment (LGIT),
- a classic ketogenic diet.

Recently, ketosis has been increasingly reported as a possibility for some forms of intermittent fasting (intermittent fasting 5:2), but usually it is not considered to be a ketosis diet (82).

THE MODIFIED ATKINS DIET (MAD)

The Atkins diet was developed by an American doctor Robert Atkins in 1972 and published under the title "Dr. Atkins diet revolution". Atkins advocated a diet in which the carbohydrate consumption was extremely limited, but one could eat proteins and fatty foods, such as meat, fish, sausage, cheese and eggs as one wished. In the initial stage of this diet, only 5 g of carbohydrates (83) are released daily.

In the modified Atkins diet, which is used as a ketogenic diet, this amount is about 10–15 g of carbohydrates per day.

This diet is more commonly used for children and adolescents in the early stages.

For adults, the daily starting amount is 20 grams of carbohydrates. No ballast substances are included in the calculation of the carbohydrate consumption, but sweet alcohols (xylitol, sorbitol, erythritol) are part of it.

KETOGENIC DIETS IN COMPARISON (57)

Diet type	Fat content %	Protein content %	Carbohydrate content %	Protein to carbohydrate ratio
Classic ketogenic diet	90	6	4	4:1
modified Atkins Diet (MAD),	64	30	6	5:1–2:1
MCT	73 (incl. 30–60% MCT oil)	10	17	1.2:1 – 1.6:1
Low glycemic index treatment (LGIT)	60	30	10	1:1
Regular diet	35	15	50	0.3:1
CKD: classic ketogenic diet;	MAD: modifed Atkins diet;	MCT: medium chain triglycerides diet;	LGIT: Low Glycemic Index-Treatment	

When doing the MAD, there is no indication as to which carbohydrates should be consumed predominantly. However, it is recommended to choose food with predominantly complex carbohydrates.

At a later stage, depending on the clinical picture, the carbohydrate consumption can be gradually increased to a maximum of 60 g/day. The protein consumption is not limited with the MAD and can reach up to 30% energy consumption (84).

The ketogenic ratio of the modified Atkins diet is 1: 1 to 1.5: 1.

The ketosis achieved with this form of diet is usually lower than with a classic ketogenic diet, but this diet is also good for the treatment of pharmacoresistant epilepsy (85). The calculation of meals is much simpler and the time spent on learning and using the diet at home is shorter because only carbohydrates are counted.

MCT DIET, USING THE MCT OIL (MEDIUM-CHAIN)

Medium Chain Triglycerides (MCT) are a type of synthetic dietary fat first produced in the pharmaceutical industry from coconut oil in the 1950s for the treatment of patients unable to digest conventional fats. Having been used so far for medical purposes, the MCT oil has also become a popular fitness supplement, which is advertised as a fat burner, muscle growth accelerator and energy source.

Medium chain triglycerides consist mainly of fatty acids with 8 and 10 carbon atoms (medium chain fatty acids, MCFA).

MCT (or MCT-fats) are triglycerides that contain fatty acids with medium chain lengths. Unlike long-chain fatty acids, MCTs can be absorbed in the intestine independently of bile acids and pancreatic lipases.

From the small intestine, they are transported through the blood directly to the liver. There they are preferably oxidised in comparison with conventional fats and have a higher ketogeneity than long-chain triglycerides (LCT).

The term "ketogenicity" refers to the rate of formation of ketone bodies, which varies depending on the initial fat [86, 87].

Fatty acids with an average chain length include capron (C 6: 0), caprilic (C 8: 0), caprilic (C 10: 0) and lauric acid (C 12: 0). No pure MCT fat is found in nature.

For example, coconut fat contains 60% MCT, milk fat 10% MCT.

Commercially available MCT fats are usually extracted from coconut fat or palm kernel oil. At the same time, MCT fat is available in the form of transparent, tasteless oil, margarine and drinkable emulsion for therapy. (Table 2).

MCT oils and fats are usually prescribed for lipid metabolism disorders, malabsorption of fats associated with diseases (e.g., pancreatic insufficiency, short intestinal syndrome) and KD. It is also prescribed for parenteral nutrition. They are also very popular among athletes.

In comparison, structurally shorter MCTs (8.4 kcal/g) have a 10% lower energy content than long-chain fats (9.3 kcal/g) [88]. On the packaging of MCT products, information on the nutritional value is given in accordance with the EU Regulation 1169/2011 with 9 kcal/g or 37 kJ/g. Fat differentiation is not provided here (89).

Since MCT fats can cause gastrointestinal discomfort, it is recommended to slowly increase their amount. Infants and young children start at 1 g/kg body weight, older children and adults start at 10–20 g/day, and then increase the amount in several steps to the desired daily dose.

As early as 1971, HuttenlocHer et al investigated the effectiveness of MCT fats in a ketogenic diet in 12 children. Patients received 60% of the required energy in the form of MCT fats.

This corresponded to more than 100 g of MCT fat per day.

Children were given MCT oil mixed with low-fat milk three times a day for the main meals. The benefits of such a diet with the MCT were described as follows: "MCT fats lead to ketosis faster, so the carbohydrate content in the diet does not need to be reduced so much, the efficiency is comparable to a ketogenic diet in a ratio of 3:1. Compatibility is better and implementation is easier. (87)

The MCT diet, according to some researchers, has a comparable effect to the classic KD (90, 91). Today, an even distribution of MCT fats is recommended for all meals (91, 92).

LOW GLYCAEMIC INDEX THERAPY (LGIT)

The term glycemic index (GI) was used in 1981 by Jenkins et al. GI is a calculated value representing the increase in the blood glucose concentration (calculated as an area under the curve) after a certain amount (50 g) of food intake compared to the reference (glucose or white bread). Consequently, products with high GI (simple sugars) result in higher blood glucose concentrations than products with low GI (complex carbohydrates) (93, 94).

A low glycemic index therapy was developed by Pfeifer and Thiele in 2002 in Boston. This form of ketogenic diet only uses food with a glycemic index of <50. Although the name suggests that no carbohydrate counting is required, this cannot be supported in practice.

With such a low carbohydrate diet, as is the case with the MAD, it is necessary to calculate and maintain the daily amount of carbohydrates.

In general, about 40–60 g of carbohydrates are allowed per day. Even with this form of the nutrient therapy, it is reported that seizures in epilepsy have decreased (95).

INTERMITTENT FASTING DIET (5:2) (IFD)

Intermittent fasting is a new trend in weight loss. With time-restricted feeding (TRF), food can only be supplied at certain intervals [96]. Instead of fasting for several consecutive days, short periods of not eating for one day (8 hours of eating, 16 hours of fasting) or for a week (diet 5: 2: 5 days of eating, 2 days of fasting) are applied.

In this case, the daily meal break, which is natural due to a night's sleep, is already an overnight-fasting (OF). The time-restricted feeding TRF (97) is achieved by expanding the overnight fasting (OF).

The best known method is the 16:8 method, which means a total of 16 hours of fasting and eating for only 8 hours per day (96).

Alternate-day fasting (ADF) offers a restriction of meals every second day. Thus, with ADF, you should fast on one day, and can eat an unlimited amount of food the next day.

The IF method: 5:2 means that a person eats regularly 5 days a week and fasts or severely limits energy for the remaining 2 days (96).

The 5:2 diet is best known due to Dr. Michael Mosley ("The fast diet"), alternate-day fasting (ADF) due to Dr. Krista Varady ("The every other day diet") and Harvie's 2-day diet due to the books which have been published since 2013 (98, 99, 100). In the case of intermittent fasting, ketosis is achieved only in some types, namely for the 5:2 type (115).

Positive metabolic changes in the case of intermittent fasting are caused by the conversion of metabolism from a predominantly carbohydrate to lipid metabolism. There is an increase in the mobilisation of free fatty acids from fatty tissue. They are used to form ketone bodies in ketogenesis. Ketone bodies are then used by some organs and the brain after the adaptation phase as an alternative energy substrate to glucose (instead of glucose).

β-hydroxybutyrate (βHB) is the main ketone body for the energy supply (101, 102). Since ketogenesis begins after a brief fasting, it is possible that the formation of ketone bodies during the intermittent fasting has a decisive influence on the positive effects proven in the studies.

This form of nutrition is also of interest in the study of hormone levels (glucagon, insulin), free fatty acids and glycogen storage.

There is growing evidence that βHB is not only an energy carrier, but also performs signaling functions on the cell surface and inside the cell, which, for example, affect gene expression, lipid metabolism, neuronal function and metabolic rate (103, 104).

Some researchers (115) believe it is possible to influence a number of diseases in humans, such as type 2 diabetes mellitus, Alzheimer's dementia, through these molecular signaling functions.

To better understand the metabolic situation of short-term intermittent fasting in humans, a nutritional intervention study was conducted that examined the effect of the intermittent fasting on ketogenesis using the 5: 2 method (115) as an example.

The study concentrated on a brief change in the βHB concentration in the capillary blood during 2 days of fasting with complete energy limitation during the intermittent fasting using the 5: 2 diet (106–108, 109-112). On the days of fasting, there was a notice-

able increase in the βHB level in the blood, which was not observed on other days.

On the second day of fasting, ketone bodies appeared in the blood (up to 2–3 mmol per liter). The data usually shows the ketone body level rise by 30–50%. Moreover, there is a rather large difference in values (113, 114, 115).

With other forms of intermittent fasting, for example, 16/8, 14/10, 24/0 there is no significant increase in the production of ketones.

Due to the high hopes that some dieticians and sports physicians have today for the intermittent fasting, it is of great interest to researchers to compare different forms of diet in terms of the weight loss (116,117,118).

Intermittent fasting helps reduce weight and can contribute to good health, but not better than regular diets (116). This is the conclusion reached by the scientists from the German Cancer Research Centre (DKFZ) and the Heidelberg University Hospital (Deutsches Krebsforschungszentrum und das Universitätsklinikum Heidelberg) in the HELENA study (HELENA-Studie) (116), the largest study in this area to date. It is obvious to researchers that there are many different ways to reduce weight, but it is important to select the right option for each patient.

The HELENA study conducted by a team of scientists from the DKFZ and the Heidelberg University Hospital examined 150 overweight and obese people for one year. The patients were randomly divided into three groups: one third had a regular diet for 12 weeks. Energy consumption was reduced by 20%. The second group was on a 5:2 diet, but they also received 20% less energy.

The control group did not follow a specific diet plan, but like all the other participants, was motivated to follow a balanced diet as recommended by the German Food Society (DGE). After the actual phase of the diet, the scientists tracked the weight and health status of the study participants for another 38 weeks.

The result was unexpected given the high expectations for the intermittent fasting: the health status improved equally in both groups (weight loss, visceral fat, and unhealthy abdominal fat and liver fat deposits).

The changes in the distribution of fat in the participants' bodies were precisely determined by a special MRI performed at the Heidelberg University Hospital. At the same time, the research has shown that with a weight loss of only 5%, about 20% of abdominal fat and even more than a third of liver fat is lost, regardless of a diet type.

There was also no difference between the two diets in all other metabolic values and in all the biomarkers and gene activity studied.

Although the HELENA study does not support euphoric expectations of for the intermittent fasting, it also shows that it is not worse than common diets for weight loss.

This method requires further observation, especially since there is evidence of a possible relationship between the intermittent fasting and family eating disorders.

THE CLASSIC KETOGENIC DIET (CKD)

According to the classic ketogenic diet (CKD) each meal including appetizers should contain a desired proportion of fat to carbohydrate/protein.

The basis of calculation for the classic ketogenic diet was designed in 1924 by Dr. Peterman, the doctor of Children Mayo Clinic. The ratio of diet composition, published by Peterman contained 1 g of protein on 1 kilo of a body weight (10 kg), 10-15 r carbohydrates per day and the rest energy was received from fat (119). To date, the proportion of fat in the ratio is given as a ketogenic proportion.

Ketogenic diet (KD), offered by Peterman has already had correlation 4:1, which meant four 4 grams of fat for every 1 gram of protein and carbohydrates. Each food intake should have a selected concentration. As a result it takes some efforts to calculate and apply the classic ketogenic diet (120).

For children older than 2 years of age the ratio 4:1 is generally recommended.

For children younger than 2 years of age as well as for children with good response to KD related to a strong ketosis or difficulties in sustaining, the ratio 3:1 may be established. (67).

In the classic ketogenic diet (CKD) weight proportion between fats and proteins is 4 to 1 (but when using this diet for weight loss the coefficient is shifted toward increasing proteins and reducing fats). Thus, the foods high in carbohydrates, such as sweet fruits and vegetables, bread, pasta, grains and sugar are removed from the ratio whereas the intake of products rich in fats such as cheeses and butters is increased.

Ketosis is explained as an increase in the level of ketone bodies in blood that reduces the incidence of epileptic seizures.

Liver in a child accumulates less glycogen than in an adult. Therefore a child body recognises earlier a deficit of glucose and resorts to fat storages. Ketone bodies in urine of newborns often show that a child is malnourished. A basic diet for children with epilepsy contains the necessary amount of proteins for growth and regeneration of the body and an adequate amount of calories to support a normal body mass (in accordance with the age and height).

Most fats consumed with the food comprise long chain fatty acids (LCFA) However, from the point of view of using KD medium chain fatty acids (MCFA) are more appropriate. In one variant of the ketogenic diet a sort of coconut oil is used, which is rich in medium chain fatty acids (MCFA) and covers a half of a daily calorie intake.

COMPARISON OF DIFFERENT VARIANTS OF DIETS

Table 1 presents different types of ketogenic ratios with their compositions proportional (%) to amount of energy. There are a classic ketogenic diet, MCT diet, modified Atkins diet, Glycemic Index diet and a diet recommended by DGE (57).

Table 1. *Legend: DGE = German Nutrition Society; KD = ketogenic diet; LGIT = Low Glycemic Index-Treatment; MAD = modified Atkins diet; MCT = medium chain triglycerides*

Diets/composition %	Fats%	Protein%	Carbohydrates %
Classic KD 4:1	90	6	4
MCT Diet	73	10	17
MAD	65	35	5
LGIT	60	30	10
Diet recommended by DGE	55	15	30

Comparison of nutrition value among different ketogenic diets and the nutrition recommended by DGE (provided from recommended protein consumption as well as recommendation amounts for carbohydrates and fats) in energy percentage (En%) (68, 69, 32).

Thus, all KD are extremely high-fat diets, which nevertheless differ on their compositions and a range of effect. In case of a milder diet it is possible to eat more protein, which simplifies the diet and makes it similar to a normal diet.

The stricter is the diet, the higher ketone level is achieved. But it should be kept in mind that assignment to such a diet affects the taste and social integration.

Special attention should be paid to regular foods that contain certain ketone values. They are shown in Table 2 (57)

UNDESIRABLE EFFECTS

Ketogenic diet is usually well tolerated by children and if administered under medical supervision in accordance with standardized protocol and supervision of an experienced nutrition team it is considered a safe diet therapy. (120).

Table 2.

Content in foods	4:1 and more
Pork fat	30,5:1
Melted butter	30,5:1
Mayonnaise (80 % fat)	23,6:1
Brazil nuts	9,6:1
Salad Mayonnaise (50 % fat)	9,5:1
Sour cream (24 % fat)	6,1:1
Macadamia nuts	5,7:1
Cream (30 % fat)	5,6:1
Mascarpone	5,4:1
Black olives	5,0:1
Coffee cream (30 % fat)	4,7:1
Content in foods	3:1 bis 3,9:1
Coconut	3,9:1
Coconut, meat	3,9:1
Walnuts	3,2:1
Pecan	3,1:1
Content in foods	2:1 bis 2,9:1
Green olives	2,9:1
Hazelnut	2,8:1
Avocado	2,6:1
Almond flour	2,4:1
Meat sausage	2,3:1
Almond drink (without sugar)	2,2:1
Meatloaf	2,1:1
Cottage cheese	2,0:1
Content in foods	1:1 bis 1,9:1
Tuna in oli	1,9:1
Gouda, min. 60 % fat	1,9:1
Meatloaf 1,8:1	1,8:1
Almond 1,8:1	1,8:1
Sesame	1,6:1
Eel	1,6:1
Boiled sausage	1,5:1
Salami	1,5:1
Gouda, min. 45 % fat	1,4:1
Black olives	5,0:1
Coffee cream (10 % Fett)	1,4:1
Herring	1,0:1

Potential side effects occurring in the beginning of the application as well as during a long-time apllication are shown in Table 4. Besides, possible causes and symptoms additional countrameasures are offered.

Especially in the initial phase such side effects as nausea, food refusal or hypoglycemia are quite common. But they seldom occur later. In general possible

side effects are not meaningful, can be easily cured and differ from person to person. (122).

The effect of a decreased appetite has been also discussed in favour of using the diet for weight loss. Obese adults assigned to a modified ketogenic diet felt less hungry and ate less. (123,124).

Of interest there are findings associated with blood lipids and blood glucose level. Improvement in triglyceride, cholesterol and glucose levels in the blood was observed in obese patients even in a 24-week study (125, 126).

Further comments on these effects are not possible until they studied further. In some cases, which are detailed described in the instruction S1, KD is strictly forbidden and causes severe and even irreversible side effects (67). Thiamine pyrophosphokinase deficiency serves as an example. A severe metabolic acidosis and irreversible neurological damage caused by KD were reported. (127). Therefere before starting a KD, a medical consultation is required.

SIDE EFFECTS

Thus, the ketogenic diet is the diet with a high content of fats and low conternt of carbohydrates. Different studies are unanimous on using KD for treatment of pharmacoresistent epilepsy when its three forms such as classic ketogenic diet, modified Atkins diet and MCT diet showed relatively equal success in reducing seisures. (57).

Anyway, significant efforts are needed to teach the application of ketogenic diet, introducing it to the daily routine since each meal has to be planned individually. For instance, a spontaneous lunch with friends at a cantine or a restaurant will be impossible without a thoroughul planning.

Due to limitation of carbohydrates contained in fruits and grains, which are allowed in very small amounts, there is a risk of developing a deficiency of vitamins and minerals.

There are no standard recommendations on substitution because the consumption is calculated individually for each patient in accordance with the protocol and when necessary a corresponding medication is prescribed. Since food preferences change often in childhood, the calculation protocol should be revised each 3-6 months. (57).

KETOGENIC DIETS FOR CANCER TREATMENT

Recently, the possiblility of using diets with low carbohydrate content for cancer patients has been widely discussed. As a justification for using ketonic diets it was indicated that metabolism of tumor cells depends on carbohydrates.

Depening on interpretation of the data, these diets either promise a direct influence on growth of the tumor or its metastasis, improve efficiency of chemotherapy or radiation therapy. Sometimes, scientists promise better tolerance of the treatment, particularly, the chemotherapy (128,129,130). However, the review carried out by Nicole Erickson and coauthors (131), showed an absolute contraindication of using isocaloric ketonic diets in cancer patients due to reduction of weight and consequent worsening of the prognosis.

The study surveyed 15 researches (8 prospective researches, 2 retrospective researches, 5 cases), which comprised 330 patients with malignant tumors.

Since there is no homogeneity in types of tumors, their localization and stages, the results of the study could not be fully compared. The points of comparison for 15 researches were: quality of life and the toleration of the diet wheras antitumor effect was not considered.

Moreover, none of the studies showed tumor regression, better survival and better tolerance of the therapy or reduction of KD side effects. (132).

The German nutrition team «Working Group on Prevention and Intergrative Oncology" (PRIO) of the German Cancer Society (DKG) (131) states that presently there are no human subject researches demonstrating that ketogenic diet or low carbohydrate diet allows (130):

- to prevent or suppress growth or metastasis in human;
- to enhance efficience of chemotherapy or radiation therapy;
- to improve tolerance of chemotherapy.

Therefore, based on the data in disposal it is not recommended to use low carbohydrate or ketogenic diet for cancer treatment.

Specialists often indicate possible side effects of such diets in cancer patients,

Documented side effects include: nausea, loss of appetite, loss of weight, constipation, hyperlipedimia, artherosclerosis, hypercholesterolemia, absence of thirst, kidney stones, pancreatitis, dehydratation, selenium deficiency and drowsiness. (133, 134, 135).

Such side effects due to the diets are hard to distinguish from consequences of cancer or its therapy. Ketogic diet carried out without supervision of an oncologist may lead to serious errors in the therapy.

Ketogenic diet and low carbohydrate diets may facilitate malnutition in cancer patients. Fine et al. observed already in 2013 reduction of weight by 4% (±6,1%) in cancer pationts who were 28 days on ketogenic diet.

Table 3. *Symptoms, Causes and solutions on administering KD (57)*

Symptoms	Causes	Solutions
Apathy, reduction of attention	Effects of ketone bodies on the central nervous system	After the adaptaion to the changes the symptoms are normally disappear.
Nausea and vomiting	Lack of carbohydrates	Under a very high levels of ketones small amount of carbohydrates can be administered
Hypoglycemia	Lack of carbohydrates	Small amount of carbohydrates can be administered.
Decreased appetite	– signs that ketone bodies produce a damping effect on hunger sensation; – another reason might deal with exposure to unfamiliar food (phase of transition)	– one missed food intake is irrelevant to success of the therapy and can be rescheduled; – food intakes should be planned according to the preferences of the patient; – to stimulate the consumption through visually appetizing food;
Constipation	insufficient intake of fiber and liquids	To ensure an adequate intake of liquids
diarrhea, stomach spasms	Excessive intake of fat or excessive consumption of TMC	Under excessive intake of long chain fatty acids one part can be replaced with MCT fats.

Table 4. *Complications during long-term KD assignment (57)*

Hyperlipidemia	Too many animal products with high fat content and consequntly too high consumption of saturated fatty acids	Careful selection of fats (training in fats /oils), preference of oils, if possible MCT fats
Kidney stone disease	Hypercalciuria, inadequate fluid intake	To control fluid intake

Tan-Shlalaby et al. reported that 73% of patients lost in average 7,5 ± 5,8 kg of body mass during a 16-week ketogenic dieting (137).

Urbain et al. (2017) reported on loss of weight by 2,0 ± 1,9 kg of the body mass in healthy volunteers on ketogenic diet, though their average consumption of energy has not changed confirming earlier evidences that isocaloric ketogenic diets facilitate loss of weight due to metabolic changes (138).

The menioned loss of weight is known due to investigations aimed at intensive medical support of patients. Nevetheless the loss might be more dangerous for patients assigned to the diets under questionable conditions (for example without blood control).

Therefore, basing on the latest data low carbohydrate or ketogenic diets can be recommended neither as a complementary therapy nor as a nutrition pattern for cancer patients.

The patients who are interested in low carb or ketogenic diets should be as early as possible informed about possible negative consequences of such diets. Besides, the cancer patients who assigned to such diets should be found and persuaded to discontinue. If patients stick to low carbohydrate or KD inspite of intensive contraargumentation, it is necessary to subject them from the very beginning to a strict interdisciplinary supervision, including control of the weight, body composition, laboratory values (131).

KETOGENIC DIET FOR REDUCTION OF WEIGHT (OBESITY)

To date, more and more specialists refer positively to the diets with low carbohydrate content due to a negative experience to the ratios recommended currently for weight loss in the practice.

Nowadays, in Germany a mediterenean diet, DASH and vegetarian diets with limitation of energy are commonly recommended in the medical practice, but the data of their efficiency is limited (139).

Besides, the diets with lower content of fats did not demostrate that they are more effective for weight loss (140). Commercially available weight loss programs show a short-time success in reduction of sugar level (in glycemic control), yet a long-term success during one year is rare (141).

The official point of view on KD and the effect of ketone bodies in terms of health benefits is rather contradictional (142).

Certain works found definite advantages of KD for weight loss, however other works did not find advantages compared to other diets. (143, 144).

Neverthelss a current consensus admits that KD decreases appetite and patients feel themselves less hungry compared to other diets based on reduction of carbohydrates. Ketosis is considered responsible for this phonomenon (145).

Well-known medical institutions have currently started ambitious researches to provide additional arguments to change the paradigm. Therefore some specialists call on their colleagues to reconsider DGE recommendations and to ignore general nutrional recommendations (146).

E.g. Sarah Hallberg from Virta Health Centre, a branch of University in Lafayette in Indiana/USA advocates a sharp decrease in carbohydrate intake. By the way, there was no suprise that her article appeared in British Journal of Sport Medicine (147). Sport doctors were the first to stress that there is no actual need in carbohydrates (148).

Already in 1980s exogene consumption of ketone bodies became a subject of sport medicine. (149, 150). Later medical use of KD has been debated and independent therapeutical investigations were carred out, including the study of Natrium-βHB for rare metabolic conditions, such as multiple acyl-CoA dehydrogenase deficiency (MADD), PDH deficiency or hyperinsulinism (151–154).

Among others, intake of dietary supplements based on exogenous ketone body was described as a supporting and at the same time facilitating therapy during clinicialy feasible KD (152).

Hallberg's team (155) has recently published an impressive „proof of principle": she compared a treatment of 262 patients with Type 2 diabetes with a limited daily carbohydrate ratio (up to 30 gram) aimed at achieving ketosis, with a conventional treatment of 87 control patients. After one yeat HbA1C values in the respondent group reduced from 7,6 to 6,3, body mass decreased by 13,8 kg. Whereas prior to the study such patients in 56,9% cases needed oral antidiabetic medications but after restructuring their nutrition the drugs were needed only 29,7% of cases.

Besides, 94% of the patients on diet did not need insulin therapy. The advantages were also evident in lipid metabolism as well as in serum creatinine and liver enzymes. In patients with daibetes who received a traditional therapy, biomarkers, medications and doses of insulin remained unchanged. (155).

Hallberg links a ratio with high carbohydrate contents to overweight, obesity, metabolic syndrome and type 2 diabetes (T2D). First of all it is meant to reduce the level of insulin secretion enabling not to suppress burning of fat but to stimulate it. Such regulation of lipolysis can be achived with the help of "very low carbohydrate/ketogenic diet"(VLCKD).

This is a kind of a medium position between a low carbohydrate content, which is also popular among healthy people, and a strict ketogenic diet, used for the treatment of epilepsy.

Thus, the ketogenic ratio with an intake of 20 to 50 grams of carbohydrates per day is discussed. Fats may contain 45% or more (it always depends on total amount of calories), proteins - about 40%. Other research groups do not focus on percentage but calculate carbohydrates, proteins and fats based on body weight.

Specialists (146) indicate that on the initial stage of transition to a ketogenic diet the use of high-protein formula diets can be helpful. They have an advantage that patients do not need to think about cooking a meal and their benefits are seen promptly. The amounts are defined individually for each patient in accordance with his body weight so that carbohydrate intake corresponds to a diet with low or very low carbohydrate content. Additionally, consumption of olive oil and vegetable juices is recommended. During several days a formula diet is gradually replaced with a low carbohydrate ration, which is stipulated by instructions.

The target can be achieved not only in T2D patients, but also in people, only susceptible to overweight. According to a recently published research in Dusseldorf (156), where the use of an intervention program aimed at changing the way of life under supervision of a medical team led to a significant loss of weight and improved metabolic control in overweight patients at risk for diabetes.

The experts from Dusseldorf drove a conclusion from the findings of their 2-year-long study that about one-third of respondents are able to follow the diet with carbohydrate depletion. Half of those who stopped dieting (one-third of the whole group) managed to continue to loose weight.

Simular positive results were reported in some reviews (157, 158). Often there were no comprehensive definitions of how low the levels of carbohydrates were, whether ketone bodies were measured, what fat or protein levels were rationed; whether calorie restrictions or a sport program were incorporated. This makes it difficult to assess the influence of VLCKD in each separate case.

As a main achievement, the adherers of ketogenic diets consider the reduction of insulin dosage in a long-term period. Insulin in overweight patients does not only suppress lipolysis but also slows down fat absorbtion. Unfortunately, insulin makes it more effectively when the body weight is higher, which was reported by a research team from Basel University Clinic (156, 159). It was shown that glucose load in patients with body mass index ≥ 30 kg/m^2 increases the level of insulin more than when BMI ≤ 25 kg/m^2.

It was long known from multiple reseaches that insulin therapy is not regarded as an effective option for patients with T2D in a long-term perspective. It refers to the general mortality as well as to cardiovascular diseases. (160–162).

This was observed by german diabetologists 30 years ago in the long-term investigation in 1987 (163).

Therefore, treatment with insulin was often doubted. Especially in Germany this issue should be cleared since practically nowhere insulin is used as a mass therapy for T2D (146).

According to the publication of the Independent Research and Consulting Institute for Health Issues (IGES) in Berlin, an individual consumption in Germany is much higher, e.g. twice as much as in France (146). It is evident that the system that stil calculates carbohydrates in "bread" units may face difficulties during transition to KD.

Reduction of triglyceride level and increase in HDL is viewed as a positive effect but at the same time there was an increase in LDL cholesterol level (164). However it is not problematic since it is explained by the transformation of small low density participles into bigger lower density participles, which are considered to be significantly less atherogenic (165).

Hallberg and authors gives a clear picture of risk factors for cardiovascular diseases: higher LDL was associated with bigger participles. Besides, ApoB remained unchanged and levels of blood pressure and inflammation markers developed favourably (166).

It is unknown, whether reduction of carbohydrates in a ratio has a positive influence on non-alcoholic fatty liver disease (NAFLD). Although the results are controversial (167, 168, 169), but the transcriptome analysis has recently showed that improved lipid metabolism in the liver may be traced even on the genetic level (170).

We have already mentioned complications caused by low carbohydrate diets.

There are such problems as apathy, decreased attention, nausea and vomiting, hyperlikemia, decreased appetite, constipation, kidney stones arising with a lower fiber intake dealt with the decreased intake of carbohydrates. (57, 171). It is important that the nutrition contains enough fiber, as, for example, in leafy green vegetables, for example. General objections are not in the least justified with tolerance of the diet by patients, who should considerably change their food preferences. Nevertheless to date, numerous formula diets have emerged with cooking recommendations as practical solutions.

Finally, the experience of using a strick KD in patients with epilepsy shows that in certain cases cooper deficiency associated with anemia was found (172). There are also findings that ketosis may prolong effects of anesthetic gases (173, 34).

And not less important: at the beginning the patients report about drowsiness, dizziness, fatique, insomnia, light nauseas, constipation — all symptoms that were named "keto-flu" but they normally lessen (26). Important: patients can continue physical training despite of limitation of carbohydrates (176, 177).

Lately a number of articles in the field of Wellness and Lifestyle has appeared on salts of ketone bodies. Main attention is paid to sport and additional heath benefits for using ketonic diet or ketone bodies as preparations (178–180).

The use of additional ketone bodies seems to be a new and interesting development at the world market.

Growing general and scientific interest to KD has given way to development of new fields of applications. We have already reported that in addition to classic ways of weight reduction and increasing working capacity a posivite effect of KD on cancer and treatment of neurodegenerative diseases (dementia, Alzheimer's disease etc) has been widely debated (181). Ablitlty of ketone bodies to reduce appetite has been often mentioned as an advantage for use as a trend diet (182).

KD, probably has a high market potential. To date, there is no data on market volume of preparations based on βHB and other co-products. Scientifically relevant monitoring of general values of vital functions such as levels of blood (βHB, glucose) and concentrations of acetone in outbreathed air could be performed during KD (183, 184).

Eventually, it should be necessary to write a review on supplements of ketone bodies based on βHB salts that are available in Europe and USA, with a focuse on their benefits and risks. Such assessment, which could review the market situation, its evolution and the degree of existing "keto-tendency", has not been conducted yet.

In addition, dietary supplements based on the ketone body β-hydroxybutyrate are interesting development that should be used with caution in KD practice. The new development of the market and the related researches has provided a great deal of new information on ketone bodies. Anyway, βHB salts should not be used without control because we still do not have the full information on spread and severity of their side effects, for example, in relation to gastrointestinal symptoms. Particularly in a long-term perspective.

A general estimation of some advertisements, concerning the benefits for neurodegenerative diseases and weight reduction has shown the necessity of further researches in many fields. This would allow referring to possible βHB effects as evidence-based facts.

There is substantial evidence of decreased appetite. That is why the use in combination with KD is considered as an interesting aspect that improves a daily diet. For instance, the limitation of daily amount of fat might considerably simplify and improve the situation on KD(154).

Not long ago such titles as "Earliar death due to low cab nutrition" shocked the adherers of low carb diets (185). In particular, there is a research of Harvard University team headed by cardiologist Prof. Dr. Scott D. Solomon (186). It was shown that diets with low and high contents of carbohydrates were linked with increase in mortality compared to moderate consumption of carbohydrates (about 50% of daily requirement in energy). This moderate option is associated with lowerest mortality level (Lancet Public Health 2018; 3 (9): e419–28).

Solomon team has analysed the data of 15428 Americans, who provided information on their ratio in 1987–1989. Then the respondents participated in the study on risk of atherosclerosis (ARIC). In the period from 1993 to 1995 the respondents were questioned again and the study was targeted on mortality level.

According to ARIC, "sweet spot" for carbohydrate intake comes to 50–55% of the total energy and associated with the lowest risk of death. Intake of carbohydrates <30% is correlated with a higher level of mortality (Hazard Ratio [HR] 1,37; 95%-confidence interval [95-%-CI] 1,16–1,63). The result was less manifested at a higher consumption of carbohydrates > 65 (HR 1,16; 95-%-KI 1,02–1,33).

Solomon and his colleagues has discovered this U-shaped connection between carbohydrates and mortality in metaanalyses of 7 further studies (carbohydrates <40%: HR 1,20 [1,09–1,32], carbohydrates > 70%, HR 1 , 23 [1,11–1,36]).

In addition, when respondents consumed more animal fats and proteins the mortality was higher (HR 1,18, 95% CI 1,08–1,29); when fats and proteins were of plant-based, the mortality level was lower. (HR 0,82 [0,78–0,87]).

Besides, a recent study of Polish researches presented at European Cardiological Conference reached a similar conclusion (187). And the press release of German Society of Cardiology warns of dangers of low carbohydrate diets. The cardiologists are supported by DGE experts (188).

On the other hand there has been a massiive methodological criticism of ARIC study.

Apart from carbohydrate intake, there is a question of the quality of nutrition in the group of the lowest carbohydrate intake. This issue has not been addressed. The critics see this as the weakest point. In 1980s and 1990s it was traditionally recommended to consume more products that rich in carbohydrates and low in fat. We may suggest that those who used the diet with low carbohydrates intake regardless the modern recommendations were not most healthy and disciplined people. Suggestively the „low level carbohydrate level", mentioned in the ARIC study had nothing to do with a modern conceipt of low carbohydrate intake. Fried chicken and hamburgers had been preferred to salmon and zucchini. Moreover, in the ARIC-study there was no data on alcohol consumption.

From the point of view of the opponents (185) none of these studies can provide evidence that a certain diet may cause long-term effect on mortality — either a positive or a negative one. To perform these prospective randomized controlled studies are necessary, but unfortunately, they are hardly conductable, in the first line because of ethical reasons.

Animal studies and short intervention researches of a controlled diet showed that diets with low carbohydrate content were favorable in terms of longevity or at least positevely influenced risks factors for cardiovascular diseases (185).

The opponents of the study consider that the current analysis has no scientific evidence to claim that low carbohydrate diet could shorten life expectancy. At least there is nothing adverse if doctors continue to recommend their overweight patients to use a mediterenean diet with low content of carbohydrates according to Prof. Matthias Blüher, Head of Clinic of Endocrinology and Nephrology for Overweight Adults, at Leipzig University (185). So far, however, the disagreement among specialists has not been resolved and the subject should be further explored.

24 experts from U.S. News Diet Ranking placed Atkins diet, Eco-Atkins and ketogenic diet in the categories "the best diet in general", "loss of weight" and "quick loss of weight". The best note was given to Atkins diet. In the category of quick loss of weight the ketogenic diet took 13th place, anyway KD only occupied 39th place in the general ranking of diets (139, 154, 189–191).

The DGE opinion in regard of KD and other unbalanced diets remains practically the same: any diet with a big amount of nutricients, either fat or carbohydrate or protein limits the choice of foods.

In this case the body is inadequately supplied with essential micronutrients, such as vitamins, minerals and fibers or, too much calories or saturated fatty acids are released. The oneside diet in long term has negative effects on health. Thus, the results of ARIC study (188) show that a shortened life expectancy due to inbalanced diets is not suprising.

Foods with high content of fiber, such as whole grains, legumes, vegetables and fruits should be eaten

without restriction, simple carbohydrates, such as mono- and disaccharides as added sugar and purified starch — only in small amounts. It is important to improve the quality of fats and carbohydrates in our western diet, as well as to avoid too high consumption of energy. This was stated by DGE in its PURE-Study (192) published in Lancet in 2018.

A well balanced, wholesame and plant-based diet formulated in 10 guidelines by DGE maintains good health, which is also confirmed by ARIC-Study (188).

CONCLUSIONS

Recent studies of ketonic diets (KD) for treatment of neurologlical, oncological diseases and diseases of the metabolic syndrome were reviewed.

Based on the literary sources a conclusion on unacceptability of using ketogenic diets in cancer patients was drawn. This was also stated by German Cancer Gesellscaft. German Nutrition Society and other researches consider it irrational to use KD for reduction of weight referring to ARIC-study, which associates carbohydrate depletion with a shorted life expectancy and possible complications.

Nevertheless, there is no doubt that the use of different ketogenic diets (KD) for treatment of epilepsy and certain metabolic deficiencies (GLUT1 deficiency and pyruvate dehydrogenase deficiency (PDHD)) remains feasible and effective.

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HIGH PREVALENCE OF NEGATIVE MOOD AND BODY DISSATISFACTION AMONG BRAZILIAN ADOLESCENTS AND WOMEN

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ABSTRACT — In Brazil, the prevalence of anxiety is the world's highest and depression ranking fifth. In the same manner, body image disorders are very frequent. This article briefly review and discusses the phenomena of body image disorder and its role on mood disorders. Body dissatisfaction is very frequent among girls, adolescents and women in Brazil which constitutes an important risk factor for both mood and affective disorders.

KEYWORDS — body image, mood disorders, suicide, depression.

INTRODUCTION

During the past decades and even today, Brazil has the highest rates of psychiatric disorders in Latin American region, with greater incidence and prevalence of anxiety, depression and mood disorders. In fact, Brazil is the world's leader of anxiety disorders prevalence and the fifth of depression prevalence [1].

Since there is no significant genetic difference in Brazilian population that explains the higher incidence of anxiety and depression, the role of social determinants must be crucial and very effective as important causes of mental disorders in Brazil.

In a nation with poor education rates, poverty, excessive freedom to social media and marketing, higher rates of social violence, increased consumption of alcohol, and drugs of abuse and addiction, and lack of effective health promotion programs for children and adolescents, it has been expected that mental health problems have a greater magnitude and incidence since the early life [2, 3].

Furthermore, excessive freedom of communication media, the cultural sexism and patriarchal gender inequality associated with a permissive culture contributes to vulgarization and exploitation of bodies of children, adolescents and women [4–6].

In a similar manner that found in other nations, mental health problems had been affected more women, especially those subjetcs with poor education, lower income and precarious working conditions [7, 8]. It is important to emphazise that higher levels of aggressiveness and anxiety had been observed in Brazilian since the childhood and early adolescence [9].

Considering that anxiety and other mental disorders are very frequent in Brazil and that this country is one of the world leaders on plastic/cosmetic surgeries, the objective of this work was to briefly discuss the association of body dissatisfaction and mood disorders.

MOOD DISORDERS AMONG BRAZILIAN FEMALES: HOW FREQUENT THEY ARE?

Mood disorders as well as anxiety and physical and psychological violence, are frequent among Brazilian children and adolescents. A study in Santa Catarina, South Brazil, reported 27.7% of intrafamiliar violence, 12.61% of anxiety, 11.71% of agressiveness behaviour, and 3.38% of depressive mood (6.2% among girls and only 2% among boys) among children [9].

In Spain, a study with adolescents reported that depression (16%), alcohol abuse (11%), bipolar disorder (7%), and drug addiction (5%) affected that population [10].

A study in Barra do Garças (MT), Central-Western Brazil, found that 86% of the adolescents had frequent mood oscilations and that 18% of them reported weekly use of alcohol and/or tabacco [11].

The ERICA study, a population-based study with Brazilian adolescents found a prevalence of common mental disorders of 30%, which was higher among girls (38.4%) than boys (21.6%) [12].

Living in a violent and sick society, it is reasonable that mood disorders should increase with age. Then, another study, with adult patients attended at a basic health unit in Rio Grande do Sul, South Brazil, the frequency of negative mood reached 40.1% [7].

BODY DISSATISFACTION AMONG GIRLS AND WOMEN

A 2012's project with adolescents from the Middle Araguaia region, Legal Amazon, observed higher body dissatisfaction and lower self-steem among adolescents, especially between girls [13].

Similar results were found regarding adult men and women. Studying 61 men and 79 women, from Barra do Garças (MT), it was verifyied that women were much more dissatisfied with their bodies compared to the men [14].

It is important to note that body dissatisfaction changes with age or generation. Elderly women presented high body satisfaction (83.33) compared with women at middle age (58.33%) [15]. It is possible that body dissatisfaction decreases with age since the social pressures to women's body also diminish. More studies are needed in this area.

A recent study covering a multiethnic population comprised by university students of the Central-Western Brazil showed that afro-american women were devalued compared to afro-american men and white men and women [16]. In the same study, both white women and men were more appreciated compared to afro-american people, but men received more positive values than women [16].

Evaluating 855 adolescents (466 female, 389 male), of 14 to 19 years-old, from the public schools of the Middle Araguaia Region, it was reported that only 60% were satisfied with their bodies, whereas the body dissatisfaction was highest among girls/women compared to boys/men [17].

A population-based study covering 4,325 adolescents of 14 and 15 years-old in Pelotas (RS), South Brazil, showed a body dissatisfaction of 51% among boys and 65.6% among girls [18].

BODY DISSATISFACTION AND MOOD DISORDERS: A RISK FOR SUICIDE?

Social media and marketing exert strongest pressures against female adolescents to lose or maintain weight at any cost, as well as they also forces males to gain weight and muscle body mass [19]. In this manner, the body dissatisfaction with a thin body was higher among men, whereas the body dissatisfaction with a heavier body was higher among women in a sample of South Brazil [20]. This female body coercion beggins during childhood and increases in the late adolescence and early adult life, causing suffering, mood disorders, lower self-steem and depression [21, 22].

Analysing 371 adolescents of 12 to 16 years-old from Juiz de Fora (MG), Southeast Brazil, Fortes et al. [23] reported that body image dissatisfaction was positively correlated with both mood state and perfectionism.

Evaluating body image during one year, Fortes et al. [24] observed that body dissatisfaction among girls increased over the time, whereas among boys it was decreased.

A longitudinal study covering 598 adolescents (269 girls and 329 boys) from Juiz de Fora, Rio Pomba and Barbacena cities (MG), Southeastern Brazil, showed that body dissatisfaction was higher among girls compared to boys, and that this dissatisfaction in girls was associated with increased prevalence of disordered eating and depressive symptoms [25].

Furthermore, body dissatisfaction and incorrect weight perception has been also associated with drive to be thiness, lower self-steem, disordered eating (anorexia and bulimia) and unecessary and irrational use of weight loss drugs and aesthetic surgeries [26–28].

A study with university students from Botucatu, São Paulo State, Southeastern Brazil, observed that one third of students had body image dissatisfaction and they also found a positive association between social anxiety disorder and body image dissatisfaction [29].

Beyond psychological sadness, body image dissatisfaction was also correlated with ideation, planning and attempt to suicide, especially among female than males [30].

CONCLUSION

Body image dissatisfaction can cause mood disorders, affective disorders and even suicide tendencies. It is urgent to improve health promotion activities which can foster positive body image deconstructing the imposed models of "beauty" and "ugliness".

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NEGLECTED TROPICAL DISEASES IN GHANA: CURRENT STATE AND THE WAY FORWARD

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ABSTRACT — Health policies are correlated with the development of the society both nationally and locally. On the other hand, the allocation of resources for health services is a priority in regions with a lower degree of local development, where the health of the population is higher because of the more difficult access to health services, the lack of information and the postponement of the decision because of the lack of personal health care resources. Under these circumstances, investment in repairers' health policies becomes absolutely necessary in order to ensure a minimum equity of healthcare systems in that state.

KEYWORDS — Health Programs; National Diabetes Program; Chronic Care Self-Care Management.

INTRODUCTION

Neglected tropical diseases are a diverse group of tropical infections that are chronic in nature and especially common in low-income populations in developing regions of Africa, Asia, and the Americas. They are caused by a variety of pathogens such as viruses, bacteria, protozoa and helminths. Neglected tropical diseases have been in existence in Ghana for many years with many projects taking place to eradicate them but till now there are still places in Ghana still endemic to these diseases. The importance of neglected tropical diseases has been underestimated since many are asymptomatic and have long incubation periods. Areas of high endemicity are often in geographically isolated areas, making treatment and prevention much more difficult.

Purpose of the study:

The aim of the research is to the study cause of prevalence and endemicity of the main neglected tropical diseases in Ghana despite various attempts to eradicate them over the years and to proposed a plan to facilitate their eradication.

Material and Method:

Analysis of data from World Health organization (annual report 2000-2017), Ministry of health Ghana annual report 1998-2017, Ghana neglected tropical diseases program (5-year strategic plan -2013-2017), scientific articles, expert analysis, statistical methods. Examined the conditions, the category of the people affected, primary health care systems, the nature of the disease in endemic communities etc.

RESULTS

Analysis index of main neglected tropical diseases in Ghana which include the following:

a)Soil transmitted helminthiasis from 2012, number of cases 540,733 representing (2,1%) of infected population, 2013: number of cases 804,472 (2.58%), 2014: 855,677 (3.1%), 2015: 830,557 (3.1%), 2016: 816,106 (3.1%). Major Soil-transmitted Helminths in Ghana are Ascaris lumbricoides, Trichuris trichuria, Necator americanus which causes malnutrition, anemia, growth retardation, cognitive impairment as well as lowering of resistance to other infections.

b) Lymphatic Filariasis Lymphatic Filariasis is currently was endemic in 98 out of the 216 districts in 8 regions of Ghana aside Ashanti and Volta regions in 2014 but currently 15 districts out of the 98 endemic districts remain endemic is still undergoing mass drug administration.

(c) Onchocerciasis: Remapping for onchocerciasis in 2009 using the rapid epidemiological mapping for oncho (REMO) methodology. The REMO results indicated 29 districts were hyperendemic (nodule prevalence ≥60%), 15 districts were meso endemic (nodule prevalence 40–59.9%), 91 districts were hypo endemic (nodule prevalence < 40%) and the remaining 81 districts were non-endemic (nodule prevalence 0%) In Ghana currently Onchocerciasis has an estimated at-risk population of 4,7 million in 3115 communities in 85 endemic districts from nine out of the ten regions and a possibility of increase to 125 endemic districts.

(d) Schistosomiasis: Urinary Schistosomiasis is also known as Bilharzia, caused by a blood fluke Schistosoma haematobium.In 2007, it was widespread and found in all regions in the Country, 47 category A

(prevalence ≥50%, high-risk) districts, 138 category B (prevalence ≥10–49.9%, moderate risk) districts and 31 category C (prevalence 1–9.9%, low-risk) districts. Results of the impact assessment indicated significant improvement in SCH in Ghana. Category A endemic districts reduced from 47 to 3 while category B reduced from 138 to 54. The results were reviewed by an expert meeting in August 2016. Out of currently 254 districts in Ghana ,83% proportion of the districts are endemic for Schistosomiasis. (GHS 2016)

CONCLUSION

Despite the various projects and attempts made by the government and international organizations to eradicate neglected tropical diseases, We found out there is still much to be done in terms of prevention of the diseases in creating awareness and educating the public on the causes and effects of these diseases. 9 out of the 14 neglected diseases are still prevalent in Ghana of which 5 are endemic in most rural communities. Access to health care in the rural areas is very necessary in eradicating these diseases. Prevention and eradication are important because "of the appalling stigma, disfigurement, blindness and disabilities caused by NTDs. Research is still in progress.

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PHYSICAL FEATURES VARIABILITY OF SPHENOID BONE ANATOMIC STRUCTURES IN ADULT POPULATION

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ABSTRACT — The article is dedicated to the variability of linear parameters, the area, the volume of the Turkish saddle and pituitary fossa of the sphenoid bone in adults, depending on the skull base type. The study was carried out through stereotopometric and craniometric methods on 87 registered skulls of mature age people (aged 21–60) from the scientific craniological collection of the Human Anatomy Department, V.I. Razumovsky Saratov State Medical University. The following parameters were identified: the basilar angle, which allowed identifying the type of the skull base and the linear parameters of the Turkish saddle, the pituitary fossa of the sphenoid bone, as well as their area and volume of the pituitary fossa. The obtained results show that the skull base of the platibasilar type features a long and narrow Turkish saddle, a small and rounded pituitary fossa with a larger area and a smaller volume; medio- and flexibasilar types of cranium can be described as having a short and wide Turkish saddle, a deep pituitary fossa of oval shape with a smaller area, yet with a large volume. There has been no typical variability of the Turkish saddle area identified.

KEYWORDS — sphenoid bone; Turkish saddle; pituitary fossa; craniotype.

INTRODUCTION

One of the important issues in neurosurgery implies the selection of proper operative access to the pituitary neoplasms adjacent to the pituitary fossa, and the involvement into the pathology of such vital anatomical structures of the Turkish saddle area as the internal carotid artery, the cavernous sinus, the oculomotor, the pathetic and the abducent cranial nerves [11]. The variability of the Turkish saddle shape and physical features allows one to make indirect judgment about the condition of the pituitary gland as well as about the pathological processes developing in there, in view of the fact that the average volume of the pituitary gland is 86% of the Turkish saddle volume [9]. The level of the Turkish saddle is where a bend in the skull base develops, the morphological basis of that being the basilar angle [16, 18]. The angle size determines the type of cranial basement (plati-, medio- and flexibasilar craniotypes) affecting the structural variability of its local structures [17, 19, 20]. Given that, establishing the variation patterns in the sphenoid bone architectonics, which is part of the anterior and middle cranial fossae, involves craniometric studies taking into account the typical features of the skull structure [3, 12, 15], since its shape variants and exact local structures dimensions generally determine the choice of surgical access to their contents [10, 13, 14].

The data on the skull morphological variability obtained by a number of authors [3–5, 8, 21–28], are used to objectively evaluate the results of the available modern diagnostic methods, as well as to develop proper approaches and carry out radical resections with the minimal number of complications [6].

Aim of study: to identify the variability of the linear parameters, the area, the Turkish saddle volume and of the sphenoid bone pituitary fossa in adults, depending on the skull base type.

MATERIALS AND METHODS.

The study was carried out on 87 registered skulls of mature age people (aged 21–60) from the scientific craniological collection of the Human Anatomy Department, V.I. Razumovsky Saratov State Medical University. The stereotopometric method, through which the device of craniosterebasiometer was used, allowed identifying the basilar angle, which in turn made it possible to detect one average and two extreme types of the skull base — mediobasilar, flexibasilar, and platibasilar [2].

Within the craniometry method, following the technique commonly employed in craniology [1], a

technical caliper with a divide value of 0.01 mm was used to measure the linear parameters of the Turkish saddle and sphenoid bone pituitary fossa — the longitudinal diameter of the Turkish saddle (length), i.e. the distance from the saddle tubercle to the saddle back base and the transverse diameter (width), i.e. the distance between the most medial edges of the carotid sulci; the longitudinal diameter of the pituitary fossa — the distance between the most distant points in the sagittal plane and the transverse diameter — the distance between the most distant points in the frontal plane; the pituitary fossa depth — a perpendicular from the line drawn between the saddle tubercle and back, to the deepest point at the pituitary fossa bottom. The area and the volume of these anatomical structures of the sphenoid bone body were identified subject to the standard mathematical calculation methods.

The statistical processing of the obtained data was done using the Statistica 6.0 application software (Windows OS). For all studied parameters, the variation statistics elements were determined — M, m, σ, Cv%, P. Since the variant distribution revealed little difference from the normal values, the statistical significance of the differences between the average values were calculated using Student's criterion. The differences were considered statistically significant at $p<0.05$.

Results and discussion. In the platibasilar type, the Turkish saddle length (14.7±0.2 mm) was 2.0 mm longer than that of the medio- (12.7±0.2 mm; $P <0.05$), and 2.6 mm over that in the flexibasilar craniotypes (12.1±0.3 mm; $P <0.01$); however, the two latter types of the skull base had no statistical difference ($P> 0.05$) in this parameter. The sphenoid bone Turkish saddle length (14.7±0.2 mm) in the platibasilar type exceeded its width (11.8±0.1 mm; $P <0.05$) by 2.9 mm, while in the medio- and flexibasilar craniotypes the width dominated (12.7±0.2 mm; 12.1±0.3 mm) over length (13.9±0.3 mm; 14.3±0.3 mm; $P <0.05$; $P <0.01$) by 1.2 mm and 2.2 mm, respectively.

The Turkish saddle area had the highest average value in the mediobasilar type (176.53 mm^2) and the smallest, equal in value, in the plati- and flexibasilar (173.46 mm^2; 173.03 mm^2) craniotypes. The predominance of the Turkish saddle area in the mediobasilar type compared to the area of the plati- and flexibasilar types was by 3.07 mm^2 and 3.5 mm^2, respectively.

In the platibasilar type of the skull base, the width of the pituitary fossa (10.1±0.4 mm) was slightly above (0.2 mm) the length (9.9±0.3 mm; $P> 0.05$) and exceeded its depth by 3.7 mm (6.4±0.3 mm; $P <0.01$). In the mediobasilar type, the width (10.3±0.4 mm) prevailed over its length by 1.5 mm (8.8±0.2 mm) and by 2.7 mm — over its depth (7.6±0.3 mm; $P <0.01$). In the flexibasilar craniotype, the fossa width (10.8±0.4 mm) exceeded its length (8.7±0.2 mm) by 2.1 mm and by 1.1 mm — its depth (7.6±0.3 mm; $P <0.01$).

A comparative analysis of the variability showed that in the platibasilar type the length of the pituitary fossa (9.9±0.3 mm) was 1.1 mm and 1.2 mm longer than that of the medio- and flexibasilar craniotypes (8.8±0.2 mm and 8.7±0.2, respectively; $P <0.01$); however, talking of the two latter skull base types, this parameter was observed to feature no significant differences ($P> 0.05$). The average pituitary fossa width of each craniotype did not have significant differences and ranged from 10.1±0.4 mm for the platibasilar to 10.8±0.4 mm for the flexibasilar craniotypes ($P> 0.05$). The average depth of the fossae in the medio- and flexibasilar craniotypes was equally variable (7.6±0.3 mm; $P> 0.05$) and the value of this parameter in the platibasilar type prevailed by 1.2 mm ($P <0.05$).

The highest average value of the pituitary fossa area was in the platibasilar type and was 78.53 mm^2, whereas the lowest value was in the medio- and flexibasilar (71.18 mm^2; 73.79 mm^2) craniotypes. The predominance of the pituitary fossa area in the platibasilar type compared with the respective area in the medio- and flexibasilar fossae was 7.35 mm^2 and 4.74 mm^2, respectively.

In the flexibasilar type, the pituitary fossa volume was on average 373.8 mm^3; in the medio- and platibasilar craniotypes — 360.68 mm^3 and 261.79 mm^3, respectively. The biggest volume difference between the flexi- and platibasilar types was 112.11 mm^3, between the plati- and the mediobasilar types — 98.89 mm^3, while it was the lowest between the flexi- and mediobasilar types — 13.22 mm^3.

The longitudinal diameter of the Turkish saddle, as our research showed, ranged on average from 12.1±0.3 mm (flexibasilar craniotype) to 14.7±0.2 mm (platibasilar craniotype), transverse — from 11.8±0.1 mm (platibasilar craniotype) to 14.3±0.3 mm (flexibasilar craniotype). The longitudinal diameter variability range of the pituitary fossa, depending on the skull base type, was from 8.7±0.2 mm (flexibasilar craniotype) to 9.9±0.3 mm (platibasilar craniotype); depth — from 6.4±0.3 mm (platibasilar craniotype) to 7.6±0.3 mm (medio- and flexibasilar craniotypes), while the transverse diameter did not have typical variability and averaged 10.1 — 10.8±0.4 mm. The available literature offers data supplied by a number of authors regarding the average values of the dimensional specifics of the sphenoid bone structure in adults, the Turkish saddle: longitudinal size — 11.6 mm,

14.0 mm; transverse — 14.5 mm; vertical — 8.5 mm, 12.0 mm; pituitary fossa — length – 12.0 mm, width — 13.0 mm, height — 3.5 mm [3, 7, 8], however comparison is not possible due to difference in the research methodologies.

As our study revealed the pituitary fossa in the platibasilar type is rounded, small, with an area of 78.5 mm^2, whereas in the medio- and flexibasilar craniotypes it is oval-shaped, deep, with an area of 71.2 mm^2 and 73.8 mm^2, respectively, which cannot be matched against the data from V.S. Maikova-Stroganova and D.G. Rokhlin [8], who, using only the correlation of vertical and sagittal dimensions, ponted at flat, round and deep types of shape. The pituitary fossa area predominated in the platibasilar type by 7.4 mm^2 compared with the medio- and 4.7 mm^2 compared with the flexibasilar craniotype.

CONCLUSION

The above means that the highest prevalence of the Turkish saddle area in comparison with the pituitary fossa area was 105.3 mm^2 for the mediobasilar type (occupies 40.3% of the area of the saddle); the lowest ratio for the platibasilar type was 95.0 mm^2 (45.2% of the saddle area), whereas for the flexibasilar type, the Turkish saddle area was 99.6 mm^2 above the fossa area (42.4% of the saddle area). A typical variability of the linear parameters of the sphenoid bone body structures, determining their configuration, was identified — the platibasilar type of the skull base features a long and narrow Turkish saddle, a small and round pituitary fossa, with a larger area and a smaller volume. For the medio- and flexibasilar craniotypes, the Turkish saddle is short and wide; the pituitary fossa is deep, oval-shaped with a smaller area, yet with a large volume. There has been no typical variability for the Turkish saddle area revealed.

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THE CORRELATION OF METABOLIC AND PLATELET INDICATORS IN PATIENTS WITH HEREDITARY THROMBOPHILIA

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ABSTRACT — Patients with the A2 polymorphism of the subunit gene of glycoprotein IIb/IIIa platelet receptors showed an increased level of endothelial dysfunction markers and increased free-radical reactions, as well as an increase in the geometric parameters of platelets, an increase in their functional activity and the appearance of young cells in peripheral blood. A correlation was found between the parameters of platelet hemostasis and the severity of free-radical reactions in women patients with thrombophilia who have A2 polymorphism in the gene of the subunits of the platelet receptor glycoprotein IIb/IIIa.

KEYWORDS — gene polymorphism, free radical reactions, platelets, thrombophilia.

INTRODUCTION

It has now been established that genetic abnormalities of hemostasis are found in patients with thrombosis in 80–90% of cases. Mutation of factor V Leiden is detected in 20% of patients, other defects in the anticoagulant system (antithrombin III deficiency, protein C and S deficiency) in 20% of cases, sticky platelet syndrome (Tp) in 14% of cases, etc. [1, 9]. In modern literature there are scattered data on the relationship of the genetic polymorphism of the Tr glycoprotein (GP) IIb/IIIa receptor gene with the development of thrombosis but the information on the relationship of this polymorphism with the development of typical obstetric pathology is very contradictory [1].

Thrombophilic complications of pregnancy and childbirth are currently considered from the standpoint of the development of polyorgan failure syndrome which is based on systemic endothelial dysfunction [2, 8, 10]. And oxidant stress acts as a provoking factor resulting in the formation of lipid peroxidation products — free radicals and atomic oxygen damaging the endothelium [7, 11, 12].

The presented mechanisms of the relationship of coagulation, angiopathy and the development of oxidative stress from the standpoint of modern concepts and their violation in patients with genetic thrombophilia (TF) have not been studied.

Defects in the Tr GP IIb/IIIa receptor gene lead to an increase in the functional properties of Tr and they contribute to an increase in the thrombogenic properties of the endothelium [3, 4, 5, 6].

The purpose of the study is a comprehensive assessment and establishment of the relationship between the level of free-radical reactions, the state of the vascular wall and changes in the parameters of platelet hemostasis in pregnant women with the genetic A^2 polymorphism of the Tr GP IIb/IIIa receptor subunit gene.

MATERIALS AND RESEARCH METHODS

226 women in the third trimester of pregnancy were treated at the obstetric department of the pathology of pregnancy at the Stavropol city hospital. The age of patients ranged from 20 to 35 years with an average of 25.2±0.6 years. Only 59 of these patients had various obstetric complications associated with TP (thrombophilia), in 152 women the pregnancy was unremarkable.

Blood sampling was carried out with the consent of the attending physician while observing the rules of the preanalytical phase of the study.

All surveyed were divided into the following groups:

I — women with physiological pregnancy, genetic carrier of the normal variant of the Tr GP IIb/IIIa receptor subunit gene (Pl^{A1}/Pl^{A1}) (n = 128) — control group;

II — healthy pregnant women with a heterozygous mutation variant (Pl^{A1}/Pl^{A2}) (n = 24);

III — patients with TP and heterozygous polymorphism (Pl^{A1}/Pl^{A2}) (n = 35);

IV — patients with TP and homozygous Pl^{A2}/Pl^{A2} polymorphism (n = 39).

Patients with other confirmed mutations in the hemostatic system genes were excluded from the study.

The determination of the genotype by the Pl^{A1}/Pl^{A2} polymorphism was carried out by an amplification-restrictional method. DNA isolation was carried out by the sorption method using the kit of DNA-Sorb B. Amplification mixtures were prepared on the basis of a universal set of reagents and preparations for PCR (polymerase chain reaction) *Ampli Sens-200-1* apparatus manufactured by CSUE (central scientific university of epidemiology) of *Rospotrebnadzor*. The primers were synthesized at Liteh LLC in accordance with the sequences described by Bojesen S.E. et al., 2000 [5].

Willebrand's factor (WF) activity level was used as an indicator characterizing the state of the endothelium of the vascular wall. The determination of the levels of WF was performed on the analyzer hemostasis *STA Compact* designed by the company *Diagnostica Stago* (France) with the help of reagents *vWF STA LIATEST* by the method of latex agglutination with monoclonal antibodies to the factor.

Malonic dialdehyde (MDA) indicators were used as markers of oxidative stress. The study was performed in serum by the method of Jagi K., Nishigaki I., Chama N. and platelets by the method according to J. Smith methods.

The quantitative indices of Tr were determined using the automatic hematology analyzer *Advia 2120 i* designed by Simens company (Germany). Four platelet parameters were analyzed: PLT — total Tp in peripheral blood and platelet indices (MPV, PCT, PDW). The study of functional activity of Tr was performed in Tr rich plasma by the Born G.V.R. method with graphic registration on a *BIOLA LA-23* laser aggregometer (Russia). ADP (200 μM), collagen (0.2 mg/ml), ristocetin (5 μM/ml) were used as inducers. To study the morphometric parameters of Tr in blood smears a computer morphometric installation *MECOS-C* (Medical Computer Systems CJSC, Moscow) was used. During the study the following geometric and color-brightness characteristics of Tr were analyzed: cell area, cell diameter, form factor, blue and red color in the preparation, the index of rejuvenation of Tr (IRTr).

The degree of reliability of differences in the studied parameters was determined by the t-criterion, the level of significance was considered reliable at $p < 0.05$.

THE RESULTS OF THE STUDY AND THEIR DISCUSSION

We judged the changes in free-radical oxidation and the state of the vascular wall in women by the indices of MDA in the serum and Tr. and the level of WF.

These changes in metabolic parameters are presented in Table 1.

A comparative analysis of the data revealed that in women without clinical manifestations of TP (groups I and II), the indicators did not go beyond the reference boundaries, however, in patients with heterozygous polymorphism a significant increase in the level of MDA Tp was found ($p < 0.001$).

Analysis of data from patients with TP revealed an increase in serum lipid peroxidation and Tp in all women compared to the control group. MDA Tp in women with the homozygous polymorphism variant (Pl^{A2}/Pl^{A2}) was most changed. The difference of this indicator in comparison with the I group was 202%. Serum MDA was increased by 110%. In the group of women with TP and heterozygous polymorphism, the indicators of serum MDA and Tp were higher by 57.1% and 103%. In healthy women with heterozygous polymorphism a significant ($p < 0.001$) increase of the MDA Tr index was also noted.

The level of WF was significantly different only in patients with TP and A^2 polymorphism ($p < 0.001$). The greatest changes in this indicator were observed in group of women IV. From the indicator of group I it was different by 88.8%.

The factual data obtained indicates that in patients with TP and A^2 polymorphism the intensification of free-radical reactions indicates a complex nature of humoral and hemostasiological disorders.

Identification of significant changes in indicators in healthy pregnant women of group II shows the effect of A^2 polymorphism on the development of signs of endothelial dysfunction even without the presence of clinical manifestations of TP which may indicate a high sensitivity of these indicators to the presence of a genetic defect.

In order to assess the effect of genetic polymorphism GP IIb / IIIa on the state of platelet hemostasis a study was made of total Tp in peripheral blood (PLT) and platelet indices (MPV, PCT, PDW), Tp aggregation activity and morphometric analysis indicators. The obtained data are presented in Table 2.

When conducting a comparative analysis of the quantitative parameters of Tr it was found that in women without clinical manifestations of thrombophilia (groups I and II) the values of Tr and platelet indices practically did not differ from each other.

Comparing the quantitative parameters of Tp in women without clinical manifestations of TP with a carrier of the normal version of the Tp GP IIb/IIIa receptor subunit gene and women with a heterozygous variant of polymorphism (Pl^{A1}/Pl^{A2}) — group III there was a significant change in the MPV and PDW, which amounted to an average of 10.9 fl and 18.2%, respectively ($p \leq 0.01$).

Table 1. *Humoral indices of pregnant women (X ± m; p≤0.001)*

Indicators, units of measure	Groups of pregnant women			
	I (n=128)	II (n=24)	III (τ=35)	IV (n=39)
MDA of serum, μmol/l	3,29±0,15	3,31±0,24	5,17±0,28*	6,91±0,37*
MDA Tr, μmol /10^{9}Tp	1,82±0,09	2,61±0,19*	3,7±0,16*	5,5±0,29*
WF,%	112,6±2,34	116,9±2,54	148,7±2,73*	212,6±2,27*

Note: ** — significance of differences compared with group I patients*

Table 2. *Changes in platelet counts of healthy pregnant women and patients with thrombophilia (X ± m; p≤0.05)*

Indicators, units measuring	Patient groups			
	I (n=128)	II (n=24)	III (n=35)	IV (n=39)
PLT,109/l	265,7±7,6	271,8±7,9	289,8±11,8	234,1±12,3*
MPV, fl	9,2±0,18	9,5±0,20	10,9±0,25*	11,2±0,27*
PCT, %	0,25±0,008	0,24±0,008	0,25±0,007	0,25±0,008
PDW, %	16,9±0,17	16,8±0,18	18,2±0,17*	18,4±0,17*
Aggregation with ADP, %	46,0±0,69	51,1±1,71*	78,4±2,97*	79,4±3,68*
Aggregation with collagen, %	42,0±0,83	45,0±1,96	65,3±2,74*	68,0±2,77*
Aggregation with ristotsetin, %	47,5±0,74	51,2±1,82	86,3±2,36*	89,1±2,11*
The average diameter of Tp, μm	2,24±0,10	2,31±0,15	2,66±0,13*	2,81±0,12*
Area Tr, μm 2	3,65±0,41	3,96±0,44	5,48±0,44*	6,33±0,44*
Form factor, cu	12,8±0,15	13,5±0,17*	15,12±0,17*	16,18±0,18*
The proportion of blue color, c.u.	0,32±0,002	0,37±0,007*	0,45±0,007*	0,52±0,007*
The proportion of red color, c.u.	0,42±0,002	0,39±0,007*	0,34±0,007*	0,31±0,007*
Common index of rejuvenation of Tr (IR Tr) c.u.	0,76±0,005	0,94±0,07*	1,32±0,07*	1,67±0,11*

Note: ** — the differences are significant compared with group 1*

In patients with a homozygous variant of the Tr GP IIb/IIIa receptor gene (Pl^{A2}/Pl^{A2}) mutations in the quantitative indices of Tr are most pronounced. There is a significant (p≤0.05) decrease in the number of Tp in peripheral blood (234.1±12.3 · 10^{9}/l, p≤0.05) an increase in the average volume of Tp (11.2±0.27 fl) and Tp anisocytosis index (18.4±0.16%) in comparison with the data of healthy puerperal (p≤0.001) of group I.

Analysis of the aggregatogram data showed that the groups of healthy puerperal significantly differed in aggregation induced by ADP (p ≤ 0.01) and there was a tendency for an increase in the degree of aggregation with collagen and ristocetin in the group of women with a heterozygous mutation in the Tr GP IIb / IIIa receptor gene. In patients with TP and Tp GP IIb / IIIa Pl^{A1}/Pl^{A2} receptor gene polymorphism there was an increase in Tg aggregation for 66% for ADP, 81% for ristocetin and 80% for collagen compared with the healthy women with normal genotype. In the group of women with the $P1^{A2}/P1^{A2}$ polymorphism the aggregation activity induced by ADP, ristocetin and collagen at the indicated concentrations which was higher by 68%, 87% and 61%.

An analysis of the morphometric parameters of Tr of healthy women revealed an increase in the functional activity of Tr in group II as evidenced by a significant (p≤0.001) increase in the index of the form factor. In addition, an increase in the number of young Tp was observed, which was manifested in an increase in the share of blue color and a decrease in the share of red color in the preparation. IR Tr was significantly higher in group II and amounted to 0.94 c.u (in group I — 0.76 in. e.), which reflects the activation of Tp in patients with Pl^{A1}/Pl^{A2} polymorphism compared with women with normal genotype. There was a tendency to an increase in the indices of the diameter and area Tr.

In patients with clinical manifestations of TP the mean diameter of Tp, cell area form factor and IR Tr were significantly increased (p≤0.001).

Thus, all geometrical and color-bright parameters changed most vividly in patients with clinical manifestations of TP and a homozygous variant of polymorphism (Pl^{A2}/Pl^{A2}). At the same time, the difference between the average diameter of Tp expressed as a percentage between patients of group V and healthy women of group I was 25.4%, between indicators of the area Tp — 73.4%, form factor — 26.4%. In patients with the Pl^{A1}/Pl^{A2} polymorphism the cell diameter was 18.7% larger, the form factor 18.1%, and the Tp area was increased by 50.9%. In women with the A1A1 genotype the diameter, area, and form factor indices were increased by 9.8%, 50.9% and 10.2% in comparison with the data of women of the first group.

The assessment of the intensity of *rejuvenation* Tr was carried out according to the degree of change in the IR Tr. The appearance of young Tp was detected in all groups of women with clinical manifestations of TP but to the greatest extent in patients with a homozygous mutation (1.67 in). In groups of women with a normal genotype and a heterozygous mutation this indicator was 1.31 c/u and 1.32 c.u.

Thus, in patients with Pl^{A2}/Pl^{A2} polymorphism there was a significant (p≤0.001) increase in geometric parameters with greater functional activity of Tp and the appearance of young cells in peripheral blood. In the groups of puerperal with the normal genotype and the polymorphism of Pl^{A1}/Pl^{A2}, similar changes were revealed but the degree of increase in the indicators was slightly lower than in patients with the polymorphism of Pl^{A2}/Pl^{A2}.

The important role of the genetic defect of the Tr GP IIb/IIIa receptor subunit gene in the development of thrombotic complications of pregnancy and childbirth is indicated by the fact that the most pronounced changes in the quantitative, functional and morphometric parameters of Tp were observed in a group of patients with homozygous polymorphism of this gene (Pl^{A2}/Pl^{A2}).

Since the heterozygous mutation variant (Pl^{A1}/Pl^{A2}) was also encountered in healthy women without clinical manifestations of TP it can be assumed that the genetic polymorphism of the Tr GP IIb/IIIa receptor subunits does not necessarily lead to the occurrence of the disease. It is possible that other provoking factors contribute to this situation. Detection of mutations and polymorphism in genes is an indication for monitoring the state of the hemostasis system since the presence of polymorphism in the Tr GP IIb/IIIa receptor subunit gene and especially when combined with other gene defects it may be associated with the risk of developing thrombosis.

There is the fact that the presence of A^2 dysmorphism of the Tp GP IIb/IIIa receptor subunit gene in pregnant women leads to an increase in free-radical reactions led us to conclude that the effects of the above disorders are indicators of platelet hemostasis in women with A^2 polymorphism.

To confirm this conclusion we carried out a correlation analysis of humoral and platelet indicators (see Table 3).

Table 3. *Correlation of humoral and platelet indicators in patients with thrombophilia*

Metabolic indicators	Hemostatic Indicators	Correlation Coefficient
MDA serum	PDW	R=0,33
	Aggregation with ADP	R=0,35
	Form Factor Tr	R=0,34
MDA Tr	MPV	R=0,42
	PDW	R=0,55
	Aggregation with ADP	R=0,61
	Area Tr	R=0,53
	Form factor Tr	R=0,42
	IR Tr	R=0,45

During the correlation analysis we established positive correlation links between an increase in serum MDA level and an increase in the heterogeneity index of the Tp population (R = 0.33) increased aggregation with ADP (R = 0.35) and an increase in the Tp form factor (R = 0.34). The indicators of lipid peroxidation (LP) in the Tr membranes were closely correlated with the parameters of hemostasis. The most significant were the association of increased LP with an increase in functional properties of Tr, as evidenced by the presence of positive correlations between the increase in Tr, the heterogeneity of the platelet population and the increased aggregation with ADP, an increase in the Tr form factor and IR Tr.

Based on the correlation analysis the dependence of the parameters of platelet hemostasis on the severity of free-radical reactions in women with TP with A^2 polymorphism in the Tr GP IIb/IIIa receptor subunit gene was revealed which is confirmed by significant correlations between metabolic and platelet indicators.

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AGE-RELATED CHANGES IN CORTICAL THICKNESS OF HUMAN ARCHICEREBELLUM

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ABSTRACT — Age-related changes in the thickness of the human cerebellar cortex in periods of growth and human development have not been studied enough. The article is devoted to the study of cortical thickness in the flocculonodular lobe of the cerebellum (115 cerebellums) received from human corpses of both sexes, aged from birth to 20 years. The material was grouped in annual intervals. Measurement of the cortical thickness was performed at the apex of the cerebellar folium on virtual images of sagittal paraffin sections, 10-μm-thick, Nissl-stained. For indicators of cortical thickness in different age groups the mean, the standard error and the confidence interval were calculated. The results have shown that the main increase in the cortical thickness in human archicebelellum is observed from birth to 3 years of age. The increase in cortical thickness in the nodule and in the right flocculus is also observed by age 7–8 years, in the left flocculus — by age 15 years. Lateral asymmetry of cortical thickness in the right and left flocculus is registered in the intervals from birth to 3 years and from 9 to 11 years of age.

KEYWORDS — flocculus, nodule, cerebellar cortical thickness, age-related changes.

INTRODUCTION

It is known that the cerebellum plays an important role in the hierarchical multi-level motor system and in several regulatory non-motor systems [1]. All areas of the cerebral cortex send information to the cerebellum; it also receives signals from all sensory systems. The cerebellum plays the role of a *central dispatcher* affecting brainstem motor centers and providing motor coordination. A special role in these processes belongs to vestibulocerebellum (flocculonodular lobe, or archicerebellum). This vestibular control is of great importance for maintaining dynamic stability and motor adaptation to environmental conditions [2]. The projection fibers from the phylogenetically old parts of the inferior olivary complex (medial part of the principal and accessory olivary nuclei) and pontine nuclei are distributed within the vestibulocerebellum providing mediated connections of the cerebral cortex, red nucleus, tegmentum of midbrain, globus pallidus and other brainstem and diencephalic structures with the cortex of flocculonodular lobe. The direct efferent projections in turn are directed from the cortex of the flocculonodular lobe to the vestibular nuclei, exerting a direct inhibitory effect, in particular, on the neurons of Deiters' nucleus which is of great importance for the motor regulation involving spinal motor centers [3, 4, 5]. Such character of direct and mediated connections of the cerebellar nodule and flocculi indicates a significant role of the cortex of vestibulocerebellum as an integrative cortical center in formation of not only basic motor functions and postural control but also visual perceptual functions, spatial and self-orientaion perception [6, 7, 8]. Motor control mechanisms and organization of motion itself with participation of the vestibulocerebellum are formed gradually in the process of child development. As was shown earlier, the cortical thickness is an integral indicator of the structural changes of the cerebellar cortex in different periods of growth and human development [9]. The purpose of this study was to study changes in the cortical thickness in the flocculonodular lobe of the human cerebellum in different periods of growth and human development.

MATERIALS AND METHODS

115 cerebellums of people of both sexes, aged from birth to 20 years, who died from injuries not associated with brain damage, were studied. Collecting of the corpse material was approved by Ethics committee of the Institute of Age-related Physiology of the Russian Academy of Education (Protocol No. 3; 23/05/1996) and was carried out in the forensic morgues of Moscow and the Moscow Region. The material was grouped according to annual intervals. Pieces of the cerebellar cortex for histological examination were taken in the area of the nodule, the right and the left flocculus, fixed in 10% neutral formalin, dehydrated in alcohols of increasing concentration and embedded in paraffin blocks. The cortical thickness at the apex of the cerebellar folium was measured on sagittal sections, 10-μm-thick, Nissl-stained (with cresyl violet), by computer morphometry using

BIOSCAN-AT and Image Tools programs (NICH, USA). The sample size for each slice was at least 10, for each preparation — at least 40, for each age — at least 160 measurements. For indicators of cortical thickness in different age groups, the mean, the standard error and the confidence interval [10] were calculated with a significance level of P = 95% ($p < 0.05$).

RESULTS

In newborns the cortical thickness in the nodule area is 505.0±22.2 μm, in the right flocculus — 495.5±28.9 μm, in the left flocculus — 403.83±17.0 μm. Thus by the time of birth, this indicator in the right flocculus and the nodule is 1.2–1.3 times greater than in the left flocculus. By the end of the first year of life, the cortical thickness in the nodule increases by 1.3–1.4 times; at age 3 years — by 1.7 times, at 7–8 years — by 1.9–2.1 times, compared to newborns. The highest values are observed in the nodule of children at the age of 8–11 years whose cortical thickness mean is from 924.4±38.2 to 965.2±41.6 μm. However, by the age of 12–13 years, a tendency towards a decrease in cortical thickness is observed in the nodule, and by the age of 14, a significant decrease in this indicator is observed. The cortical thickness in the nodule of teenagers at the age of 14 and 15 is 830.7±29.9 and 848.4±34.4 μm, respectively. In adolescents after 15 years of age, the cortical thickness mean in the nodule does not change, and by the age of 20, it is 820.8±54.3 μm (Fig. 1).

The cortical thickness in the right flocculus increases from birth to 12 months by 1.2 times, at 2 years — by 1.4 times, at 3 years — by 1.6 times, and at 7 years — by 1.9 times, compared to newborns. In children of age 9–11 years, the greatest rates of cortical thickness in the right flocculus are observed, the means are from 914.8±28.9 to 960.6±38.4 μm. By the age of 12, in the right flocculus, as well as in the nodule, there is a tendency towards a decrease in the cortical thickness, and by the age of 13 years, a significant decrease in this indicator is revealed. By the age 13 and 14, the cortical thickness in the right flocculus is 817.5±43.1 and 821.2±50.2 μm, respectively. By the age of 15, the cortical thickness in the right flocculus is 894.6±32.8 μm; it grows, compared to 13 years. After age of 15 years, the cortical thickness in the right flocculus stabilizes and by the age of 20 its mean is 875.8±33.2 μm (Fig. 2).

The cortical thickness in the left flocculus from birth to 12 months increases by 1.6 times, at the age of 2 years — by 1.9 times, at 6–7 years — by 2.1 times, compared to newborns. Thus the cortical thickness in the left flocculus from birth to 6 years grows more intensely than in the right flocculus. The cortical thickness in the left flocculus up to 3 years of age remains 1.1 times greater than in the right flocculus. Only by the age of 4 years the differences between them disappear due to increase in the cortical thickness in the right flocculus. By the age of 6 years, the cortical thickness in the left flocculus is 855.6±29.7 μm, and in the right flocculus — 854.3±39.8 μm. At the age of 7–8 years, the cortical thickness in the left flocculus remains stable, from 9 to 11 years it shows a clear tendency to decrease. At the age interval from 9 to 11 years, the cortical thickness in the left flocculus becomes 1.1–1.2 times thinner than in the right flocculus. By the age of 12–14 years the differences in the cortical thickness in the left and the right flocculus are gradually smoothed out. By the age of 15, the cortical thickness in the left flocculus is 896.44±41.4 μm, in the right flocculus — 894.56±34.6 μm. After 15 years of age, the cortical thickness in the left flocculus, as well as in the right, stabilizes, and by the age of 20 its mean is 880.5±24.3 μm (Fig. 3).

DISCUSSION

Each of the analyzed archicerebellar cortical zones, despite their phylogenetic homogeneity, is characterized by a peculiarity of the timing of changes in the cortical thickness. The peculiarities of the age-related changes in the cortex at the apex of the cerebellar folium in the region of arhicerebellum are well illustrated by polynomial curves with a high degree of confidence of the approximation (Fig. 1–3).

The obtained data allow identifying a number of gradual stages of age-related changes in the cerebellar cortical thickness in all investigated cortical loci: I — synchronous growth of the cortical thickness from birth to 3 years; II — heterochronic and heterodynamic growth from 4 to 9–11 years; III — asynchronous specialized changes in the cortical thickness from 12 to 14–15 years; IV — stabilization of cortical thickness after 15 years. It is interesting to compare the revealed periods of age-related changes with the data on coordination abilities, parameters of which, closely related to vestibular regulation, are most intensively formed in children and adolescents at the age from 8 to 15 years [11, 12]. This is also evidenced by data on improvement of postural regulation in children and adolescents with age, as well as by data on the strengthening of the cerebellum role in adolescents, compared to children, in recognizing vestibular signals with head movements during orientation in space [13, 14, 15].

CONCLUSION

In the vestibulocerebellum high growth pace of the cortical diameter in thickness is observed in the period from birth to 3 years of age, as well as by the

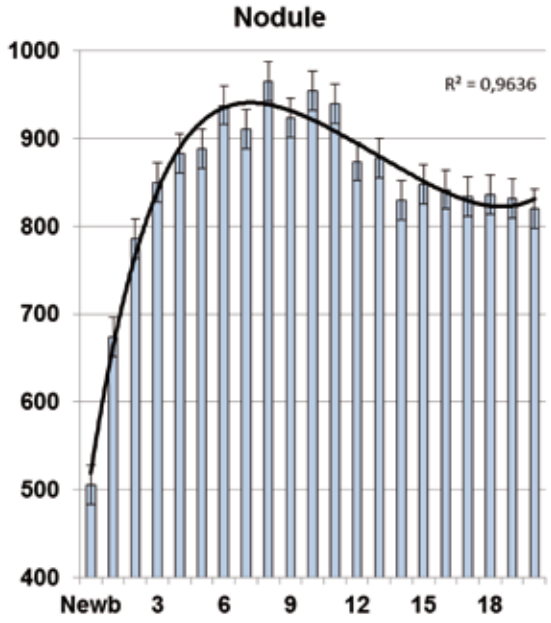

Fig. 1. *Changes in the cortical thickness in the human cerebellar nodule from birth to 20 years of age*

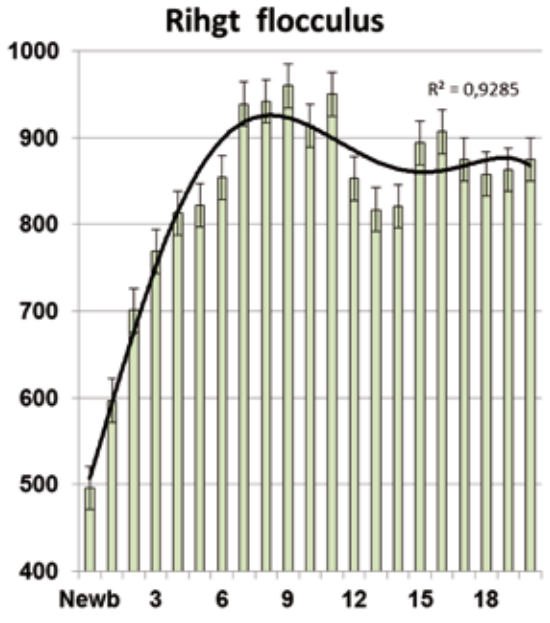

Fig. 2. *Changes in the cortical thickness in the right flocculus of the human cerebellum from birth to 20 years of age*

Here and on Fig. 2 and 3: *on the x-axis – age in years, newb – newborns; on the y- axis – cortical thickness in μm.*
The bars represent the group means, the line represents the approximating curve of polynomial function, R2 indicates the level of significance of approximation, the vertical segments represent the standard error of the mean.

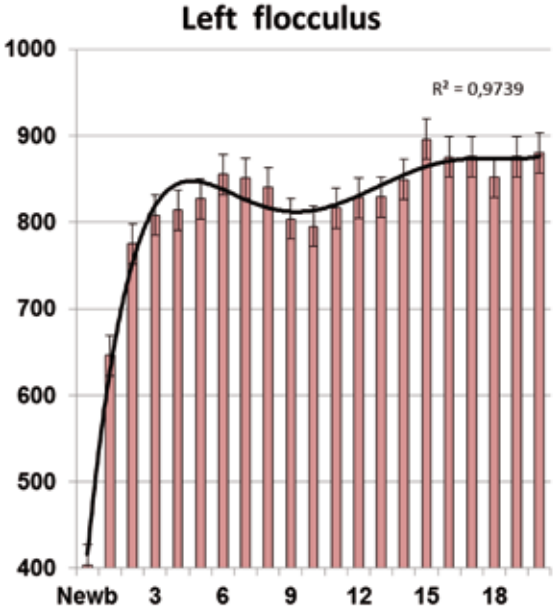

Fig. 3. *Changes in the cortical thickness in the left flocculus of the human cerebellum from birth to 20 years of age*

age of 7–8 years – in the nodule and the right flocculus, and by the age of 15 years – in the left flocculus. From birth to 3 years and from 9 to 11 years, there is a lateral asymmetry in the cortical thickness in the right and the left flocculi. After 15 years of age, the cortical thickness in the cerebellar flocculonodular lobe does not change on average but varies individually. The findings indicate the heterochronic maturation of hierarchically distributed multimodal integrative neural networks of the brain, involved in the motor regulation, in which the flocculi and the nodule are specifically included.

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BLOOD AND LYMPH CIRCULATION UNDER NORMAL CONDITIONS AND IN CIRCULATORY HYPOXIA

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ABSTRACT — The aim of the study was to assess the indices of blood and lymph microcirculation in the skin in healthy men under normal condition and in circulatory hypoxia. Thirty healthy men, aged 19 to 27, were examined. Evaluation of blood and lymph circulation was carried out by laser Doppler flowmetry (LDF) of the skin — the palmar surface of the base of thumb. The results showed that in occlusal period an increase in the lymph flow and decrease in blood flow were revealed, compared to normal conditions. In post-occlusal period a rapid recovery of blood circulation and slight decrease in lymph circulation were observed. Conclusion. Indices of blood and lymph circulation differ in normal conditions, in occlusal test and in post-occlusal period. Different reactivity of blood and lymph microvessels is observed in circulation recovery. The data obtained can be used for the diagnosis of blood and lymph flow disorders and assessment of local toxicosis severity under various pathological conditions.

KEYWORDS — blood microcirculation, lymph microcirculation, laser Doppler flowmetry, palm skin.

INTRODUCTION

The issues of prevention and treatment of various circulatory disorders constitute an urgent problem of modern medical practice [3, 4]. Microcirculatory — tissue system is a structural-functional complex consisting of parenchymal elements, cellular and non-cellular component of connective tissue, blood and lymph microvessels, nerve fibers, combined into a whole system by regulatory mechanisms [12]. The lymphatic system is closely connected with the venous part of bloodstream, providing additional to veins drainage of organs. In the middle of 20th century it was proved that every part of human body has a regional lymphatic apparatus, consisting of prelymphatic pathways, lymph vessels and lymph nodes and providing local detoxification and the maintenance of water and immune homeostasis [10]. Malfunction of the regional lymphatic apparatus causes accumulation of metabolic products and xenobiotics in intercellular space with the development of local endotoxicosis and exacerbation of chronic and acute pathologic conditions. In this regard, examination of the local blood and lymph flow in normal and pathological processes is important for development of diagnostic criteria for local and general endotoxicosis and severity of drainage-detoxification malfunction.

Laser Doppler flowmetry (LDF) is a modern non-invasive method for evaluating microcirculation. Registered Doppler frequency shift of the reflected signal is proportional to the speed of movement of the microvasculatory particles [3, 6, 11, 14].

Functional tests in LDF allow identifying the adaptive reserves of microcirculation, as well as regulatory mechanisms and functional condition of microvascular bed. One of the most used in the clinical practice is the occlusal test [9]. Since in clinical pathology ischemia is the dominant mechanism underlying general pathological processes, assessment of its tolerance allows, on the one hand, identifying the individual reactivity of vascular bed, and on the other hand, assessing the contribution of local and systemic mechanisms to its regulation [7].

The aim of the study was to examine the indices of blood and lymph microcirculation in the skin in healthy men under normal conditions and in circulatory hypoxia.

MATERIALS AND METHODS

Thirty healthy men aged from 19 to 27 years, were examined. Registration of changes in blood and lymph circulation in the microvasculature was carried out.

Examination of blood and lymph circulation in the skin was performed using LDF — standard probe holder put on the palmar surface of the base of thumb (tenar) in sitting position.

The study was performed using laser analyzer "LAZMA MC-1" (LLC NPP "LAZMA", Moscow, Russia; registration certificate of Roszdravnadzor № RZN 2015/3142 dated 10.28.2015), intended for evaluation of tissue blood and lymph circulation. The index of tissue blood circulation, determined by LDF method, is proportional to the product of the erythro-

cytes number and the average speed of their movement (range 0.5–5 mm/s). The index of lymph circulation is proportional to the product of the number of diffusers in the lymph flow and the average speed of their movement (10–40 μm/s) [3 ,4, 6].

The design of the study was as follows: we recorded the initial level of tissue blood and lymph circulation for 60 seconds (normal conditions), and then the recording was stopped. In the pressure cuff, put on the middle third of the shoulder, air was pumped up to 250 mmHg, and then the recording was continued. After 1 min, with release of the pressure, reaction of microcirculation was recorded during restoration of blood flow over the next minute. The total time of the test was 3 minutes. LDF registration and occlusive test were performed by the same person (physician) with the same instrument (LAZMA MC-1 analyzer; manometer).

The perfusion indices were calculated using the software attached to LAZMA MC-1 analyzer. The dynamics of average microcirculation index in perfusion units (PU), its standard deviation (or flux level – σ), and the variation coefficient were determined. The microvascular reactivity index, characterizing post-occlusal hyperemia, was calculated in % in post-occlusal period; indices in occlusal period were taken as 100%. Blood (B) and lymph (L) circulation indices were processed using methods of variation statistics, statistical significance was determined using Student's criterion. Threshold for statistical significance was determined as 0.05 [8].

RESULTS

Characteristic quantitative changes of blood (B) and lymph (L) circulation indices were revealed. The indices are shown in the Table 1.

hyperemia in the skin blood microvessels was 228%, and the lymph index dropped by 27%, compared to the occlusion level. The indices of blood and lymph circulation in occlusal test were statistically significant.

Thus, the data obtained indicate different dynamics of blood and lymph circulation in occlusal test and post-occlusal period.

DISCUSSION

In occlusal test not only the brachial artery is involved in the compression area but also the deep and superficial veins of the shoulder. This allows us to suggest transient hypoxia in the distal parts of the upper limb.

Earlier studies by other authors have not shown the extensive changes in the lymph flow on the first day of arterial ischemia. There are numerous data on the compensatory-adaptive activation of the lymph flow in the development of local venous hypertension [1]. According to Starling, the increasing lymph flow in venous stasis is caused by the following factors: high blood pressure in the veins increases capillary pressure; increased hydrostatic pressure in the blood capillaries leads to ultrafiltration in the interstitium, and resorption in the venous part of capillary becomes difficult. Excess fluid is absorbed from the interstitium into the lymph capillaries increasing the lymph flow [1, 4].

Thus, some increase in lymph production and lymph flow in our study seems to be a mechanism of compensation for the venous insufficiency in occlusal period. Based on the literature data and our results, it can be concluded that the functional test revealed the fact of lymph filing in the tissues in local venous hypertension.

In the post-occlusal period we found a rapid recovery of blood circulation with index exceeding the

Table 1. *Indices of blood and lymph circulation in the tenar skin in normal conditions and in circulatory hypoxia (M+m)*

Area of examination	Normal conditions (PU)		Occlusal test (PU)		Post-occlusal period (PU)		Reactivity of microvessels %	
	B	L	B	L	B	L	B	L
Skin in the tenar area	11,82+ 1,70	0,29+ 0,06	3,17+ 0,56*	0,46+ 0,05*	12,33+ 1,56	0,38+ 0,05	+288	-27

** the differences are statistically significant if P<0.05.*

In occlusal period the index of blood circulation decreased by 73% compared to normal conditions, and the index of lymph circulation, on the contrary, increased by 58%. In the re-perfusion period, the index of blood circulation increased, compared to normal, by 4%, while the lymph circulation index decreased by 31%. The index characterizing reactive post-occlusal

initial level by 4%, while the lymph circulation index decreased by 31%.

In this connection, it can be assumed that even partial occlusion of the upper limb leads to vein hypertension and hypoxia that contribute to increase in lymph production, lymph tissue filling and increase in interstitial pressure. This seems to cause a slower re-

covery of microcirculation indices in the post-occlusal period.

Our data are supported by a number of studies showing that tissue fluid balance depends on many factors, including angioarchitecture, intensity of tissue blood flow and rheology of blood, but the key role is played by directed transport of protein molecules from the bloodstream to the lymph flow [3 , 13].

CONCLUSION

The results of our study - blood and lymph circulation assessment using the LDF method - enable us to conclude that indices of blood and lymph circulation differ in normal conditions, in occlusal test and in post-occlusal period. Different reactivity of blood and lymph microvessels is observed in circulation recovery. The data obtained can be used both to diagnose disorders of the blood-lymph flow and to assess the severity of local toxicosis in tissue microdistricts under various pathological conditions.

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TYPOLOGICAL STRUCTURE OF HEMODYNAMICS IN AGE SCALE

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ABSTRACT — The aim of the study was to clarify the features of adaptation of the cardiovascular system (by hemodynamic parameters) to the conditions of the earth's gravity in different periods of ontogenesis.
Methods: The analysis was conducted for the number of age groups: up to 8 years (n=55), 9–14 years (n=68), 15–21 years (n=226), 22–35 years (n=326), 36–55 years for women and 36–60 years for men (n=658), up to 70 years (n=413) and over 70 years (n=198). The study of the cardiovascular system was carried out on the hardware and software complex "ANTHROPOS-CAVASCREEN" created by us on the basis of tetrapolar thoracic and regional rheography. Three orthostatic types of hemodynamics (hypokinetic, eukinetic and hyperkinetic) are determined by the ratio of minute blood volume standing/lying.
Results: The age limit of transition to the increase in the severity of hyperkinetic organization of blood circulation (corresponding to the orthostatic type III of hemodynamics) is the first reproductive age (22–35 years). The age dynamics of the typological structure indicates that the orthostatic type of hemodynamics is a dynamic characteristic of the organization of blood circulation and it must be taken into account when assessing the circulatory state of the cardiovascular system.

KEYWORDS — age, hemodynamics, orthostatic type.

INTRODUCTION

In clinical practice, diagnostic studies of the cardiovascular system are carried out in the supine position, that is realizing in conditions of minimal manifestation of the gravitational effect on blood circulation [2, 3]. It can dramatically affect the reference values of the studied parameters [3]. At the same time, there are practically no standards and diagnostic approaches that take into account the typical human circadian rhythm of the natural positions of the body and the characteristic of blood circulation by the gravitational (hydrostatic) factor (orthostatic type of hemodynamics).

Interaction with the earth's gravity is a permanent factor of the environment, which has a systemic gravitational effect on blood circulation. It manifests itself in a characteristic human stage adaptation to the conditions of upright walking throughout postnatal ontogenesis [1–3]. Accordingly, the relevant parameters for a person are standing and lying down, as well as their ratio *standing / lying* in the systemic characteristics of the functional state of the person by the main hemodynamic mechanisms. This creates the prerequisites for clarifying the features of adaptation of the cardiovascular system (by hemodynamic parameters) to the conditions of the earth's gravity in different periods of ontogenesis, which was the aim of the work.

METHODS

The analysis of the data on circulatory state of the cardiovascular system was carried out by age samples, which were formed in accordance with the proposed classification of the stages of ontogenetic adaptation to Earth gravity (*anthropogenetic model*) in the process of formation and activity in the human-specific conditions of walking. The analysis was conducted for the following age samples (total for men and women): up to 8 years (n=55), 9–14 years (n=68), 15–21 years (n=226), 22–35 years (n=326), 36–55 years for women and 36–60 years for men (n=658), up to 70 years (n=413) and over 70 years (n=198). The study of the cardiovascular system was carried out on the hardware-software complex "ANTHROPOS-CAVASCREEN" created by us on the basis of tetrapolar thoracic and regional rheography [1]. A complex of hemodynamic parameters for central and peripheral blood circulation in standing and lying positions was recorded. To do this, the following ***orthostatic types of hemodynamics*** are determined by the ratio of minute blood volume standing/lying:

– ***Type I***, or ***hypokinetic state***, with a decrease in cardiac output in the standing position compared to the lying position (less than 94%). it is considered the best option ;

– ***Type II***, or ***eukinetic state***, the magnitude of cardiac output lying and standing is not different (94–106%);

– ***Type III***, or ***hyperkinetic state***, in which the cardiac output in the standing position increases (more than 106%) compared to the lying position.

Calculations were performed by specially designed software on the basis of a statistical software package Microsoft Excel 2010.

RESULTS

We have considered the age representation of orthostatic types of hemodynamics. The dynamics of the typological structure of the organization of blood circulation was determined by the ratio of minute blood volume in standing/lying positions (Fig. 1).

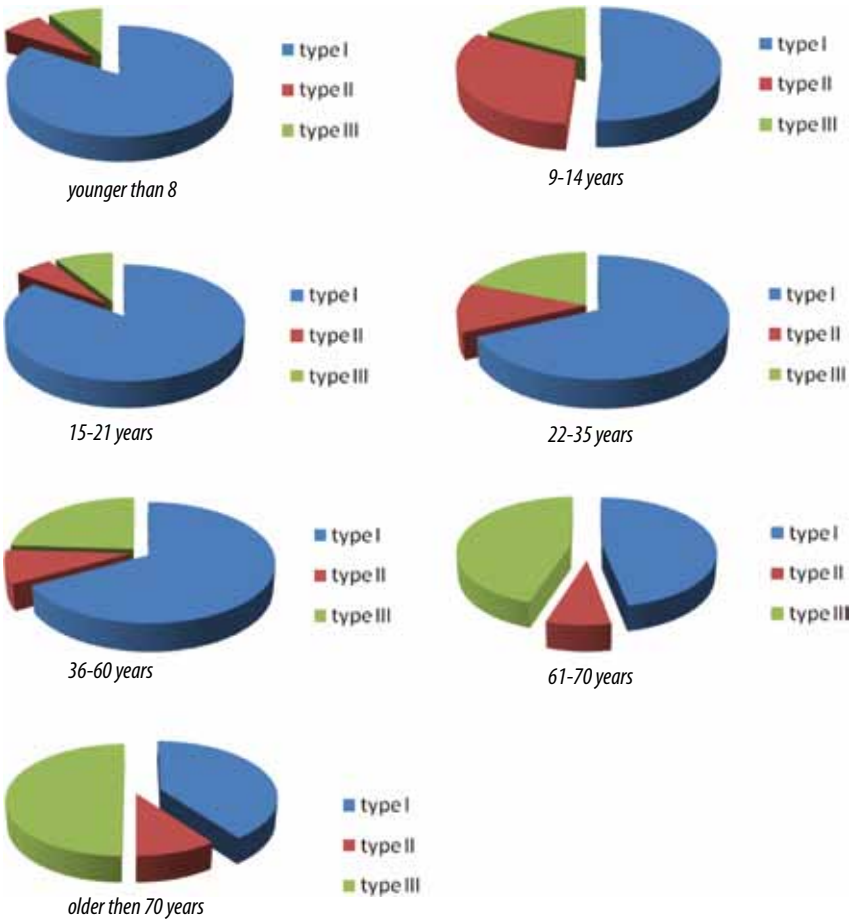

Fig. 1. *Typological structure of hemodynamics state in different age groups*

It was found that in children under 8 years the structure of orthostatic types of blood circulation is mainly present by I type (84%, $p<0.05$), or hypokinetic state. The instability of the typological structure of hemodynamics in adolescents compared to younger children was noted as they grew up (in the puberty period). A significant increase in the representation of type II (eukinetic) of hemodynamics in comparison with children up to 8 years was determined (up to 32%; $p<0.05$). Also, the proportion of persons with type I of hemodynamics (from 84% to 51%) significantly decreases ($p<0.05$), whereas type III showed almost double growth (9% to 17%). At the same time, the proportion of people classified by type I remains prevalent compared to type II ($p<0.05$) and, especially, with type III ($p<0.01$). The state of hemodynamics in children aged 9–14 years can be assessed as a transitional nature of the dynamics of the typological organization of blood circulation, where there was no significant predominance of any type in the sample.

At the end of the growth (15–21 years) and physical maturity (22–35 years old and 36–60) of the typological organization of the blood circulation is getting stable. At these age stages, the share of type I reaches 85%, 68% and 67%, respectively, significantly different from the representation of this type in younger people (9–14 years; $p<0.01$). In the older age groups (60–70 years and older than 70 years), the proportion of type I decreases to 47% and 40%, along with a parallel increase in the proportion of persons with type III to 45–50%, which is considered as a manifestation of the non-optimality of the typological structure of blood circulation. It should be noted that, unlike the sample of adolescents (9–14 years) with a transitional typological structure of the organization of blood circulation, there were no significant differences between the representation of I (40–47%) and III (45–50%) types at the postdefinitive stage (older than 60 years). However, here is a significant ($P<0.01$) increase in the proportion of type III compared with the first (22–35 years) and second (up to 60 years) reproductive age.

CONCLUSION

The age limit of transition to the increase in the severity of hyperkinetic organization of blood circulation (corresponding to the orthostatic type III of hemodynamics) is the first reproductive age (22–35 years). The age

dynamics of the typological structure indicates that the orthostatic type of hemodynamics is a dynamic characteristic of the organization of blood circulation and it must be taken into account when assessing the circulatory state of the cardiovascular system.

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CHANGES IN PERIPHERAL BLOOD AND CYTOGENETIC INDICATORS DURING THE APPLICATION OF COMPOUNDS OF THE COPPER-ORGANIC COMPLEXES TO IRRADIATION

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INTRODUCTION

One of the priorities of the contemporary radiobiology is the search for new efficient radioprotective compounds. In this field metal-organic complexes of high antioxidant activity are of special interest. According to literature sources [1–4] and our early researches [5–9] such complexes have low toxicity and obvious radioprotective properties.

With a view to releave possible radiation protective actions, we studied compounds of copper-organic complexes: [Cu $(PTA)_4$] BF_4 and [Cu(PCN)(HBP_{23})] BF_4, in which legands are: PTA-1,3,5-triaza-7-phosphaadamantane and PCN-tris(cyanoethyl)phosphine HBP_{23}-trispyrazolilborato (hereinafter referred to as "PTA" and "PCN") which were synthesized in Camerino University (Italy) under the supervision of Professor Carlo Santini.

MATERIALS AND METHODS

The experiments were carried out on reproductive, white, outbred rats of both genders with middle-weight of 180–200 g. An hour before general, single-shot radiography of the animals with dose of $LD_{50/30}$ — 5,3Gy and $LD_{100/30}$ — 7,0Gy, by PYM-17 equipment, PTA and PNC Cu-organic complexes were injected abdominally with dose of 20mg/kg in the form of an aqueous suspension. Radioprotective activity of these compounds was estimated by survivability of average life expectancy which showed dynamics of experimental rats death during 30 days of monitoring.

Since ionizing radiation is essential disturbing factor of cytogenetic status of the organism, we analyzed radiation-induced clastogenesis of the bone marrow cells (BMC) as one of the most informative indicators. Generally accepted method of cytogenetic analysis of white rats' BMC indices was used. Proliferative activity (mitotic index-MI), chromosome aberration (CA), cell ploidy (CP) was taken into consideration. We also performed hematological analysis of peripheral blood: red blood cell count, leucocyte count, level of hemoglobin.

Survival experiments were performed on 60 rats, 10 animals per group. Groups: net radiation with dose of 5,3Gy and 7,0Gy (2 g) (control group); PTA+radiation with dose of 5,3Gy and 7,0Gy (2 g) and PCN + radiation with dose of 5,3Gy and 7,0Gy (2 g).

The results were also compared with the norm group — a group of non-irradiated animals and not receiving the tested complexes.

Statistical processing of digital material carried out using a number of computer programs. Microsoft Excel spreadsheet and specialized statistical packages Statsoft, SPSS, and StatGraphics Plus were used [10, 11].

RESULTS

Biomaterial was taken from 40 individual subjects: bone marrow was taken from femoral bone and periferic blood from tail vein. Indicators of survival and average life expectancy in the irradiated rats injected with the PTA and PCN complexes were higher than in animals from the control group (only irradiated) which revealed the radioprotective properties of the tested complexes (Table 1).

A survival analysis was also conducted using the regression method, which makes it possible not only to describe the dynamics of changes in indicators, but also the possibility of prediction. The logarithmic regression equations for survival were obtained for the control group (exposure to 5.3Gy-Y1) and groups of animals after the introduction of the PTA (Y2); PCN (Y3) of compounds (20 mg/kg before irradiation) Y1 = 120.69-50.61lg (x); Y2 = 107.62-20.01lg (x); Y3 = 110.9-21.5lg (x), where x is the number of days after irradiation at 5.3Gy.

Were also obtained regression curves and the corresponding equations describing the survival of groups

Table 1. *Survival and average life expectancy of rats*

Indicators	Survival (%)		Average life expectancy in days	
Doses / Groups	5,3Gy	7,0Gy	5,3Gy	7,0Gy
Control	60	30	19,4	14,3
PTA+ irradiation	80	60	26,8	25
PCN+ irradiation	80	60	27,3	25,9

sion of proliferative activity in the control groups (net radiation with doses of 5,3 and 7,0Gy) is somehow descending (table 3). Comparison of proliferative activity of PTA and PTC groups with control groups showed that their significance is trustingly higher than that of the control groups.

A comparative analysis between the control groups and groups with experimental complexes showed that, regardless of radiation dose, significant decrease in the level of aberrant cells was observed. The

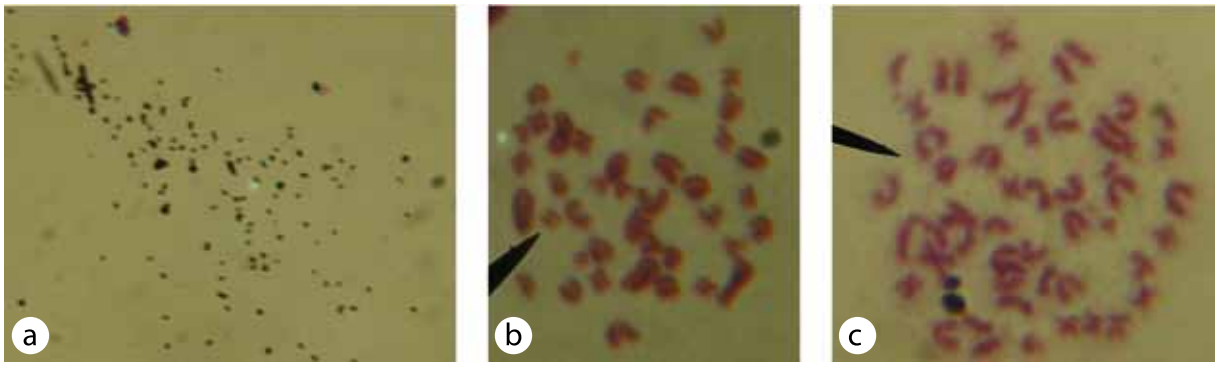

Fig. 1. *Death of cells in the form of karyoclasis (a). Chromosome aberrations in the form of double fragment (b) and acentric circle (c).*

of animals irradiated with a dose of 7.0 Gy (Y4 is the control group), to which, prior to exposure, PTA (Y5) were introduced; PCN (Y6) ;: Y4 = 124.86-70.5 lg (x); Y5 = 113.4-30.6lg (x); Y6 = 116.37-31.8lg (x), where x is the number of days after irradiation at 7.0 Gy.

Cytogenetic analysis were performed on the 3rd and 30th days post radiation exposure. The first period of analysis of the cytogenetic parameter showed that both radiation doses cause bone marrow syndrome: radiation block of mitoses and reproductive death of BMC leading to the devastation of the bone marrow (Fig. 1a). This syndrome is stronger expressed with the dose of 7,0Gy. However, statistically significant higher levels of proliferation is noted (Table 2) when comparing MI groups, which received Cu-containing complexes, with control groups.

Analysis of the karyotype showed that chromosome aberrations in the form of fragments, acentric circles and translocations (Fig. 1 b, c) in all research groups with both doses of radiation significantly exceed the level of aberration of the intact group. When comparing CA group which received PTA and PCN with control groups, it should be noted that their aberration is significantly lower than the level of control values (Table 2).

Analisis of the cytogenetic indices on the 30th day of experiment showed that earlier noted depres-

number of polyploids with an exposure dose of 5,3Gy in PTA and PCN groups is somehow below the control. With an exposure dose of 7,0Gy, this indicator is significantly lower in both groups with complexes compared with control ones.

Hematological indices were analysed in dynamics during the whole period of experiment. High radiation sensitivity of BMC which led to depression of hemapoiesis and death of formed elements just in blood vessels are the cause of severe erythro- and leukopenia and anemia. These three indicators in the early period (3rd and 7th days) of a research were significantly below the sizes of the relevant standards. The exception makes hemoglobin level for the 7th day at rats with the injected complexes with a dose of 5,3Gy.

As can be seen from table 4, on the last dates of observation (the 14th and 30th days) there was an increase in the number of erythrocytes and correspondingly there was an increase in the level of hemoglobin. When we compare the data standards in all groups, except control groups with dose of 5,3Gy on the 14th day and with dose of 7,0Gy on the 14th and 30th days, unreliable deviance from the standards is noted (Table 4).

Number of leukocytes on the 14th day of analysis, despite the significant increase of its level in all groups,

Table 2. *Cytogenetic indices on the 3rd day post radiation*

Indicators \ Groups	3rd day. Radiation with dose of 5,3Gy			3rd day. Radiation with dose of 7,0Gy		
	Control	PTA+radiation	PCN+radiation	Control	PTA+radiation	PCN+radiation
MI %	6,5±0,5	8,8±0,72*	8,6±0,8*	4,2±0,4	7,2±0,68*	6,9±0,6*
CA %	13,2±1,4	7,6±0,7*	8,2±0,74*	17,5±1,5	11,2±1,2*	12,8±1,3*
CP %	4,2±0,38	3,8±0,4	4,0±0,36	6,4±0,6	6,0±0,62	5,9±0,47

Table 3. *Cytogenetic indices on the 30th day post exposure*

Indicators \ Groups	30th day. Radiation with dose of 5,3Gy			30th day. Radiation with dose of 7,0Gy		
	Control	PTA+radiation	PCN+radiation	Control	PTA+radiation	PCN+radiation
MI %	10,2±1,2	16,8±1,48*	17,4±1,42*	8,4±0,74	15,5±1,2*	16,3±1,4*
CA %	7,2±0,64	4,8±0,5*	5,0±0,4*	12,2±1,2	7,1±0,53*	6,9±0,5*
CP %	3,2±0,34	2,8±0,22	2,6±0,22	4,6±0,38	3,0±0,24*	3,2±0,27*

Table 4. *Dynamics of hematological indices post irradiation*

Indicators	Norm	Groups / days	Radiation with dose of 5,3Gy			Radiation with dose of 7,0Gy		
			Control	PTA+radiation	PCN+radiation	Control	PTA+radiation	PCN+radiation
Erythrocytes $x10^{12}/l$	6,2±0,35	3	3,9±0,4 *	4,22±0,42 *	4,16±0,38 *	3,0±0,26 *	3,4±0,32 *	3,36±0,26 *
		7	4,22±0,5 *	4,94±0,48 *	4,88±0,4 *	3,3±0,3 *	4,0±0,38 *	3,87±0,36 *
		14	5,0±0,52	5,86±0,54	5,69±0,52	4,16±0,43 *	4,8±0,5	4,77±0,42
		30	5,2±0,56	6,0±0,56	5,96±0,5	4,4±0,5 *	5,44±0,52	5,42±0,54
Leukocytes $x10^9/n$	8,6±0,68	3	0,45±0,05 *	0,5±0,06 *	0,52±0,05 *	0,29±0,026 *	0,32±0,04 *	0,3±0,032 *
		7	0,9±0,07 *	1,24±0,22 *	1,28±0,14 * **	0,5±0,04 *	0,78±0,08 * **	0,76±0,06 * **
		14	2,2±0,3 *	3,7±0,36 * **	3,58±0,33 * **	1,6±0,15 *	2,85±0,26 * **	2,9±0,3 * **
		30	4,8±0,4 *	6,2±0,54 * **	6,0±0,52 *	3,5±0,34 *	5,2±0,5 * **	5,22±0,53 * **
Hemoglobin g/l	158±14,6	3	92,4±9,5 *	110,0±11,2 *	109,2±10,3 *	81,3±7,4 *	94,6±7,5 *	93,8±8,2 *
		7	100,0±9,8 *	130,0±12,6	129,4±12,2	92,2±9,3 *	106,0±9,7 *	104,5±10,0 *
		14	115,6±12,0 *	142,0±14,3	140,9±13,8	106,4±10,2 *	128,5±11,5	127,6±12,2
		30	137,0±13,2	150,4±14,6	150,0±14,4	118,0±11,5 *	142,5±13,7	140,0±14,0

* — *statistically significant deviance of indices from the norm*
** — *statistically significant deviance of indices from the control*

authentically remained below the standard level. When comparing the complex injected groups with control ones, statistically significant increase of this indicator was observed (Table 4). On the 30th day this indicator in all groups still remains authentically below the standard (norm), but there is a persistent trend towards normalization.

CONCLUSIONS

Basing on the survival results, average life expectancy, cytogenetic and hematological indicators, it can be concluded that study complexes demonstrate noticeable radioprotective properties. Both complexes showed identical effects on experimental animals according to all observed criteria.

In eruptive phase, on early dates of analysis (3rd and 7th days) both compounds soften the damaging effects of radiation with both doses which is especially obvious in significant shifts of mitotic index and CA in comparison with control data. On the last dates of observation (14th and 30th days) many data of analysis (MI, red blood readings) approached the standard data (norm), CA and number of leucocytes in comparison with the control data, demonstrate statistically significant difference.

Based on the results obtained, it can be assumed that the studied Cu-ligands effectively promote reparative processes in bone marrow cells and have the properties of pronounced radiomodifiers.

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IRON METABOLISM UNDER SELECTIVE INDIVIDULIZED CORRECTION

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ABSTRACT — The aim of the study was to study the effect of individually formed vitamin and mineral complex is containing iron on the metabolism of this trace element. Methods: we examined healthy persons (n=314), is randomizing into 2 groups. The main group (n=116) received a vitamin and mineral complex with the necessary amount of iron (the dose was calculated based on the results of the initial laboratory examination of the patient) during 30 days, and the comparison group (n=198) got a similar complex without iron-containing component. Prior to administration of the complex and immediately upon completion of the full course, the total iron concentration in the blood, serum iron level and ferritin content in the blood were determined. Results: the randomized controlled one-center study confirmed the positive effect of the course of personalized correction of iron metabolism on a number of its biochemical indicators (total iron level in the blood, serum concentration of this trace element and the amount of ferritin).

KEYWORDS — iron, blood level, personalized correction.

INTRODUCTION

Iron deficiency is one of the most common problems of the population of large cities [1, 2, 5]. Numerous epidemiological studies indicate, on the one hand, a sufficiently high frequency of iron deficiency anemia and associated pathological conditions [1–3] and, on the other hand, indicate a significant proportion of persons with subclinical manifestations of metabolic disorders of this trace element [6]. Thus, according to the results of our previous studies, up to 27% of the adult population of the metropolis have either full-fledged or subclinical (at the level of the lower quartile) serum iron deficiency [4]. These facts clearly indicate the feasibility of targeted detection and personalized correction of iron deficiency. In this regard, the aim of the work was to study the effect of individually formed vitamin and mineral complex is containing iron on the metabolism of this trace element.

METHODS

The study was designed as an open prospective, randomized controlled trial. It included 314 people belonging to the category of *practically healthy people*. The inclusion criteria were the age from 20 to 50 years, the absence of acute or chronic in the acute stage of pathology, as well as the presence of subclinical or clinical serum iron deficiency. All participants in the study signed informed consent prior to the initial survey. Further, the examined persons were randomized into 2 groups: the main group (n=116), representatives of which received a vitamin and mineral complex containing the necessary amount of iron (the dose was calculated based on the results of the initial laboratory examination of the patient), and the comparison group (n=198), which received a similar complex, the only difference of which was the absence of an iron-containing component. The duration of reception of the complex was 30 days.

Prior to administration of the complex and immediately upon completion of the full course, the total iron concentration in the blood (in μg/l), serum iron level (in μmol/l) and ferritin content in the blood (in μg/l) were determined. All these parameters were evaluated by standard methods.

The data were processed in the software package Statistica 6.1.

RESULTS

It was found that the majority of the studied parameters in the second control point of observation did not change relative to the first one (Fig. 1–3).

Thus, the total concentration of iron in the blood and its serum level remained at the initial values (Fig. 1 and 2), while the amount of the main iron-transport protein of the blood — ferritin — even showed a downward trend (-2.6%; $p<0.1$).

On the contrary, the personalized correction of the metabolism of the microelement under consideration contributed to the increase of all the studied parameters (Fig. 1–3). In particular, there was an increase in the total concentration of iron in the blood by 13.4% compared with the baseline ($p<0.05$), exceeding the value characteristic of the comparison group by 10.0% ($p<0.05$). A similar dynamics was recorded for serum iron level (an increase of 16.8% relative to baseline values and 8.3% — to the comparison group ($p<0.05$ for both cases))

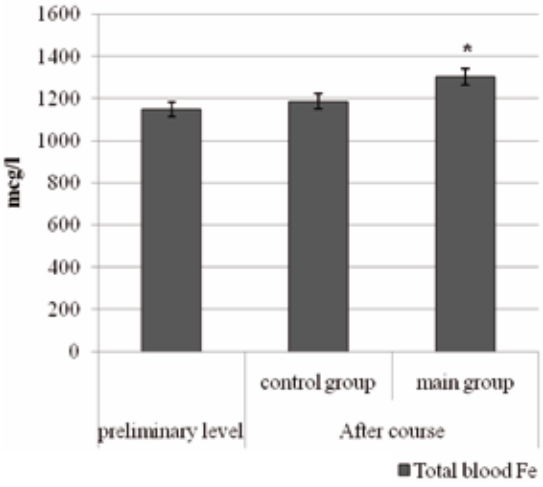

Fig. 1. Total blood level of the iron before and after the administration of vitamin and mineral complex

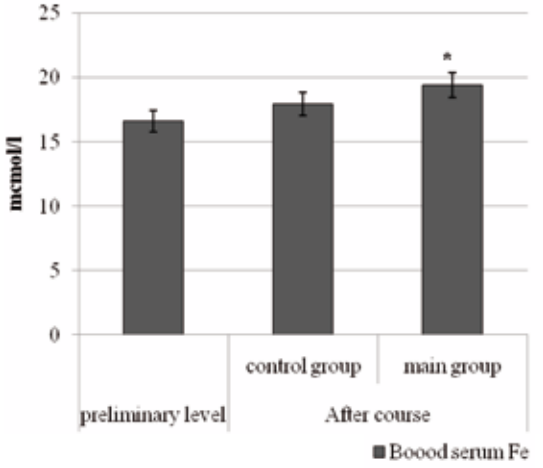

Fig. 2. Blood serum level of the iron before and after the administration of vitamin and mineral complex

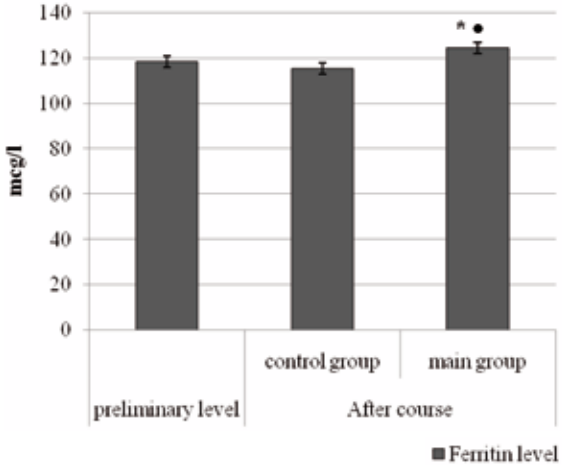

Fig. 3. Blood level of the ferritin before and after the administration of vitamin and mineral complex

The changes in ferritin levels were significant too (Fig. 3). It was found that the concentration of this iron transport protein in the representatives of the main group increased by 5.1% compared to the first control point and by 7.7% - relative to the comparison group ($p<0.05$ for both cases)

CONCLUSION

Thus, the randomized controlled one-center study confirmed the positive effect of the course of personalized correction of iron metabolism on a number of its biochemical indicators (total iron level in the blood, serum concentration of this trace element and the amount of ferritin).

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THE ONTOGENY OF CIRCADIAN RHYTHMS OF SEVERAL PHYSIOLOGICAL PARAMETERS IN MALE WISTAR RATS

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ABSTRACT — The rhythmicity of biological processes is one of the fundamental properties of living matter. Rhythmic effects from the external environment are the main stimulators of the biorhythms of the organism, which ensure their formation in the early stages of ontogenesis and determine the level of their intensity throughout all subsequent life. Among the environmental factors the leading is photoperiodicity. Exposure to light at night has become an essential part of the modern lifestyle and it is accompanied by many serious behavioral and health disorders, including cardiovascular diseases and cancer. To make a more complete picture of the patterns of temporary organization in mammals it occurs currently important to study the characteristics of the daily dynamics of some physiological parameters of male Wistar rats in ontogenesis.
The study was conducted on Wistar male rats at the age of 3, 6 and 12 months. Each age group was divided into 2 subgroups, comprising 40 animals in each of them. The rats of first subgroup of each studied age were housed under a fixed illumination, animals of second subgroup of each of studied ages were investigated under the same experimental conditions except for the light regime, representing constant light. The daily rhythmic of blood biochemical parameters and rhythmic of rectal temperature were studied. For the analysis of characteristics of circadian rhythm of the studied substances the cosinor-analysis carried out by means of the Cosinor Ellipse 2006-1.1 program was used. A study of the characteristics of the daily rhythm of a number of parameters of Wistar albino rat males at the age of 3, 6 and 12 months at a fixed light regime and under constant light conditions was conducted. Keeping of rats in conditions of constant illumination leads to significant violations in the structure of the chronoarchitecture of the organism. This is manifested in a change in the nature of the daily dynamics of rectal temperature, a significant reorganization of the circadian rhythm of glucose, a disturbance of the circadian rhythm of the total protein and albumin, which together indicates the development of desynchronosis and stress of adaptation mechanisms. It was established that the stability of the circadian system of the rat organism manifests different degrees of maturity in the studied periods of ontogenesis, and also reacts differently to changes in the light regime. The animals at the age of 6 months are the most resistant to the effects of constant lighting.

KEYWORDS — circadian rhythm, ontogenesis, temperature, albumin, glucose, total protein

INTRODUCTION

The rhythmicity of biological processes is one of the fundamental properties of living matter. Rhythmic effects from the external environment are the main stimulators of the biorhythms of the organism, which ensure their formation in the early stages of ontogenesis and determine the level of their intensity throughout all subsequent life. Thus, the exposures from the external environment are the factor that set the biological clock of organism and determines the specifics of their rate. For living organisms of different levels of organization the presence of evolutionarily determined endogenous circadian rhythm is characteristic. Each mammalian cell is a potential oscillator, as it contains the necessary elements that determine the molecular genetic mechanisms of circadian biological clock (BC), which can be activated under certain conditions. Balsalobre (2002); Kim et al. (2018).

The chronoperiodic system, being present at all levels of the organization of a living organism, generates oscillations of its own activity with frequencies that are close to the frequencies of the main external geophysical cycles (daily, monthly, annual), and is capable of capturing external timers (Zeitgeber) and thus synchronizing its own activity with external rhythmic changes. Carter et al. (2016); Dibner, C., Schibler, U. (2015); Erkekoglu and Baydar (2012); Halberg (2005).

Among the environmental factors the leading is photoperiodicity. Photoperiodicity (the duration of daily and/or seasonal illumination) is the main pacemaker for mammals (time-setting cue or external synchronizing factor) Wehr (2001); Foster (2008); Boyce (2014).

It is known that information about the illuminance enters the organism through the retina. Currently, it has been shown that, in addition to retinal receptors, ganglion and bipolar cells also take part in the perception of the photoperiod. In these cells, the action of the light waves of the blue spectrum leads to the induction of cry genes that produce cryptochrome proteins from the group of flavoproteins. The accumulation of such proteins

can provide the primary perception of photoperiodic information in living organisms. Tang et al. (2010); Schmidt et al. (2011); Nasir-Ahmad et al. (2019).

Then the photoperiodic information goes along the optic nerve, which contains the retinohypothalamic tract, reaching the suprachiasmatic nuclei. There is also an indirect path through which light information reaches the suprachiasmatic nuclei - the geniculohypothalamic pathway. This path is a projection from parts of the lateral geniculate nuclei that receive retinal information, as well as from the ventral parts of these nuclei. Photoperiodic information comes to the geniculate nuclei by direct projections from the retina which comprise into the main part of the optic nerve. It is assumed that the geniculohypothalamic tract contributes to suprachiasmatic nuclei's obtaining of modulating signals from other sensory and motor systems of the organism. Rusak (1992); Harrington (1997); Ding (2018).

The key role of the suprachiasmatic nuclei of the hypothalamus in the chronoperiodic system of mammals is unquestionable.

Suprachiasmatic nuclei have all the properties of oscillators. Hastings et al. (2019):

— they are endogenous pacemakers, i.e. oscillators with intrinsic circadian frequency of neuronal activity;
— they are capable to capture the rhythm of an external synchronizer — a photoperiod — through direct and indirect communication with the retina of the eye and can synchronize their own rhythms with an external photoperiod;
— they are the main synchronizers for many endogenous innate rhythms of mammals.

SCN fully or partially synchronize locomotor activity, food and water consumption, reproductive behavior, temperature regulation, sleep-wake cycle, daily rhythm of heart contractions. At the same time, suprachiasmatic nuclei implement a synchronizing effect on the circadian rhythm of the endocrine system. Martino and Young (2015). By means of various connections, the SCN can manage the temporal organization of homeostatic systems, synchronizing their rhythms. In addition, the SCN can regulate circadian activity by affecting the centers of the autonomic nervous system through the caudal parts of their efferents. Chiesa et al. (2015); Moller et al. (2009) Sympathetic fibers, in turn, provide innervation to the pineal gland, another important link in the mammalian photoperiodic system. Iovino et al. (1986).

In addition to the adrenergic innervation, the pineal body also receives a variety of non-adrenergic nerve fibers of both peripheral and central origin, but their effect remains little studied.

The formation of melatonin in mammals almost exactly corresponds to the activity of postganglionic sympathetic neurons, which depends primarily on the photoperiod phase; all cells of the organism can react to the state of the external zeitgeber by the level of melatonin in the circulating blood. Therefore, melatonin is sometimes referred to as an *internal zeitgeber*, and the pineal body is called a neuroendocrine transducer, which converts information on the duration of a photoperiod, encoded in nerve impulses, into a humoral response in the form of circulating melatonin. Melatonin is produced in the dark, and its main function is chronoregulation, although melatonin can simultaneously perform various other functions Robert et al. (2015); Touitou et al. (2006).

The circannual part of the photoperiodic system of the brain has been studied significantly less. It has been established that in many organisms the circadian rhythms are involved in the control of circannual changes, since the former take part in the mechanisms of photoperiodic time measurement, perceiving the rhythms of external pacemakers.

The circadian (context-independent) system can only inform the organism about the difference between the light and dark periods of the day or between low and high melatonin levels, but not about the seasonal reduction or lengthening of the photoperiod Yurt et al. (2018).

It is supposed that the suprachiasmatic nuclei can not only be a circadian oscillator (pacemaker), but also take part in seasonal photoperiodic *timekeeping*, that is, conduct a seasonal estimate of time, be a photoperiodic *counter* of time Schwartz et al. (2001).

It is also possible to consider the involvement of the nuclei of the anterior hypothalamus (anterior, suprachiasmatic, preoptic and others) in the transmission of information from the circadian system to the photoperiodic comparator. And the disappearance of photoperiodic reactions due to the disruption of the activity of the anterior hypothalamic nuclei is associated with blocking the transmission of information from the circadian system to a such comparator.

The septohippocampal system is proposed as a such comparator. The assumption is due to the facts that:

— one of the neuroanatomical memory substrates is the hippocampus;
— the hippocampus together with the septal complex forms a joint morpho-functional septohippocampal system, which can function both in the direction of the "medial septal nucleus – hippocampus – the lateral septal nucleus – medial septal nucleus", and, possibly, in the opposite direction;
— the septohippocampal system, due to its diverse connections, has an ideal arrangement for the comparator;

— taking into account the morphofunctional features of the structure, the septo-hippocampal system is already considered a comparator, but with a different function — acting as a generator of consciousness and attention;
— there is evidence of involvement of the hippocampus and the entire hippocampal formation in the occurrence of photoperiodic reactions. In addition, it has been demonstrated that the adaptation effects of the pineal body are realized through the changes in the functional state of the hippocampus. Arushanyan (2001); Serrano et al. (2006).

Currently a fairly large number of people in the world are exposed to light pollution (in other words, night lighting). Such an impact may be due to the profession, or due to habits and lifestyle. Exposure to light at night has become an essential part of the modern lifestyle and is accompanied by many serious behavioral and health disorders, including cardiovascular diseases and cancer Ha (2005); Jasser et al. (2006); Knutsson (2003). Inhibition of the function of the epiphysis (pineal gland) in constant light contributes a carcinogenesis, while an absence of light suppresses carcinogenesis.

According to the hypothesis of *circadian disruption*, the effect of light at night violates the endogenous circadian rhythm, suppresses the melatonin secretion by epiphysis, which leads to a decrease in its concentration in the blood Wang et al. (2014). Disturbance of CR during shift working leads to increased risk of cardiovascular diseases, metabolic syndrome, type II diabetes. Fonken et al. (2010). Animal studies have shown that replacing of total darkness with dim night lighting causes metabolic disturbances and obesity Vinogradova et al. (2013); Gubin et al. (1997). Thus, the violation of the CR negatively affects eating behavior and metabolism.

The temporal organization of biological processes in mammals undergoes complex ontogenetic transformations, which reach their maximum during aging, and in general can be characterized as age-related desynchronosis, which plays a significant role in the development of aging processes and the development of diseases associated with it. In the process of aging, there is a gradual loss of synchronism of factors involved in the modulation of the amplitude-phase characteristics of phenotypic rhythms (physiological, biochemical processes, behavioral reactions, etc.). These factors can be molecular-genetic, tissue-organ and systemic. Phenotypic manifestations of desynchronosis are formed according to the principles of pleiotropy. It is assumed that the general manifestation of age-related desynchronosis on the part of the rhythm architectonics is extracircadian dissemination (ECD) — a violation, and most often — a decrease in the amplitude of the circadian rhythm (CR) with the background of the loss of its phase stability and an increase in oscillations in adjacent frequency ranges Gubin et al. (1999); Gubin et al. (2001); Gubin et al. (2006).

To make a more complete picture of the patterns of temporary organization in mammals it occurs currently important to study the characteristics of the daily dynamics of some physiological parameters of male Wistar rats in ontogenesis.

MATHERIALS AND METHODS

Animals

The study was conducted on Wistar male rats at the age of 3, 6 and 12 months. Animals were taken from the Stolbovaya nursery (the *Stolbovaya* affiliate of the Federal State Budgetary Institution of Science "Scientific Center for Biomedical Technologies of the Federal Medical and Biological Agency"). Each age group was divided into 2 subgroups, comprising 40 animals in each of them.

Treatment design

Animals of each of the studied ages were initially kept under a fixed light regime.

During the whole experiment, the rats of first subgroup of each studied ages were housed under a fixed illumination, L:D 10:14 (±180 lux, respectively; 8:00 AM lights on) in a temperature-controlled environment with ad libitum access to tap water and food (rat chow).

Animals of second subgroup of each of studied ages were investigated under the same experimental conditions except for the light regime, representing constant light (LL ±180 lux). Both the first and second subgroups of animals were kept at the specified light regime for 2 weeks.

After two weeks, euthanasia of the animals in the carbon chamber was performed at 0900 hours, 1500 hours, 2100 hours and 0300 hours, blood was collected for biochemical studies.

All animal experiments were performed in according to the compliance with EC Directive 86/609/EEC and with the Russian law regulating experiments on animals.

Biochemical studies

In the blood plasma the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, cholesterol, triglycerides, total protein, glucose, albumin and uric acid were deter-

mined using the StatFax-3300 (USA) analyzer and corresponding *Spinreact* kits (Spain).

Rectal temperature studies

For measurement of rectal temperature, the BIO-TK8851 thermometer (Bioseb, USA) was used.

Statistical Analysis

The obtained data, analyzed using Graph Pad Prism 6.0, were expressed as Mean±SD. The statistical difference was determined using Student t-tests. A p value of < 0.05 was considered statistically significant.

For the analysis of characteristics of circadian rhythm of the studied substances the cosinor-analysis carried out by means of the Cosinor Ellipse 2006-1.1 program was used. The cosinor analysis allows to analyze the chronobiological data, to reveal the rhythm of various functional parameters, and also to calculate the parameters of the established rhythms Cornelissen (2014). The presence of a reliable circadian rhythm, and also its acrophase and amplitude were determined. Acrophase is the measure of peak time of the total rhythmic variability in a 24-hour period. Amplitude corresponds to a half of the total rhythmic variability in a cycle. The acrophase is expressed in hours; amplitude values are expressed with the same units as the documented variables.

RESULTS

The daily dynamics of rectal temperature in rat's groups of different ages at a fixed light regime and in conditions of constant lighting.

The study of rectal temperature of animals at the age of 3 months have found a circadian rhythm with acrophase at 0132 hours, low amplitude – 0.18° C – and 35.68° C mesor. Transition to a constant lighting leads to the destruction of the rectal temperature rhythm (Fig. 1).

In rat's group aged 6 months we observed a circadian rhythm of temperature, characterized by acrophase, falling at 1615 hours, with a mesor of 35.43° C and an amplitude of 0.68° C. At the same time, the transition to keeping animals in conditions of constant illumination leads to breakdown of circadian rhythm.

In rat's group of the third studied age, both at fixed light regime and under constant illumination the circadian rhythm of temperature is marked. In the first case it is characterized with acrophase at 0458 hours, and at constant light the acrophase shifts to 1308 hours. At the same time, the amplitude of the rhythm increases from 0.62°C to 0.77°C, with almost unchanged mesore – 34.92°C and 34.33°C, respectively.

The daily dynamics of plasma glucose level in rat's groups of different age at a fixed light regime and in conditions of constant lighting.

Analysis of the daily dynamics of blood glucose at a fixed light regime revealed the presence of a reliable circadian rhythm in rats of all studied ages (Fig. 2). It is noteworthy that consideration of its changes shows a tendency towards directional displacement of its acrophase (1600 hours in group of 3 months age, 1157 in 6-months-old group and 0837 hours at 12 months). The rhythm amplitude is 0.49 g/l at 3-months-old group, 0.9 g/l at 6 months and 0.76 g/l at 12 months. The rhythm mesor was 7.58 g/l in 3-months-old rats, 8.26 g/l at 6 months and 6.97 g/l for 12 months.

Under the constant light conditions the circadian rhythmicity of plasma glucose level was detected at 3- and 6-months-old groups. Acrophase of rhythms shifted at 1920 and 2030 accordingly, the rhythm amplitude at 3-months-old rats was 1.15 g/l and 1.57 g/l at 6-months-old. Mesor is respectively equal to 6.87 g/l and 8.53 g/l.

The daily dynamics of total plasma protein in rat's groups of different age at a fixed light regime and in conditions of constant lighting.

At a fixed light regime in animal's group aged 3 months no reliable circadian rhythm of total plasma protein was detected. At 6 months the rhythm is characterized by acrophase of 0230 hours, with an amplitude of 7.52 g/l and mesor of 73.44 g/l. At 12 months, the acrophase of rhythm is noted at 0055, the amplitude decreases, amounting 6.48 g/l with a mesor of 60.23 g/l. In conditions of constant illumination in group of 3 months, the rhythm with acrophase at 2100 hours, an amplitude of 7.52 g/l and mesor of 73.44 g/l is observed. At 6 months in the same conditions the acrophase of rhythm is noted at 1242, the amplitude sharply increases to 24.85 g/l with a mesor of 60.23 g/l. In animals at the age of 12 months circadian rhythm is not detected (Fig. 3).

The daily dynamics of plasma albumin in rat's groups of different age at a fixed light regime and in conditions of constant lighting.

At a fixed light regime in rat's group aged 3 months acrophase rhythm falls on 0010 hours, the amplitude is 8.86 g/l, mesor – 21.13 g/l. At 6 months the rhythm acrophase shifts to 1500, the amplitude is reduced to 2.95 g/l, the mesor is 8.46 g/l. At 12 months the acrophase of this rhythm falls at 0615 hours, the amplitude is 0.76 g/l, and the mesor is 4.95 g/l.

At constant light a reliable circadian rhythm is noticed at all ages. At the same time in 3 months it is characterized with acrophase at 1108 hours, an amplitude of 5.71 g/l and a mesor of 11.48 g/l. At 6 months rhythm acrophase shifted to 1125 hours, the ampli-

tude was 0.55 g/l, and mesor – 4.27 g/l. At 12 months, the acrophase is noted at 1845 hours, the amplitude is equal to 0.29 g/l, and the mesor is 3.14 g/l (Fig. 4).

DISCUSSION AND CONCLUSION

Consideration of the results of changes in the daily dynamics of rectal temperature in ontogenesis suggests that at rat males of all investigated ages at a fixed light regime the reliable circadian rhythm is noted, while keeping animals in conditions of constant illumination leads to the establishment of reliable circadian rhythm only in animals aged 12 months. We can suggest that in the first two investigated ages (3 and 6 months) lack of circadian rhythm of temperature is associated with the violation of photoperiodicity and relative dysmaturity of functional systems maintaining homeostasis in animals of these groups, and detecting of circadian rhythm of rectal temperature at constant light at 12 months may due to the fact that during this period of ontogenesis under these conditions the change of light and darkness is not the lead pacemaker, but some kind of another factor is.

Information received by us is confirmed by a number of studies, according to which the optimal biorhythmological structure of body temperature of mammal starts to form in childhood and up to mature age persists in close boundaries (both on the mesor and on daily amplitude). In the future, the level of body temperature as a whole and of its circadian amplitude decreases. Especially this trend is expressed in old age. In general, since old age, the chronodesm of temperature homeostasis in humans changes abruptly. Its boundaries in the senior age groups are significantly lower down at the temperature scale. In our opinion, a decrease in the temperature level homeostasis and its diurnal rhythm is due to a decrease in energy potential of the cells of the organism. The deviation from the energy optimum of organism undoubtedly leads to a decrease in balance of biosystem as a whole, followed by an entropy growth and change of the most important indicator of the level of viability — the amplitude of the daily rhythm. Thus, the body temperature undergoes a daily rhythm synchronous in profile and amplitude to the structure of the daily rhythm of energy and plastic metabolism parameters established at the cellular and tissue levels.

In analysis of the circadian rhythm of glucose at a fixed light regime it is noticeable the presence of age drift of acrophase to the early morning hours. Changing of the lighting regime leads to a rerun of the circadian rhythm at the age of 3 and 6 months and its absence in 12 months. The level of total plasma protein has the circadian rhythm at fixed light regime only from the age of 6 months, while in conditions of constant illumination the daily rhythm of this parameter is noted at 3 and 6 months and collapses at 12 months. The circadian rhythm of albumin is noted as at a fixed light, and at constant illumination in groups of all studied ages, but in both lighting regimes there is a significant decrease in albumin rhythm amplitudes. Thus, studies have shown that keeping of rats in constant light conditions leads to significant violations in the structure of the chronoarchitecture of the organism. It manifests in a change in the nature of the daily dynamics of rectal temperature, significant alteration of the circadian rhythm of glucose, violation of the circadian rhythm of total protein and albumin, which is cumulative talking about the development of desynchronosis and stress of mechanisms of adaptation. The most resistant to constant lighting are animals at age of 6 months.

A study confirms the fact that the violation of such an important factor as normal photoperiodism causes stress of mechanisms of adaptation of mammals and serves the cause of the formation of a general adverse background, conducting to a development of pathologies and pathological conditions.

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Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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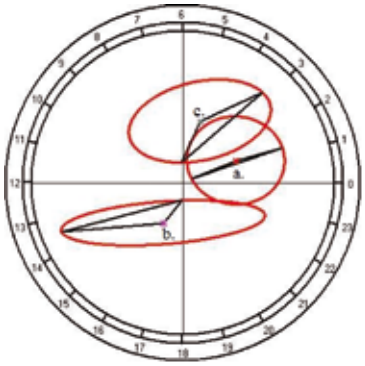
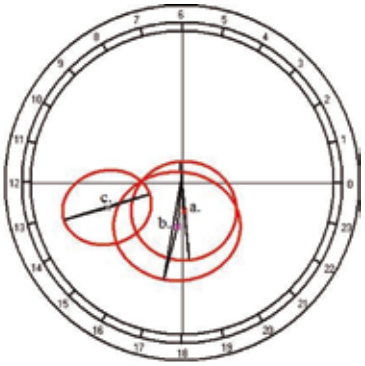
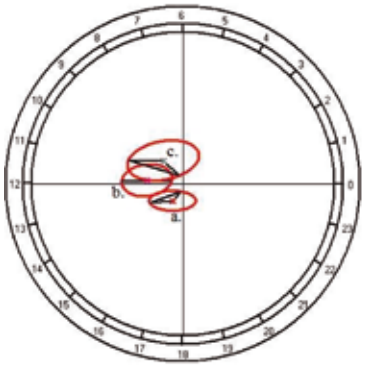


a



b

Fig. 1. *Cosinor analysis of daily temperature dynamics at a fixed light regime (A) and under constant light conditions (B). Hereinafter: a — parameters of 3-month-old animals, b — parameters of 6-month-old animals, c — parameters of 12-month-old animals*



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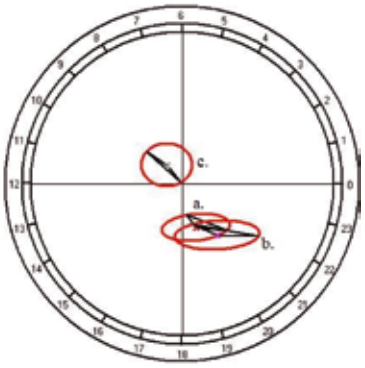

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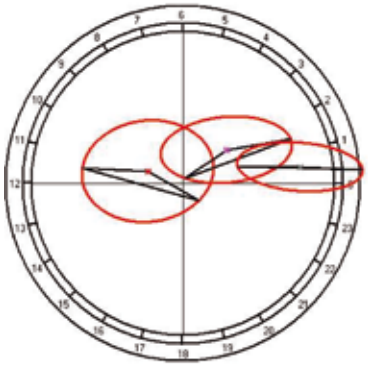
Fig. 2. *Cosinor analysis of the daily dynamics of plasma glucose at a fixed light regime (A) and under constant light conditions (B)*



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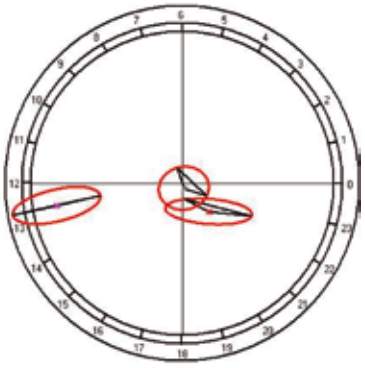
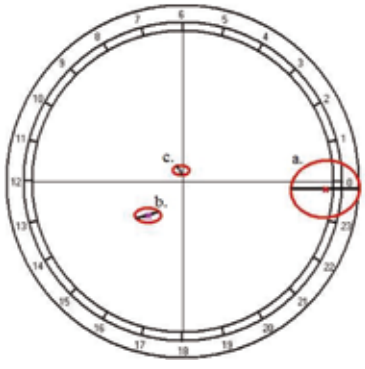


b

Fig. 3. *Cosinor analysis of the daily dynamics of total plasma protein at a fixed light regime (A) and under constant light conditions (B)*



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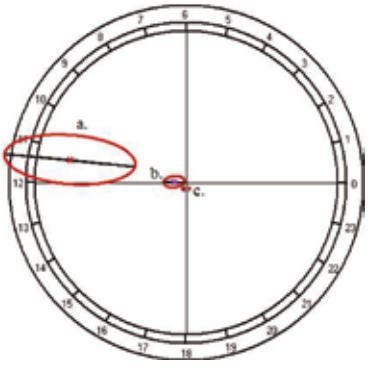

b

Fig. 4. *Cosinor analysis of the daily dynamics of plasma albumin at a fixed light regime (A) and under constant light conditions (B)*

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VARIANT ANATOMY OF TEMPOROMANDIBULAR JOINT MAJOR BONE STRUCTURES IN ADULT POPULATION

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INTRODUCTION

There is a lot of special emphasis placed currently on studying the morphological and functional features of the temporomandibular joint in normal and pathological conditions [1–3]. Basic data concerning the temporomandibular joint morphological structure and function can be obtained from works by many experts [4–6]. At the same time, there has been interrelation identified for occlusal, muscular and articular factors, joining in a single balanced mechanism, which, if disturbed, contributes to pathology development, including in the temporomandibular joint [7–12]. Therefore, awareness of the major dimensional features and the shape types of the basic bone structures appears relevant.

Aim of study

to identify the dimensional features and shape types of the basic bone structures in the temporomandibular joint in adults.

MATERIALS AND METHODS

The structural features of the temporomandibular joint major bone elements were studied on 103 adult skull preparations from the scientific craniological collection of the Human Anatomy Department, V.I. Razumovsky Saratov State Medical University. Measurements were made for the articular tubercle, the mandibular fossa and the mandible head.

RESULTS AND DISCUSSION

The inner-outer dimension of the articular tubercle (the distance from the inner to the outer poles) was 23.95±0.12 mm, while the antero-posterior size (the distance between the anterior and posterior sides) was 9.28±0.13 mm. The inner-outer size was 2.5 times that of the antero-posterior one.

The articular tubercle height was 10.31±0.15 mm. The height of the articular tubercle was 1.5 times the height of the postglenoid tubercle. The minimum height of the articular tubercle was 5.5 mm, the maximum height being 15.8 mm. Depending on the articular tubercle height, the following types of its shape were identified: flattened (up to 7 mm), moderately prominent (from 7 to 12 mm), prominent (more than 12 mm).

The moderately prominent type of the articular tubercle (9.92±0.10 mm), which accounted for 65.1% of the observations, was the most common. The prominent type of the articular tubercle (13.21±0.13 mm) was detected in 23.3% of the cases. Less common was the flattened type of the articular tubercle (6.56±0.08 mm), which accounted for 11.6% of the cases.

The inner-outer size of the mandibular fossa was 23.5±0.27 mm, while the antero-posterior was 14.24±0.11 mm. Depending on the mandibular fossa width in the anterior-posterior direction, the following shapes were identified — narrow (up to 12 mm), medium-width (from 12 to 15 mm), wide (more than 15 mm). The mandibular fossa depth reached 10.31±0.15 mm. Depending on the mandibular fossa depth, the following types of its shape were identified — shallow (up to 7 mm), medium-depth (from 7 to 12 mm), deep (more than 12 mm).

The inner-outer size of the mandible head was 20.50±0.45 mm, whereas the antero-posterior size was 9.51±0.11 mm. The inner-outer size was 2.1 times that of the antero-posterior. The minimum width of the mandible head in the antero-posterior direction was 5.3 mm, and the maximum — 14 mm.

Depending on the mandible head width in the antero-posterior direction, the following types of its shape were identified — small (up to 8 mm), medium-width (from 8 to 11 mm), large (more than 11 mm). The most common was the medium-width mandible head, which accounted for 66.1% of the cases. The small type of the mandible head was observed in

18.4% of the cases. Less frequent was a large mandible head, which was to be observed in 15.5% of the cases.

CONCLUSION

Given the above, the temporomandibular joint of an adult person features certain regularities in its structural elements shape and size, which may be different, while knowing these could allow more accurate pathology diagnostics, as well as planning and assessing the treatment effectiveness.

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CRANIOFACIAL LINE OF TELERADIOGRAPHY AND ITS MEANING AT CEPHALOMETRY

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INTRODUCTION

Cephalometric analysis is an integral part of diagnostics in orthodontics and prosthetic dentistry [8]. A special emphasis is placed on the spatial location of the jaws regarding the major anatomical craniofacial points. This principle lays the basis for classifications of anomalies of the maxillofacial area and the differential diagnostics of gnathic, dentoalveolar, and articular pathologies [3, 10]. Cephalometric analysis of head teleradiography in the lateral projection is performed both manually and by machine (computer). The analysis is based on measurements between common coordinate points. The said points serve a guideline for constructing horizontal planes (lines), each of them being of a diagnostic value and serves as a guideline for carrying out angular measurements [7]. The horizontal lines divide the craniofacial area into sections. The spinal plane, for instance, separates the face gnathic part, while the occlusal plane separates the jaws between themselves. These studies are of both applied and clinical value. There has been a relationship detected between the size of the craniofacial complex and the dental arches parameters, both in physiological and pathological occlusion [1, 4, 5, 6]. Such studies often determine orthodontic and prosthetic treatment tactics [2, 9, 11, 12]. However, we saw no marks that would allow delineating the border between the facial part of the skull and the cranial one. Since there is no reference point then there is no data regarding the relation between the craniofacial plane and the other teleradiography reference points, which determined the objectives of this study.

Aim of study

To identify anatomical marks for constructing the craniofacial plane and to evaluate its relationship with the teleradiography major planes.

MATERIAL AND METHODS

While analyzing lateral telegraphs, we employed anatomical reference points that are commonly accepted in clinical and anthropometric studies.

To build the craniofacial line, we used the nasal point (N) located at the junction of the frontal and nasal bones. Another mark was the point of condylion (Cond), sprawling on the upper point of the articular head of the mandible condylar process. The line ran diagonally and separated the facial and cranial parts of the skull. Given that, we proposed to mark this line as a craniofacial one. We identified the interrelation between the craniofacial line and the mandibular plane. The resulting angle was marked as maxillofacial. In addition, the location of the angle in relation to the skull structures was identified. The results were evaluated in groups of people with different types of the facial area growth, namely with a neutral, a horizontal and a vertical type. The statistical processing was performed following common requirements.

RESULTS AND ITS DISCUSSION

People with a neutral growth trend in the facial part had the mandibular angle varying within the range of 119° to 124°. A decrease in the angle resulted in the horizontal type of growth, whereas an increase – in the vertical one. The mandibular angle correlated with the size of the maxillofacial angle, which, in case of a neutral type of growth, ranged from 40° to 45°. Notable is the spatial arrangement of the angle. In case of the vertical growth, the angle shaped by the intersection of the craniofacial and mandibular lines was located on the skull occipital bone and descended in case of the neutral and, even more, the horizontal growth type.

CONCLUSION

The above means that the craniofacial plane of the lateral telegraphy can be used to construct the

maxillofacial angle, which, in turn, may be used as a criterion for identifying the facial area growth type. The proposed method can be used in clinical orthodontics to diagnose various anomalies, as well as to determine the tactics for comprehensive treatment, and to check its efficiency.

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REPRESENTATIVES OF NEUROPEPTIDES — SELANK AND PRO-GLY-PRO-LEU AS MODULATORS OF IMMUNOREACTIVITY IN CONDITIONS OF "SOCIAL" STRESS

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ABSTRACT — This study is devoted to the experimental study of the immunocorrecting action of neuropeptides Selank (Thr-Lys-Pro-Arg-Pro-Gly-Pro) and Pro-Gly-Pro-Leu on the model of "social" stress. Functional activity of the immune system was assessed on the basis of standard immunopharmacological tests: delayed-type hypersensitivity reaction (DTH), direct agglutination test (DAT), latex test for studying the phagocytic activity of peripheral blood neutrophils, evaluation of changes in the leukocyte formula. As a result of the experiment, it was found that, under the influence of "social" stress, changes in the immune response are multidirectional, which confirms the theory of "immune disbalance" under the action of stressors. As a result of studying the effect of neuropeptides under the conditions of "social" stress, it was found that Selank and Pro-Gly-Pro-Leu proved to be an effective correctors, restoring the cellular and humoral immunogenesis reactions, the phagocytic activity of neutrophils and indicators of leukocyte formula.

INTRODUCTION

Studies concerning the mechanisms of the dysfunctional changes in the immune system under stress, as well as the development of methods for their correction, are one of the main directions of development of modern immunology and pharmacology. The greatest achievement of molecular biology and medicine was the possibility of synthesizing bioregulators, in particular of neuropeptides, and the creation on their basis of new highly effective drugs, exhibiting, among other things, stress protective properties [7, 10]. It is important to emphasize that molecules in the form of short fragments of peptide compounds are characterized by a high degree of safety due to their complete proteolytic degradation [1]. It should be noted that at the moment a representative of neuropeptides is actively used in clinical medicine — Selank synthesized at the Institute of Molecular Genetics of the Russian Academy of Sciences. This drug was created by attaching a tripeptide Pro-Gly-Pro to the C-termini of an unstable regulatory peptide taftsin which solved the problem of in vivo stabilization and supplemented it with the effects of Pro-Gly-Pro itself [6]. Along with the already registered drug Selank, glyproline Pro-Gly-Pro-Leu is of considerable interest from the standpoint of the promise of practical implementation in clinical pharmacology [3, 5].

The aim of research

To study of the immunomodulatory effects of Selank and Pro-Gly-Pro-Leu on the model of "social" stress.

MATERIAL AND METHODS

White non-linear rats (males, 6–8 months old) were used as experimental animals. In order to create a "social" stress in the experiment a model of intermale confrontations was chosen. Animals were placed in pairs in experimental cells separated by a septum which prevents physical contact but has openings that provide sensory contact. Every day the partition was removed for 10 minutes which overwhelmingly led to agonistic collisions (confrontations) [2, 8, 9]. Groups of animals with alternative types of behavior were formed: aggressive type — in case of repeated victories experience (winner, aggressor) and submissive type - in case of defeats (victim). Laboratory animals were divided into 5 groups (n = 10): a group of intact males; a group of animals that were exposed to stress for 20 days (sensory contact); a group of individuals treated intraperitoneally with Selank at a dose of 100 μg/kg/day under conditions of 20-day stress exposure (sensory contact) in a course of 20 days; a group of rats treated intraperitoneally with Pro-Gly-Pro-Leu at a dose of 100 μg/kg/day under conditions of 20 days of stress exposure (sensory contact) in a course of 20 days. Functional activity of the immune system of laboratory animals was assessed on the basis of standard immunopharmacological tests: delayed-type hypersensitivity reaction (DTH), direct agglutination test (DAT), latex test for studying the phagocytic activity of peripheral blood neutrophils, evaluation of changes

in the leukocyte formula. Particle T-dependent antigen (the erythrocytes of the ram) was used as an antigenic stimulus in DTH and DAT [4].

The experiment results were statistically processed using the following programs: Microsoft Office Excel 2007 (Microsoft, USA), BIOSTAT 2008 Professional 5.1.3.1. To process the obtained results, a parametric method was used with the Student t-test with the Bonferroni correction. Statistically significant differences were considered at $p<0.05$.

RESULTS AND ITS DISCUSSION

In the course of the experiments, it was found that long-term inter-male confrontations caused the suppression of DTH and DAT in animals with both aggressive and submissive types of behavior compared to control animals. The delayed-type hypersensitivity reaction index in aggressors decreased by more than 45% (p<0,01), in victims — by more than 30% (p<0,05). In relation to the humoral immunity in animal aggressors, more pronounced changes in indicators were observed: a decrease in antibody titer in the aggressors — by more than 80% (p<0,001), in victims — by more than 50% (p<0,001) compared with the control indicators (Table 1). As can be seen from the results presented in Table 1, the neuropeptides contributed to the restoration of the indices of both immunity units. The index DTH in aggressors increased in conditions of introduction of Selank by 80% (p<0,01), Pro-Gly-Pro-Leu — by 60% (p<0,05), in victims — by an average of 30%, but statistically significant indicators were only in the group of animals that were administered Selank (p<0,05). With regard to the formation of anti-erythrocyte antibodies in DAT, the hemagglutinin titer indices increased in animals with aggressive type of behavior on average more than 5 times (p<0,001), in animals with submissive type of behavior — on average almost 2 times (p<0,01) (Table 1).

When studying the indicators of phagocytic activity of peripheral blood neutrophils in animals exposed to "social" stress, an increase in the phagocytic index (PhI) and phagocytic number (PhN) was found in rats with both aggressive and submissive behaviors. There was an increase in the phagocytic index by 20% in aggressors (p>0,05) and almost 30% in victims (p<0,05), phagocytic number — by 40% in aggressors and by 20% in victims (p>0,05), which indicates a hyperreactivity of the nonspecific element of the immune system (Table 2). When assessing phagocytosis in the group of animals treated with neuropeptides on the background of the impact of "social" stress, it was found that the introduction of these compounds leads to the restoration of the parameters of nonspecific immunoreactivity. The phagocytic number decreased in aggressors and victims by an average of 30% (p<0,05) under the conditions of administration of Pro-Gly-Pro-Leu, in the group of animals treated with Selank — by 20% (p>0,05). With respect to the phagocytic index, there was a tendency to a decrease in this indicator in animals with aggressive and submissive types of behavior (Table 2).

An important stage of our work was to determine the total number of leukocytes, as well as the study of indicators of leukocyte formula. Under the conditions of "social" stress, there was a decrease in the total number of leukocytes by an average of 30% (p<0,05) in both the aggressors and the victims, relative to the control group. The leukocyte formula in stressed animals showed a decrease in the percentage of eosinophils by 30% (p<0,05) in aggressors and more than 40% (p<0,01) in victims. It should also be noted a statistically significant increase in segmented neutrophils by an average of 2 times (p<0,001), band staple — by more than 50% (p<0,01) in aggressors and almost 2 times in victims (p<0,01) (Table 3). It was established that neuropeptides on the background of stress contributed to an increase in the total number of leukocytes: in the group of aggressors the introduction of Selank almost 2 times (p<0,001), Pro-Gly-Pro-Leu — 1,5 times (p<0,01); in the group of victims the introduction of Selank — by more than 1,5 times (p<0,01), with the introduction of Pro-Gly-Pro-Leu there was only a tendency to increase (p>0,05). When assessing the number of eosinophils in aggressors under the influence of Selank and Pro-Gly-Pro-Leu, there was only a tendency to increase this indicator. In leukocyte formula in rats with a submissive type of behavior, the increase in the percentage of eosinophils was determined, which averaged 30% (p<0,05). In addition, in individuals with an aggressive type of behavior, the introduction of Selank and Pro-Gly-Pro-Leu led to a decrease in the band neutrophils by an average of 40% (p<0,05 and p<0,01 respectively). With the introduction of Selank and Pro-Gly-Pro-Leu in animals with a submissive type of behavior, the number of band neutrophils significantly decreased on average by 50% (p<0,01 and p<0,001 respectively). The percentage of segmented forms of neutrophils decreased in all groups by more than 40% relative to stressed animals (Table 3).

CONCLUSION

As a result of the experiment, it was found that under the influence of "social" stress, changes in immunoreactivity are multidirectional, which indicates the formation of an immune imbalance, manifested by activation of some and suppression of other parts of the immune system. As a result of studying the effect

Table 1. *The effect of neuropeptides on the formation of DTH and DAT under the conditions of "social" stress*

(M ± m) Experimental groups (n = 10) / Indicators	Index DTH, %	Titer of antibodies in DAT, log2
Animals with an aggressive type of behavior		
Control	30,83 ± 3,52	224,77 ± 23,27
"Social" stress	16,57 ± 1,75**	40,46 ± 5,81***
"Social" stress + Selank (100 mcg /kg/day)	30,38 ± 3,48##	210,56 ± 22,54###
"Social" stress + Pro-Gly-Pro-Leu (100 mcg /kg/day)	26,47 ± 3,61#	181,65 ± 20,37###
Animals with a submissive type of behavior		
Control	30,83 ± 3,52	224,77 ± 23,27
"Social" stress	20,78 ± 2,54*	103,55 ± 11,64***
"Social" stress + Selank (100 mcg /kg/day)	28,26 ± 2,66#	231,19 ± 34,91##
"Social" stress + Pro-Gly-Pro-Leu (100 mcg /kg/day)	26,40 ± 2,86	205,51 ± 23,27##

Note: ** — p<0,05; ** — p<0,01;*** — p<0,001 — comparing with control; # — p<0,05; ## — p<0,01; ### — p<0,001 — comparing with stress (Student's t-test with Bonferroni amendment for multiple comparisons).*

Table 2. *The effect of neuropeptides on the phagocytic activity of neutrophils under conditions of "social" stress*

(M ± m) Experimental groups (n = 10) / Indicators	Phagocytic index	Phagocytic number, %
Animals with an aggressive type of behavior		
Control	17,7 ± 1,68	53,3 ± 3,66
"Social" stress	21,0 ± 1,85	74,3 ± 7,37*
"Social" stress + Selank (100 mcg /kg/day)	16,3 ± 1,87	57,6 ± 4,23
"Social" stress + Pro-Gly-Pro-Leu (100 mcg /kg/day)	17,4 ± 1,68	49,2 ± 4,04#
Animals with a submissive type of behavior		
Control	17,7 ± 1,68	53,3 ± 3,66
"Social" stress	22,9 ± 1,61*	63,7 ± 4,73
"Social" stress + Selank (100 mcg /kg/day)	18,4 ± 1,58	50,5 ± 4,65
"Social" stress + Pro-Gly-Pro-Leu (100 mcg /kg/day)	19,2 ± 0,96	45,8 ± 5,15#

Note: ** — p<0,05; ** — p<0,01;*** — p<0,001 — comparing with control; # — p<0,05; ## — p<0,01; ### — p<0,001 — comparing with stress (Student's t-test with Bonferroni amendment for multiple comparisons)*

of neuropeptides under the conditions of "social" stress, it was found that Selank and Pro-Gly-Pro-Leu proved to be an effective correctors, restoring the cellular and humoral immunogenesis reactions, the phagocytic activity of neutrophils and indicators of leukocyte formula.

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Table 3. *The effect of neuropeptides on the leukocyte formula of animals under "social" stress*

(M ± m) Experimental groups (n = 10) / Indicators	Total number of leukocytes, x10⁹/l	Eosinophils, %	Band neutrophils, %	Segmented neutrophils, %	Lymphocytes,%	Monocytes, %
Animals with an aggressive type of behavior						
Control	11,7 ± 0,93	2,8 ± 0,33	2,2 ± 0,23	12,7 ± 1,59	81,5 ± 5,95	0,83 ± 0,15
"Social" stress	8,3 ± 0,82*	2,0 ± 0,21*	3,4 ± 0,25**	26,7 ± 1,81***	67,1 ± 4,27	0,71 ± 0,10
"Social" stress + Selank (100 mcg /kg/day)	15,7 ± 1,24###	2,4 ± 0,20	2,1 ± 0,36#	16,0 ± 2,10##	78,7 ± 4,87	0,86 ± 0,11
"Social" stress + Pro-Gly-Pro-Leu (100 mcg/kg/day)	12,0 ± 0,76##	2,3 ± 0,40	1,9 ± 0,42##	14,6 ± 1,36###	79,9 ± 4,11	1,0 ± 0,10#
Animals with a submissive type of behavior						
Control	11,7 ± 0,93	2,8 ± 0,33	2,2 ± 0,23	12,7 ± 1,59	81,5 ± 5,95	0,83 ± 0,15
"Social" stress	8,4 ± 0,77*	1,6 ± 0,11**	4,1 ± 0,40**	27,1 ± 2,11***	66,4 ± 4,77	0,71 ± 0,10
"Social" stress + Selank (100 mcg /kg/day)	13,1 ± 0,58###	2,1 ± 0,22#	2,1 ± 0,37##	16,1 ± 2,57##	78,9 ± 4,87	0,86 ± 0,11
"Social" stress + Pro-Gly-Pro-Leu (100 mcg/kg/day)	9,6 ± 0,77	2,1 ± 0,18#	1,6 ± 0,30###	13,9 ± 1,51###	81,6 ± 6,04	0,86 ± 0,11

Note: ** — p<0,05; ** — p<0,01;*** — p<0,001 — comparing with control; # — p<0,05; ## — p<0,01; ### — p<0,001 — comparing with stress (Student's t-test with Bonferroni amendment for multiple comparisons)*

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INFLUENCE OF SEMAX ON THE INTENSITY OF REDOX REACTIONS IN IMMUNOCOMPETENT ORGANS IN THE CONDITIONS OF "SOCIAL" STRESS

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ABSTRACT — The experiment investigated the effect of Semax (Met-Glu-His-Phe-Pro-Gly-Pro) on the intensity of redox reactions in immunocompetent organs (thymus and spleen) on the model of "social" stress. The intensity of redox processes was assessed by determining the intensity of lipid peroxidation (LPO) in immunocompetent organs (thymus and spleen) and catalase activity. "Social" stress, formed in the experiment, is accompanied by an increase in the peroxidation processes in immunocompetent organs, which contributes to the development of stress-induced functional disorders of the immune system. Under the influence of "social" stress, the activity of the investigated antioxidant enzyme in the thymus and spleen increased in comparison with the corresponding indicators in intact rats. Against the background of Semax administration under "social" stress, its pronounced corrective effect on lipid peroxidation rates is observed, as evidenced by a decrease in spleen and thymus tissue homogenates of male rats in the baseline level of TBA-reactive products, speed of spontaneous and ascorbate-dependent lipid peroxidation, and catalase activity.

INTRODUCTION

In modern medicine, one of the most pressing problems is the effect of various types of stress on the functioning of various body systems, in particular, immune. It is known that the pathogenesis of post-stress disorders on the part of the immune system is closely associated with a change in the balance between oxidative and antioxidant processes in tissues [9]. The study of the effects of stressing factors of different nature on the functional activity of immunocompetent organs, by determining the products of lipid peroxidation, makes it possible to assess the degree of their participation in the pathogenesis of stress-induced changes in the functions of the immune system and is the basis for finding ways to correct these changes [5, 6, 7, 10].

A promising approach to the correction of impaired protective functions of the body due to stressful effects is the study and subsequent implementation of peptide drugs in practice [1]. As a promising means of correcting stress-induced immunity disorders, the drug Semax, which is a synthetic heptapeptide — an analogue of ACTH 4–10 (Met-Glu-His-Phe-Pro-Gly-Pro), is devoid of hormonal activity [3, 8]. Despite the fact that Semax has been used in clinical practice for more than 20 years, the full range of pharmacological effects of this peptide has not yet been determined.

The aim of research:
to study the effect of Semax on the intensity of redox reactions in the thymus and spleen under the conditions of "social" stress.

MATERIAL AND METHODS

White non-linear rats (males, 6–8 months old) were used as experimental animals. In order to create a "social" stress in the experiment a model of intermale confrontations was chosen. Animals were placed in pairs in experimental cells separated by a septum which prevents physical contact but has openings that provide sensory contact. Every day the partition was removed for 10 minutes which overwhelmingly led to agonistic collisions (confrontations) [2]. Groups of animals with alternative types of behavior were formed: aggressive type — in case of repeated victories experience (winner, aggressor) and submissive type - in case of defeats (victim). Laboratory animals were divided into 3 groups (n = 10): a group of intact males; a group of animals that were exposed to stress for 20 days (sensory contact); a group of individuals treated intraperitoneally with Semax at a dose of 100 μg/kg/day under conditions of 20-day stress exposure (sensory contact) in a course of 20 days. The intensity of redox processes was assessed by determining the intensity of lipid peroxidation in immunocompetent organs (thymus and spleen) and catalase activity [4].

The experiment results were statistically processed using the following programs: Microsoft Office Excel 2007 (Microsoft, USA), BIOSTAT 2008 Professional 5.1.3.1. To process the obtained results, a parametric

method was used with the Student t-test with the Bonferroni correction. Statistically significant differences were considered at $p<0.05$.

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RESULTS AND ITS DISCUSSION

Against the background of "social" stress, an increase in the rate of spontaneous and ascorbate-dependent lipid peroxidation, as well as the initial level of malondialdehyde (MDA) in the thymus, was noted. The initial level of MDA significantly increased in the aggressors by almost 40% ($p<0,01$), in victims — by 20% ($p>0,05$). The rate of spontaneous lipid peroxidation in animals with an aggressive type of behavior increased by 40% ($p<0,001$), in animals with submissive — more than 30% ($p<0,05$). "Social" stress also led to an increase in the rate of ascorbate-dependent lipid peroxidation by 30% ($p<0,05$) in the aggressors and 50% ($p<0,01$) in the victims. Along with the increased severity of peroxidation processes on the background of "social" stress, it should be noted that catalase activity in the thymus increased by more than 30% ($p<0,05$) in aggressive animals and by almost 60% ($p<0,01$) in submissive compared with intact individuals (Table 1).

With the introduction of Semax under stress, a decrease in the rate of spontaneous and ascorbate-dependent lipid peroxidation and the initial level of MDA was observed in the thymus homogenate. The initial level of TBA-reactive products was significantly reduced in the aggressors, almost 40% ($p<0,01$) relative to the stress group. Under the influence of Semax, animals with an aggressive type of behavior also noted a decrease in the rate of spontaneous and ascorbate-dependent lipid peroxidation by more than 30% ($p<0,01$) relative to animals subjected to stress. In addition, a decrease in the rates of ascorbate-dependent and spontaneous lipid peroxidation in animals with a submissive type of behavior was observed by almost 40% ($p<0,01$) and 30% ($p<0,05$), respectively. It should be noted that the studied drug contributed to a decrease in the initial level of MDA in the thymus in victims — by 30% ($p<0,05$). When evaluating the effect of Semax under stress on catalase activity, a decrease in this indicator was observed in aggressors and victims by 30% ($p<0,05$) compared with stressed rats (Table 1).

An increase in the level of TBA-reactive products under conditions of experimental stress was also observed in spleen homogenate. Social stress led to a significant increase in the rate of spontaneous lipid peroxidation in the spleen by an average of 45% ($p<0,01$) in aggressive and submissive animals. The initial level of MDA in the spleen homogenate in male aggressor rats increased by more than 50% ($p<0,01$), and in victims almost 70% ($p<0,001$). "Social" stress also led to an increase in the rate of ascorbate-dependent lipid peroxidation by 30% in the aggressors ($p<0,05$) and 20% in the victims ($p>0,05$). It should be emphasized that under the influence of stress, there was an increase in catalase activity in the spleen of rats in aggressive and submissive animals by 60% ($p<0,01$) and 40% ($p<0,05$) respectively relative to the "control" group (Table 2).

Under the influence of Semax on stressed animals, the indices of the initial level of MDA in the spleen of both experimental groups decreased on average by 40% ($p<0,01$), the rate of ascorbate-dependent lipid peroxidation decreased in aggressors by 35% ($p<0,01$), in victims — more than 20% ($p>0,05$) relative to the stress group. This drug also corrected the rate of spontaneous lipid peroxidation in aggressive and submissive animals, reducing it in both groups by almost 40% ($p<0,01$). In addition, under the influence of Semax, a decrease in the level of catalase was observed on average by 30% ($p<0,05$) in aggressors and victims as compared with the stress group (Table 2).

Table 1. *The effect of Semax on lipid peroxidation and catalase activity in the thymus of male rats under the conditions of "social" stress*

Experimental groups (n = 10)	Lipid peroxidation indicators (M ± m)			Catalase activity, %
	The initial level of MDA, M ± m, nmol / g tissue	The rate of spontaneous lipid peroxidation, M ± m, nmol / g · h	The rate of ascorbate-dependent lipid peroxidation, M ± m, nmol / g · h	
Animals with an aggressive type of behavior				
Control	2,7 ± 0,3	3,0 ± 0,2	2,4 ± 0,2	50,7 ± 4,1
"Social" stress	3,7 ± 0,3*	4,2 ± 0,3**	3,1 ± 0,2*	66,8 ± 5,4*
"Social" stress + Semax (100 mcg /kg/day)	2,3 ± 0,2##	2,8 ± 0,3##	2,1 ± 0,2##	47,2 ± 3,9#
Animals with a submissive type of behavior				
Control	2,7 ± 0,3	3,0 ± 0,2	2,4 ± 0,2	50,7 ± 4,1
"Social" stress	3,2 ± 0,3	4,0 ± 0,3*	3,6 ± 0,3**	80,3 ± 7,2**
"Social" stress + Semax (100 mcg /kg/day)	2,3 ± 0,2#	2,8 ± 0,3#	2,3 ± 0,2##	56,4 ± 5,1#

Note: ** — p<0,05; ** — p<0,01;*** — p<0,001 — comparing with control; # — p<0,05; ## — p<0,01; ### — p<0,001 — comparing with stress (Student's t-test with Bonferroni amendment for multiple comparisons)*

Table. *The effect of Semax on lipid peroxidation and catalase activity in the spleen of male rats under the conditions of "social" stress*

Experimental groups (n = 10)	Lipid peroxidation indicators (M ± m)			Catalase activity, %
	The initial level of MDA, M ± m, nmol / g tissue	The rate of spontaneous lipid peroxidation, M ± m, nmol / g · h	The rate of ascorbate-dependent lipid peroxidation, M ± m, nmol / g · h	
Animals with an aggressive type of behavior				
Control	8,4 ± 0,7	9,8 ± 0,8	10,5 ± 1,1	14,2 ± 1,0
"Social" stress	12,8 ± 1,0**	14,6 ± 1,3**	13,8 ± 1,2*	22,3 ± 1,9**
"Social" stress + Semax (100 mcg /kg/day)	8,0 ± 1,2##	9,2 ± 0,9##	9,0 ± 0,8##	15,0 ± 1,6#
Animals with a submissive type of behavior				
Control	8,4 ± 0,7	9,8 ± 0,8	10,5 ± 1,1	14,2 ± 1,0
"Social" stress	14,1 ± 1,3***	13,9 ± 1,1**	12,6 ± 0,9	19,5 ± 1,7*
"Social" stress + Semax (100 mcg /kg/day)	8,2 ± 0,9##	8,5 ± 0,9##	9,8 ± 1,0	13,8 ± 1,4#

Note: ** — p<0,05; ** — p<0,01;*** — p<0,001 — comparing with control; # — p<0,05; ## — p<0,01; ### — p<0,001 — comparing with stress (Student's t-test with Bonferroni amendment for multiple comparisons)*

CONCLUSION

"Social" stress, formed in the experiment as a result of inter-male confrontations, is accompanied by an increase in peroxidation processes in immunocompetent organs, which contributes to the development of stress-induced functional disorders of the immune system. Under the influence of "social" stress, the activity of the studied antioxidant enzyme in immunocompetent organs (thymus and spleen) increased compared with the corresponding indicators in intact rats. This testified to the imbalance in the system of antioxidant protection of the body and, possibly, its depletion.

Against the background of Semax administration under "social" stress, its pronounced corrective effect on lipid peroxidation rates is observed, as evidenced by a decrease in the spleen and thymus tissue homogenates of male rats in the initial level of TBA-reactive products, spontaneous and ascorbate-dependent levels of lipid peroxidation, and also catalase activity.

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DIAGNOSIS AND TREATMENT OF NECROTIZING ENTEROCOLITIS IN NEWBORNS
LITERATURE REVIEW

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ABSTRACT — A literature review of ulcerative necrotizing enterocolitis, which is one of the most urgent problems in the structure of pathology of newborns surgical profile is presented. The relevance is due to the high prevalence of ulcerative – necrotizing enterocolitis, difficulties in its diagnosis, conservative and surgical treatment, high mortality and frequent complications by perforation to the abdominal cavity and other organs.

KEYWORDS — NEC, newborns, review, treatment, diagnosis

Determination, pathogenesis and prevalence of ulcerative-necrotizing enterocolitis in newborns

Ulcerative necrotizing enterocolitis (NEC) is an inflammatory disease of newborns which is accompanied by damage to the gastrointestinal tract (GIT) with the development of perforations and intestinal necrosis. Despite the progress made in diagnosis and treatment, the mortality of patients with NEC after surgery is high, and reaches 60% and does not tend to decrease [8, 11, 14, 17, 18, 26].

To date, the etiology and pathogenesis of NEC has been the subject of extensive scientific discussion. The main efforts are aimed at finding potential markers to identify NEC in the early stages, to determine the nature of the course and prognosis of the disease [3, 8].

Despite the progress achieved in the diagnosis, the analysis of the immediate and long — term results of treatment of NEC shows that in 16–40% of children the course of the disease is complicated by perforations of the gastrointestinal tract, the mortality in which reaches according to different authors from 40–90% [8, 11, 14, 27, 18, 19, 23, 27]. It becomes quite obvious that the success of treatment depends not only on adequate surgical correction, timely and rational prescription of antibiotics, but also is determined by a complex of diagnostic and therapeutic measures aimed at early detection of signs of complicated course of NEC [2, 3, 4, 9, 10, 21, 22, 24].

It is known that in NEC any parts of the gastrointestinal tract (GIT) can be damaged. The prevailing pathological process in more than 90% of cases is inflammation and coagulation necrosis of the intestinal wall. The proven risk factors in the pathogenesis of NEC are prematurity, early onset of feeding, asphyxia, intestinal ischemia, immune disorders and infection, and low gestational age [1, 7, 20, 25, 29].

The role of other risk factors, their interrelation and consistency, remains unclear.

In the 1960s, when describing a series of newborns and infants with gastrointestinal perforations, it was found that most patients had clinical signs of neonatal hypoxia, which was considered the main cause of NEC the development of NEC Further accumulation of data suggested that possible producing factors of NEC in premature infants are intestinal hypoperfusion caused by congenital defects of the cardiovascular system, hypoxic-ischemic lesions of the Central nervous system, polycythemia, persistent circulatory disorders in the upper mesenteric artery [20, 28].

Endothelial cells (EC) play a key role in the regulation of blood flow, permeability and intercellular metabolism. In experimental models of NEC, EC damage has been revealed as a result of edema, cytoskeleton disorders, depolarization and increased permeability of cytomembranes caused by active oxygen forms and proinflammatory mediators, the excess production of which is the result of stimulation of the immune system, which leads to microcirculatory dysfunction in NEC.

The mechanisms of development of ischemic intestinal damage in NEC are directly related to inflammatory changes in the intestinal wall due to the development of acute inflammation manifested by leukocyte infiltration. Interstitial neutrophils appear to be the main producers of active types of oxygen, proteases and inflammatory cytokines, which contribute to further damage to the intestine, increasing immune inflammation. Interestingly, neutrophil infiltration are largely restricted to post-capillary venules, which confirms the observation that only 0.6% of leukocytes pass through the arterioles in comparison with 39%

in venules. In addition, the endothelium venul is more sensitive to the effects of leukocyte producers than the endothelium of arterioles and capillaries [29, 30, 32].

Thus, ischemia is not the only initiating factor in the development of NEC in most cases, but it plays an important role at all stages of development of NEC. Sharp changes in hemodynamics after birth and immature control system of mesenteric blood circulation, especially in premature infants, makes mesenteric vessels vulnerable. Postpartum stress easily disrupts the function of the endothelium, which leads to microcirculatory dysfunction of the intestine, manifested by vasoconstriction of arterioles, violation of the endothelial barrier in the capillaries, inflammatory cell infiltration of the intestinal wall. With prolonged exposure to damaging factors, microcirculation disorders progress to coagulation necrosis, ultimately determining the direction of NEC [6, 13, 29, 31].

Experimental studies on cell cultures from resected drugs of the intestine of newborns with NEC has allowed to establish that fetuses and preterm infants excessive inflammatory response to postnatal microbial colonization, accompanied by damage of the epithelial intestinal cells [6, 13].

Excessive intestinal damage due to inadequate inflammatory response associated with abnormal intestinal flora is now considered to be the most likely basis for the pathogenesis of necrotizing enterocolitis [7, 10, 13].

The main objective of the examination of children with NEC is to identify symptoms that allow to determine indications for surgery as early as possible before the development of intestinal perforations and peritonitis [5, 12, 15, 16, 18].

Currently, the absolute indications for surgical treatment of newborns with NEC, taking into account all signs of the disease, can be distributed in the degree of importance and frequency of occurrence as follows:

1. Inflammatory changes in the abdominal wall, pronounced subcutaneous venous network, tension and soreness in palpation of the abdomen, which usually indicate the presence of peritonitis, gangrene of the intestine adjacent to the abdominal abscess;
2. Pneumoperitoneum — a direct sign of perforation in combination with other signs of NEC;
3. Specific x-ray picture, indicating the gangrene of the intestine — the presence of a static loop of the intestine, uneven inflation of the intestinal loops, common pneumatosis of the intestinal wall, gas in the portal vein, signs of suddenly arisen ascites;
4. Laparocentesis data — cloudy brown or greenish effusion with a high content of leukocytes and extracellular bacteria;
5. Inflammatory infiltrate in the abdominal cavity with signs of abscess or intestinal obstruction, indicating the presence of conglomerate soldered together necrotizing intestinal loops;
6. Laboratory data — acute thrombocytopenia, hemocoagulation disorders, severe hyponatremia and persistent metabolic acidosis.

Relative indications for surgery are:

1. Clinical deterioration of the patient;
2. Severe thrombocytopenia;
3. Profuse bleeding from the lower gastrointestinal tract;
4. Persistent tension of the anterior abdominal wall;
5. A sharp decrease in gas filling of intestinal loops in the presence of signs of ascites.

Despite the clearly defined indications for surgical treatment, the results of clinical observations indicate that the choice of terms and optimal methods of surgical treatment of NEC is the subject of discussion. One of the priority directions of research of the pathogenesis of NEC is the search for reliable molecular markers that can adequately determine the severity of the disease in order to determine the outcomes and the choice of individual treatment tactics [30].

Thus, the above shows that there is no single algorithm of medical diagnostic measures in NEC in newborns. Optimization of early diagnosis and treatment of NEC is an urgent task of neonatologists and pediatric surgeons.

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THE INFLUENCE OF COMORBID PATHOLOGY ON THE CARDIOVASCULAR CONDITION IN YOUNG SPORTSMEN

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ABSTRACT — 74 young athletes aged 8–10 years underwent cardiovascular assessment. Additionally, levels of MB — creatine phosphokinase, troponin - T, parameters of heart rate variability were studied. It is stated that the syndrome of vegetative dysfunction and undifferentiated connective tissue dysplasia are the adverse background contributing to the development of disturbances of cardiac activity, arrhythmias and cardiomyopathies in young athletes.

KEYWORDS — children involved in sports, biochemistry indicators: creatine phosphokinase, troponin-T, spectral indicators of heart rate variability, syndrome of vegetative dysfunction, undifferentiated connective tissue displasia, comorbid pathology

In recent years the concept of comorbidity has been included in clinical pediatric practice. There are scientific papers on comorbid pathology in children with bronchopulmonary dysplasia, congenital pathology of the musculoskeletal system, undifferentiated connective tissue dysplasia, nephropathy and other [1,2 ,4,5,6]. The evaluation of disorders of the cardiovascular system without comorbidity among young sportsmen should not be objective.

In children (adolescents) involved in sports, undoubtedly, various violations of the vegetative regulation affecting the condition of health can occur with great frequency. There is not seldom the combination of manifestations of undifferentiated connective tissue dysplasia with autonomic dysfunction. The comorbidity of these symptoms and cardiac abnormalities among young sportsmen remains to be not enough investigated.

It is generally considered that to estimate the degree of myocardium damage (cardiomyocytes), the determination of the activity of the MV fraction of creatinine phosphokinase (MB-CPK) of the troponin-T level (Tr-T), etc. may be used [3,4,7,8].

The study of the state of the spectral parameters of heart rate variability (HRV) is not only a significant addition to the electrocardiographic data but also allows to quantify the vegetative influence on the work of various regulatory systems.

The utilized activities of MB-CPK and the main parameters of heart rate variability allow to identify and evaluate various signs of impaired cardiac activity in young sportsmen. The search of clinical, biochemical and instrumental criteria for myocardial dysfunction and heart rhythm disturbances in comorbid pathology in young sportsmen is an important unsolved problem in pediatrics.

The aim of investigation:
is to establish the value of comorbid pathology in the development of various forms of cardiac abnormalities in young sportsmen.

THE CHARACTERISTIC OF CHILDREN

Under the supervision there were 74 children, aged 8–10 years (average age 9±1), engaged over 3 years and more in handball. The sportsmen involved in other sports with organic pathology of the cardiovascular system and having been undergone the acute respiratory diseases 1–1.5 months before the examination were excluded.

According to results of the clinical examination the respondents were divided into three groups. The first group included 28 children with signs of undifferentiated connective tissue dysplasia (UCTD).

The second group included 19 children with the manifestation of the syndrome of vegetative dysfunction (SVD) of the mixed character in the compensation stage. 13 children had vagotonia (asimpathicotonia).

In the third group, there were 27 young sportsmen with signs of UCTD combined with ADD of mixed character in the period of acute attack of the disease.

THE METHODS OF INVESTIGATION

The investigation was conducted on the base of the Regional Curative Physical –Culture Dispensary of Astrakhan. It included the collection of data for medical history about the peculiarities of child development and diseases of the circulatory system. The pediatrician and pediatric cardiologist monitored all

children for 2–3 times a year. Alongside with the clinical evaluation of the cardiovascular system, ELISA and troponin-T content there were studied all the observed levels of MB-CPK activity using the BeringerMaugeim enzyme-test system.

From instrumental methods, standard electrocardiography (ECG), echocardiography (ECHO-CG) were used with the interpretation of the data obtained according to standards. To study the vegetative and energy characteristics of the organism, there were used the definition of the main spectral parameters of heart rate variability (HRV) on the Polyspectra-12E apparatus of the Neyrosoft Company. The obtained data were processed according to generally accepted methods of variation statistics.

RESULTS AND DISCUSSION

USTD in the first group was characterized by the presence of asthenic physique (11), hypermobility of joints (8), chest deformity (5), scoliosis (6), flatfoot (5). From the visceral signs on the echocardiogram there were determined: mitral valve prolapse with I–II degree regurgitation, an additional chord of the left ventricle; prolapse of the tricuspid valve with regurgitation of grades I–II, 5 had diastolic disfunction, 7 had the tendency to decrease in systolic function. These visceral manifestations can be interpreted as dysplastic heart. The boundaries of relative cardiac dullness did not change significantly. During auscultation, in 9 observables there were determined muffled tones of the heart (4), attenuation of I tone at point V (2). On the ECG 13 persons showed signs of impaired repolarization.

The activity of MB-CPK ($p<0,05$) and levels, Tr-T ($p<0,05$) were moderately elevated in contrast to the norm. These clinical-biochemical and instrumental data given above allowed to make the diagnosis of secondary cardiomyopathy. It was caused by the comorbid state of USTD and the influence of physical activity in sports (Table 1).

In the second group young sportsmen had no complaints. Clinical signs of SVD were detected during careful examination: red dermographism, skin moisture, lethargy, fatigue and bradycardia at rest. The boundaries of relative cardiac dullness in 9 sportsmen were moderately widen to the left. Heart sounds were moderately muffled in 10, soft systolic murmur at point V (5). In 17 children sinus bradyarrhythmia was determined, in 7 children there were ventricular premature beats. CM ECG confirmed these data. In this case bradyarrhythmia occurred in one third part at night from 40 to 54 beats per minute.

The levels of biochemical indicators of myocardial damage did not significantly differ from the norm ($p>0,05$, $p>0,05$) (Table 1).

In comparison of findings of clinical and instrumental examination, the diagnosis of cardiac rhythm disturbance, sinus bradyarrhythmia (8%), over ventricular (10%) or ventricular premature beats (5%) on the background of SVD was made. The occurrence of these disorders should be regarded as comorbide pathology of SVD and heart rhythm disturbance in physical exertion and emotional stress.

In the 3rd group (27) children had complaints of recurrent heart pain. Phenotypic signs of UCTD were noted — hypermobility of joints, flat-footedness, violation of posture, asthenic physique, deformity of auricles, nails, etc. Each observed person had up to six or more signs in combination with 2–3 visceral ones from the side of the heart (red dermographism, lethargy, fatigue, bradycardia at rest, skin moisture) of the SVD had mixed the character with marked manifestations.

The boundaries of relative cardiac dullness were moderately widen to the left in 7 children. Muffled heart sounds at 8, soft systolic murmur at the apex and at point V (7). The sinus bradyarrhythmia in 8, supraventricular premature beats in 5, ventricular in 3. The levels MB-CPK, troponin-T were high in comparison with data the firat and second groups (Table 1).

On ECG disorders of repolarization in 15, rhythm disturbances confirmed the clinical data. At the CM-ECG supraventricular extrasystoly was recorded in 5, ventricular in 3 and in 8 cases-sinus bradyarrhythmia.

On the ECHO-CS the contractility of the myocardium was not changed; in 7, the decrease of left ventricular diastolic function was determined.

These data indicate the presence of combinations of comorbide diseases in this group such as: UCTD and SVD. The influence of prolonged physical exertion in combination with emotional stress they have become the cause of the development of cardiac pathology: secondary cardiomyopathy and cardiac arrhythmia.

Thus the presence of undifferentiated connective tissue dysplasia, autonomic disfunction syndrome in sportsmen is an unfavourable background for the development of cardiovascular pathology during athletic exercises in children. Prognosticallyunfavourable sign of the development of cardiac abnormalities is autonomic disfunction syndrome. The asympathicotonia may contribute to the formation of cardiac arrhythmia or the manifestation of existing latent forms.

The combinations of UCTD with autonomic disfunction syndrome in young sportsmen often lead to the development of cardiac abnormalities.In these cases the combinations of myocardial disfunction with cardiac rhythm disturbances of functional and organic

Table 1. *The value of laboratory and instrumental data in young sportsmen*

Indicators	Groups		
	First (n=28)	Second (n=19)	Third (n=27)
MB-CPK (F/l)	54,3±0,4**##	34,5±0,3*##	58,5±0,4**
Troponin-T (mg/l)	0,044±0,006*##	0,045±0,005*##	0,052±0,006**
TP (ме^2)	2510±544*#	3150±554**##	2120±560*
VLF (ме^2)	1234±418*#	2910±560**#	1240±420*
LF (ме^2)	920±134**##	950±610**#	915±136*
HF (ме^2)	640±152*#	690±148*##	651±144*

Legend: *p* in the comparison of all groups with the norm p*>0,05 p**<0,05*
p — in comparing the first and second groups with the third p>0,05 p<0,05

nature are possible. The physical and emotional stress in sportsmen should lead to the development of functional disorders of the heart or the manifestation of latent disfunction. The presence of comorbid pathology: UCTD and SVD may be the risk factors for the development of cardiac abnormalities in the formation of arrhythmia and cardiomyopathy in young sportsmen.

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PECULIARITIES OF METABOLISM IN PATIENTS WITH A HIGH RISK OF COMPLICATIONS IN THE POSTOPERATIVE PERIOD

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ABSTRACT — In order to develop a methodology for assessing the risk of postoperative complications in surgical patients, the biochemical parameters of 254 patients were studied in the laboratory of the Tver State Medical University scientific center. The patients were aged 16 to 35 years and underwent planned surgical treatment in hospitals of Tver and Moscow. Investigations were performed before surgery, on the first day after surgery and on the fifth day after surgery. It was revealed that if the ratio of the level of matrix metalloproteinase I to the level of magnesium of erythrocytes on the fifth day after surgery is 20% or more higher than the same indicator before surgery, then this patient is more likely to develop complications in the postoperative period. Based on the study results, a patent for an invention (RU) was obtained.

KEYWORDS — postoperative complications, matrix metalloproteinase I, erythrocyte magnesium

INTRODUCTION

The development of postoperative complications remains one of the main problems of surgery [2]. Despite the improvement of surgical methods, the creation of new equipment and new materials, the frequency of complications after surgery is up to 18% [2, 3]. Most complications significantly reduce the quality of life of patients [5]. In our previous studies, it was found that complications more often occur in patients with signs of MASS (Mitral valve, Aorta, Skeleton, Skin) syndrome (MIM 604308) [6]. External signs of MASS syndrome (MIM 604308) are too diverse and do not allow an unambiguous conclusion about the diagnosis. In the surgeon's daily work, conducting a genetic study of the patient may be too costly.

The aim of the study is to develop a methodology for assessing the risk of postoperative complications in surgical patients based on an analysis of changes in biochemical parameters of blood serum.

MATERIALS AND METHODS

We examined 254 patients who were undergoing planned surgical treatment in hospitals in Tver and Moscow. Pairwise comparison method was used. The main group included 177 men and women, aged 16 to 35 years, who had complications after planned operations. They were operated on for inguinal hernia, cholecystitis, vascular pathology of the extremities and pathology of the retroperitoneal space. A long pain syndrome, complications from the surgical wound, a prolonged temperature reaction and patient complaints about a prolonged deterioration in well-being were taken into account as complications. Each patient from the main group corresponded to surgical patients from the control group of the same age, gender and having a similar diagnosis. Biochemical blood testing was done in the laboratory of the scientific center of Tver State Medical University (License No. FS-69-01-000780 dated 23/04/2015). Investigations were performed before surgery, on the first day after surgery and on the fifth day after surgery. The level of alkaline phosphatase, sialic acids, fibrinogen, erythrocyte magnesium, type I C-terminal telopeptide (Cross Laps), matrix metalloproteinase I, matrix metalloproteinase IX in blood serum were determined. The data was processed with the STATISTICA (Stat Soft Russia) and BIOSTAT application software.

RESULTS

Before the operation, biochemical parameters in both groups were in a minor way different. In patients of the main group, the median levels of alkaline phosphatase, sialic acids, fibrinogen, and erythrocyte magnesium were slightly higher than in the control group.

A day after the operation, a gradual change in a number of indicators was revealed. On the fifth day after the operation, changes in the median serum levels of alkaline phosphatase, sialic acids, fibrinogen, erythrocyte magnesium, type I C-terminal telopeptide (Cross Laps), matrix metalloproteinase I, matrix metalloproteinase IX in the main and control groups were significant and reliable.

The most pronounced changes were identified in relation to the level of matrix metalloproteinase I and the level of matrix metalloproteinase IX. The

median level of matrix metalloproteinase I decreased to 9.97±4.21 ng/ml, while in the control group it was 21.04±4.09 ng/ml (p<0.01). The median value of the level of matrix metalloproteinase IX decreased to 412.41±51.07 ng/ml, while in the control group it was 558.11±87.54 ng/ml (p<0.01). It should be noted that the level of erythrocyte magnesium increased to 8.39±0.98 (mmol/L), while in patients without postoperative complications it remained at the level of 5.78±0.79 mmol/L.

CONCLUSION

A mathematical analysis of the data revealed the following pattern: if the ratio of the level of matrix metalloproteinase I to the level of erythrocyte magnesium on the fifth day after surgery is 20% or more higher than the same level before surgery, then this patient is more likely to develop complications in the postoperative period. A systematic analysis of the obtained data suggests that an analysis of the dynamics of the level of matrix metalloproteinase I, the level of matrix metalloproteinase IX and the level of erythrocyte magnesium before surgery and 5 days after surgery can serve as a reliable criterion for the risk of postoperative complications during planned surgical interventions. Based on the results of the study, there was obtained the patent "Method for assessing the risk of developing complications in the distant postoperative period in patients with signs of connective tissue dysplasia" [4].

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LONG-TERM RESULTS FOR SIMULTANEOUS SURGICAL TREATMENT OF GLAUCOMA COMBINED WITH CATARACT USING THE SUPRACHOROIDAL AUTOSCLERAL DRAINAGE METHOD

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ABSTRACT — The development of cataract in patients with glaucoma poses an important task for ophthalmologist to sought pathogenetic feasible and effective treatment of this combined pathology. This is due to the fact that glaucoma and cataract are one of the main causes of blindness and low vision [1].
In comparison with traditional methods, sutureless cataract surgery and new methods for anti-glaucoma operations are widely used in clinical practice due to their high records of safety. The latter has led to an increasing use of combined treatment for patients with combined pathology among the population [2]. The combined operation allows normalization of ophthalmotonus and restore visual functions, which is exceedingly important in reducing the amount of time required for visual, professional and social rehabilitation [3].
The utilization of drains in ophthalmic surgery is the most relevant and propitious direction in ophthalmic surgery, in view of the fact that they cause a longer and stable hypotensive effect. Currently, methods of activation of the uveoscleral outflow pathway by introducing drains from different materials into the suprachoroidal space, as well as a combination of operations aim at activating various aqueous humor outflow pathways [4]. The existence of numerous surgical aids (their modifications), various drains and drainage devices indicate that there is no universal surgical method for treatment of glaucoma [8].
Currently, the most promising methods are: (I) the activation of uveoscleral outflow pathway by introducing drains from different materials into the suprachoroidal space, and (II) the combination of operations aim at activating various aqueous humor outflow pathways [5].

PURPOSE

Evaluation of long-term functional results of combined surgical method for treatment of glaucoma in combination with cataract using the suprachoroidal autoscleral drainage.

MATERIAL AND METHODS

The study included 84 patients (84 eyes). Out of the 84 patients 61 were women and 23 — men. The observation period lasted from March 2018 to January 2019. The mean preoperative intraocular pressure (IOP) was 31.4 ± 1.3 mmHg. Assessment of ophthalmic status included conventional clinical examination: determination of visual acuity, tonometry, biomicroscopy, ophthalmoscopy, keratorefractive, and perimetry. All patients in the preoperative and postoperative period underwent applanation tonometry by A.N. Maklakov, with a weight of 10.0 grams. The B.L Polyaka's ruler was used for calculation. The state of the uveoscleral outflow pathways activated by autoscleral drainage of the suprachoroidal space after impenetrable deep sclerectomy in combination with cataract was assessed using an optical coherence tomography (OCT) on the Zeiss Visante OCT device (Germany).

The indication for carrying out non-penetrating deep sclerectomy with drainage of suprachoroidal space autoclaves with cataract phacoemulsification of complicated cataract was combined with uncompensated (b-c) glaucoma stage II–III.

OPERATION TECHNIQUE

The surgical treatment was carried out as follow: From the limb make a 5mm incision of the conjunctiva from 10 to 1 h. Separate the conjunctiva and the tenon's capsule from the sclera, from the limb. Coagulate the sclera vessels. With the help of a scleral knife cut out a rectangular scleral flap base to the limb on 2\3 sclera thickness, size 3×4. Furthermore, on the surface of the remaining 1\3 of the

sclera, longitudinal and parallel incisions are made to form four (4) strips of length and width, 4mm and 1mm respectively. Cut the distal strip up to the flat part of the ciliary body. With a spatula make a tunnel (width — 2 mm and length — 4 mm) into the suprachoroidal space, where the previously separated distal sclera strip leads to a depth of 3.5 mm. Thus, the self-drainage in this method is the patient's own sclera. Of the remaining three scleral strips, the median is separated completely, exposing the surface of the ciliary body. Further, in the drainage system of the anterior chamber angle of the eye, the outer wall of the Schlemm's canal is excised, the corneal tissue is also excised 2.0 mm above the sinus, using iris forceps remove the pigment epithelium from the inner wall of the Schlemm's canal, where further filtration of the intraocular fluid from the anterior chamber will occur, creating a filtration zone. Subsequently, cataract extraction is performed by standard phacoemulsification with intraocular lens implantation. Corneal incision 2.2 mm. Rectangular flap sutured with two nodal sutures. A continuous suture is performed over the conjunctiva.

RESULTS AND DISCUSSION

Complications were not recorded in the intraoperative and the early postoperative period of the operation. In all cases, mild inflammatory reaction was recorded, as well as first degree mild inflammatory reaction, according to the classification of S. N. Fedorov — E. V. Egorova [9]. When all patients were examined after 6 months, parameters such as intraocular pressure (IOP) remained within the statistical norm. In the late postoperative period (1.5 years), the patients with normotony (mean IOP, 16.0 ± 1.8 mm Hg) were 69 in total. In 15 cases, there was an increase in IOP, for which hypotensive therapy (β-blockers) was prescribed. In the evaluation of OCT data, preservation and increase in size of the uveoscleral slit with a functioning filtration zone was observed.

Currently, there is no single approach on the tactics of managing patients with primary open-angle glaucoma combined with cataract, although there are quite a lot of works that give preference to simultaneous cataract extraction and anti-glaucoma surgery in recent literatures [6]. The main requirements that apply to the anti-glaucoma component in combined operations are a long hypotensive effect and stabilization of the glaucoma process and maximum safety of the intervention. One possible solution to this problem could be operations aim at activating uveoscleral outflow, which normally provides evacuation of 4–27% of fluid [7].

CONCLUSION

We conclude that since it is a one-stage surgery, it could be advantageous in reducing a patient's length of stay in the hospital and also solving economic, social, moral, psychological, and financial issues. Our proposed method is less traumatic, safe, contributes to the stabilization of intraocular pressure, and does not require the use of donor materials, including xenotransplantation. This type of surgery can be performed at any stage of glaucoma.

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DISTURBANCES OF FOLATE METABOLISM IN MEN WITH INFERTILITY

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ABSTRACT — Aim. To study the association between the male infertility and the -1298A/C (rs1801131) polymorphisms of methylenetetrahydrofolate reductase (MTHFR) gene with male infertility in the Moscow region.
Methods. Study group of infertile men (n=70) includes 26 non-obstructive azoospermic patients, 23 astenospermic and 21 patients with teratospermia and 68 healthy controls with normal sperm parameters who had one child and more. Genotyping was performed by generated amplicons from melting curve analysis after real time PCR.
Results. We did not receive significant differences in the frequencies of MTHFR genotypes (A1298C) in infertile and fertile men. In the subgroup of men with pathospermia, carriers of the minor allele 1298C (genotype AC + CC) were found with a frequency of 61%, in the group of fertile men the frequency of carriers of the allele 1298C was 53% ($\chi^2 = 1.01$; $p = 0.31$). However, the frequency of the minor allele 1298C of the MTHFR gene in the subgroup of men with asthenozoospermia was 50%, in the subgroup with teratozoospermia it was 33% and in the subgroup of men with azoospermia — 39% ($\chi^2 = 8.67$; $p = 0.003$).
Conclusions. Genetic polymorphism of the locus A1298C (rs 1801131) of folate metabolism gene MTHFR is associated with risk of violations of motility of sperm in men of the Moscow region of Russia.

KEYWORDS — Folate metabolism, male infertility, asthenozoospermia, gene polymorphism, methylenetetrahydrofolate reductase (MTHFR) gene

INTRODUCTION

The frequency of male infertility reached 17.2% in Russia [1]. Today, all authors tend to assume that the most frequent endogenous cause of male infertility is oxidative stress [1, 3, 7]. Oxidative stress occurs in 30–80% of cases in men with infertility [4]. The development of scientific and technological progress has led to environmental degradation, the growth of chronic diseases, which leads to the accumulation of reactive oxygen species (ROS). In a healthy man, the level of ROS and antioxidant system are balanced [1, 4]. Folate deficiency leads to an imbalance of the methyl groups involved in the synthesis and methylation of DNA, and increased oxidative stress [3, 6] According to Kurzawski M. et al. polymorphism of the folate metabolism gene methylenetetrahydrofolate reductase — MTHFR 1298A> C (rs1801131) is associated with a decrease in enzyme activity by 30% [2]. The association of polymorphisms of the MTHFR 1298A> C (rs1801131) and patospermia was not studied in the Moscow region.

Aim

To determine the association of polymorphic locus A1298C (rs 1801131) of the folate metabolism gene MTHFR with the risk of male infertility in the Moscow region.

METHODS

The study included 70 infertile patients (according to the conclusions of the spermogram, WHO 2010) — 1 group of patients, and 68 fertile men (with one or more children) — the control group. All men were aged 30±6 years, and lived in the Moscow region, agreed to the study. All patients underwent standard clinical examination, determination of karyotype and presence of deletions of AZF-region of Y-chromosome, mutations of CFTR gene.

To study the association of MTHFR A1298C (rs 1801131) gene polymorphism, we isolated DNA from peripheral blood leukocyte mass. Polymorphism of folate exchange genes were detected by polymerase chain reaction (PCR) in real time (Real-Time-PCR). For real time PCR used ready-made kits to identify polymorphisms in genes of folate metabolism (the company "Synthol", Russia). Genotyping was performed by generated amplicons from melting curve analysis after real time PCR on the basis of Tag Man method of probes with respect to fluorescence (RFU) of each probe. The study was performed at laboratory of Biology and General genetics department of the Peoples ' Friendship University of Russia. The genotypes of homozygotes for the reference allele (AA), heterozygous carriers with genotype (AC) and homozygotes for the minor allele of the (CC) were detected.

The frequency of alleles of the polymorphisms studied in the groups of patients and fertile men was compared using the χ^2 criterion. The significance of the differences was assessed by the nonparametric criterion

"Obs/Exp", the differences were considered significant at p<0.05. The data obtained were subjected to treatment with the use of STATISTICA 6.0

RESULTS

The most number of patients of the first group were with impaired sperm motility (Fig.1). The percentage of progressive motile sperm of category a+b was significantly lower in men with pathospermia compared to the control group: 18.6+5.7% versus 29.6+2.8% (p<0.05). The percentage of normal morphological forms of sperm also significantly differed between the first and second group of men: 2.8+2.3% and 21.7+9.1% respectively (p<0.05).

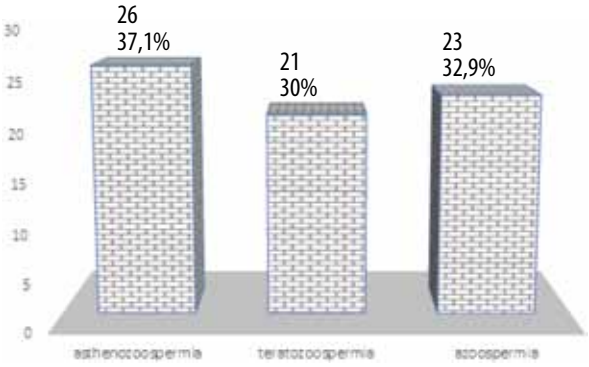

Fig. 1. *Distribution of patients of the first group in terms of sperm*

All the men we examined had normal karyotype, no mutations in the CFTR gene and microdeletions of AZF locus in the Y chromosome. It was excluded the infertility of their wives.

We compared the allele frequency distribution of the studied MTHFR gene polymorphism in both groups of men: infertile and fertile. For A1298C polymorphism of MTHFR gene, the frequencies of A/A, A/C, C/C genotypes were 38.57%, 42.85%, 18.57% among infertile patients and 47.05%, 42.64%, 10.29% in the group of fertile men (χ^2 = 2.20; p=0.33).

In the subgroup of men with pathospermia, carriers of minor allele 1298C (genotype AC+CC) were detected with a frequency of 61%, in the group of fertile men the frequency of allele carriers 1298C was 53% (χ^2 = 1.01; p=0.31).

Since we have not received significant differences in the genotypes frequencies of MTHFR (A1298C) in infertile and fertile men, we decided to conduct a comparative analysis of genotypes frequencies of the locus A1298C (rs 1801131) of folate metabolism gene MTHFR in subgroups of patients with different forms of pathospermia (Table 1). The frequencies of heterozygous carries of minor allele 1298C of the MTHFR gene (genotype AC) in the subgroup of men with asthenozoospermia was 50%, in the subgroup with teratospermia was 33% and in the subgroup of men with azoospermia — 39% (χ^2=8.67; p=0.003).

Table 1. *Distribution of the frequencies of polymorphic locus A1298C (rs 1801131) of the MTHFR gene in infertile men with different types of pathospermia*

Genotype / Sperm values	Homozygoues for the reference allele (AA)	Heterozygous carriers (AC)	Homozygoues for the minor allele (CC)
Asthenozoospermia (n(%)	6(23%)	13(50%)*	7(27%)*
Teratozoospermia (n(%)	10(48%)	7(33%)	4(19%)
Azoospermia (n(%)	11(48%)	9(39%)	3(13%)

** p<0.05 in carriers of minor allele with impaired sperm motility compared to other sperm parameters*

DISCUSSION

Folates are nutrients that are involved in many metabolic processes in the human body. In men, folates are necessary for the synthesis of sperm DNA and are involved in maintaining the integrity of the genome [6]. Folate deficiency can worsen the DNA methylation, lead to the accumulation of homocysteine, which further leads to excessive oxidative stress and disruption of DNA repair [3].

To date, published data on the association of the polymorphism of the folate metabolism gene MTHFR A1298C (rs1801131) with the risk of male infertility are inconclusive. This association has not been identified for some areas of China [5]. In some European populations there were not detected any significant differences between fertile and infertile men according to MTHFR gene polymorphisms [2].

It was shown by Shen O. et al. that MTHFR A1298C was associated with a significant increased risk of azoospermia when comparing homozygotes (CC vs. AA) (OR = 1.66) [6]. The authors believe that the C allele of MTHFR is a genetic risk factor for male infertility [6]. The results of our study demonstrate the association of folate gene polymorphism MTHFR A1298C with different forms of pathospermia (p=0.003) for infertile men. Consequently, it can be assumed that the A1298C polymorphism of the MTHFR gene may be involved in the etiology of male infertility in patients of the Moscow region. Probably, genetic variations in the genes of enzymes associated with folates metabolism may be associated with male infertility.

CONCLUSIONS

Polymorphic locus A1298C (rs 1801131) of the MTHFR gene for folate metabolism is associated with the risk of asthenozoospermia in men in the Moscow region of Russia. We believe that in infertile men, especially with impaired sperm motility, it is necessary to carry out molecular genetic measures to identify the carriers of the allele 1298C of the MTHFR gene in order to choose a personal therapeutic tactics in overcoming the factor of infertility.

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THE PREVALENCE OF PATOSPERMIA IN MEN AFTER HERNIA REPAIR

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ABSTRACT — Aim: to assess the prevalence of patospermia in men after inguinal hernia repair
Methods. The study was retrospective. We've analyzed the medical documentations of 472 men, who went to andrologist with complaints about the absence of pregnancy of the wife. We've evaluated the presence/absence of inguinal hernia repair in history, duration and nature of infertility, the main parameters of the spermogram (according to WHO criteria, 2010) in all patients.
Results. After inguinal hernia repair the pathospermia in patients persists for more than 3.6+1.2 years. After inguinal hernia repair the obstructive form of male infertility prevails (56,7%). The most severe abnormalities in the semen analysis develops after bilateral hernia repair.
Conclusions. Inguinal non-tension hernia repair is a risk factor for male infertility in 14.1% of men of reproductive age.

KEYWORDS — Inguinal hernia repair, male infertility, infertility obstructive azoospermia

INTRODUCTION

Hernias of the anterior abdominal wall are the most common human pathology that requires surgical treatment [2, 5, 7]. Every year more than 600 thousand operations on hernia reconstruction are performed in Russia.

According to the latest recommendations of the Guideline modern hernia repair involves the utilization of synthetic grafts to strengthen the posterior walls of the inguinal canal [8]. The effectiveness of mesh implants in the surgical treatment of inguinal hernias has now been proven and is due to a decrease in the frequency of hernia recurrence to an average of 4% [2, 5]. For this reason, today the effectiveness of surgical treatment of inguinal hernias is evaluated not only by medical indicators (relapse, hematoma, seroma, etc.), but also by social criterias – indicators of life quality after surgery [2, 5]. Most recent publications are devoted to the formation of pain syndrome in patients during the postoperative period in the area of operation, of different intensity and duration [1, 8].

Only a few articles indicate a change in reproductive function after inguinal hernia repair [7]. Animal studies have shown that non-tension inguinal hernia repair results in obstruction of vas deferens and impaired spermatogenesis in the testicle [3]. Currently, in Russia there are no reliable statistical indicators of the prevalence of male infertility in patients undergoing hernia repair in the groin region.

Aim: to evaluate the prevalence of patospermia in men after inguinal hernia repair.

METHODS

The study was retrospective. We've analyzed the medical documents of 472 men who went to the andrologist with complaints about the absence of pregnancy of the wife during the 2017. Inclusion criteria: changes in the spermogram, age 18-50 years, agreement with the processing of personal data. Exclusion criteria: varicocele, hydrocele, testicular tumor, changes in karyotype.

We evaluated the presence/absence of inguinal hernia repair in history, the duration of the postoperative period, the duration and nature of infertility, the main parameters of the spermogram (according to who criteria, 2010): the volume of ejaculate, the concentration of germ cells, the percentage of total mobile (category a+b), active mobile (category a) and normal morphological forms of sperm in all patients.

The statistical analysis was performed using spreadsheets "EXCEL" and "STATISTICA 6.0". The significance of differences between quantitative indicators was assessed using the Mann–Whitney test. Differences were considered significant at $p < 0.05$.

RESULTS

The age of all men was 32.4+8.5 years. According to medical records, 67(14.1%) men had a history of inguinal hernia repair. The duration of infertility in these patients was 3.6+1.2 years. Right-sided inguinal hernia was diagnosed in 35(52,2%) patients, left-sided — in 26(38,8%); double-sided in 6(8,9%). In men who had previously had inguinal hernia repair, obstructive form of infertility was revealed more often — 38(56.7%). Sperm counts are presented in Table 1.

The dependence of sperm parameters on the side of surgery is shown on the Fig. 1. After herniation on

Table 1. *Indicators of ejaculate in men with inguinal hernia repair in history*

Indicators of ejaculate	Patients after inguinal hernia repair (n=67)	WHO, 2010	P value
Semen volume (ml)	2,1+0,8	>1,5	p>0,05
Sperm concentration (million/ml)	9,3+2,7	>15	p<0,05
Progressive motility (a) %	19+8,5	> 32%	p<0,05
Total motility (a+b) %	23,6+6,2	>40%	p>0,05
Morphology (normal form %)	1,4+0,8	>4%	p<0,05
Leukocytes (million/ml)	0,5+0,3	<1	p>0,05
MAR-test (%)	12+6	<50	p>0,05

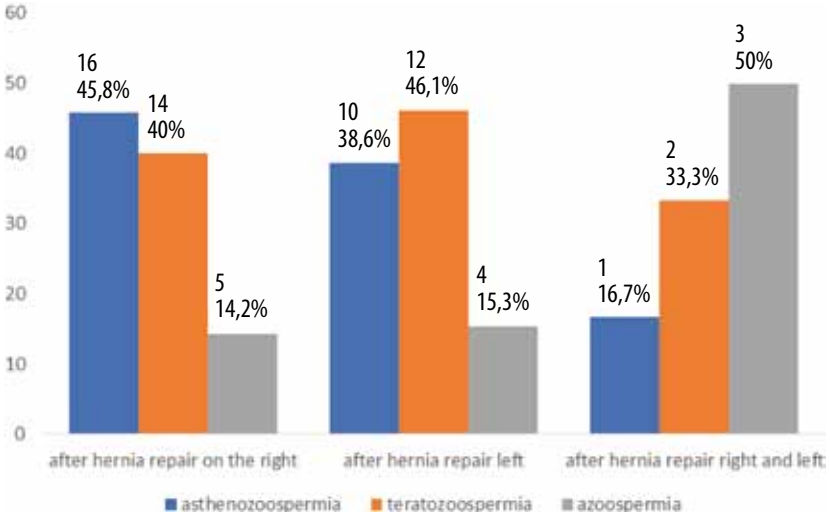

Figure 1. *The dependence of sperm parameters on the side of hernia repair is performed (*p<0.05 azoospermia frequency in bilateral hernia repair compared with unilateral).*

both sides, the parameters of the ejaculate were significantly worse in patients, in comparison with the performed hernia repair on the right or left sides (p<0.05). There was no significant difference in the criterion of mobility and the number of normal morphological forms of sperm between patients with inguinal hernia repair on the right and left sides (p >0.05).

DISCUSSION

The modern methods of surgical treatment of hernias, including inguinal hernias, involves the operation using a synthetic implant (allogernioplasty), for prosthetics of functionally incompetent anatomical structures of the posterior wall of the inguinal canal [5, 8]. The question of the effect of inguinal hernia repair on spermatogenesis is still under discussion among specialists. The results of a large epidemiological study (n=34 267) for ten years in Sweden show that male infertility develops only in 0.7% of men after unilateral inguinal hernia repair [2]. However, the authors recognize that patients with bilateral hernia have an increased risk of infertility almost 5 times [2]. At the same time, the results of another randomized study showed that men after non-tension hernia repair had a tendency to reduce the concentration of sperm (p<0.05) [9]. Study by Khodari M. et al. herniation in the inguinal region is shown to cause obstructive azoospermia in 7.8% of cases [4]. According to Maciel L. C. et al. inguinal hernia repair is an operation that presents a potential risk of iatrogenic lesions of the vas deferens, especially in recurrent hernias.

Our study showed that after surgical treatment of inguinal hernia male infertility develops in every 7 men. We believe that the cause of pathospermia in men after inguinal non-tension hernia repair is edema of the spermatic cord, orchitis, inflammation of local tissues, which leads in most cases to obstruction of the vas deferens. Violation of spermatogenesis in men is global in Russia and around the world. Therefore, men of reproductive age after surgery in the groin need dynamic control of the spermogram and the supervision of not only the surgeon but also the andrologist.

CONCLUSIONS

Inguinal non-tension hernia repair is a risk factor for male infertility in 14.1% of men of reproductive age. After inguinal hernia repair the pathospermia in patients persists for more than 3 years (which means that not only in the early but also in the late postoperative periods). After inguinal hernia repair the obstructive form of male infertility prevails. The most severe abnormalities in the semen analysis develops after bilateral hernia repair.

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DIAGNOSIS OF GENETIC FORMS OF AZOOSPERMIA

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ABSTRACT — Aim: To identify the main genetic causes of azoospermia.
Methods. The study included 92 patients with azoospermia. In all patients we carried out genetic tests — karyotyping, PCR-diagnosis of blood.
Results. Genetic disorders were found in 35 (38%) men. Of these, the majority of men were with Kleinfelter syndrome — 21 (60%) and deletions in the AZF regions of the Y chromosome — 11 (31.4%).
Conclusions. Patients who have genetic abnormalities should receive comprehensive medical and genetic advice.

KEYWORDS — Male infertility, azoospermia, chromosomal abnormalities, Kleinfelter syndrome, AZF microdeletions

INTRODUCTION

Reduced sperm fertility occurs on average in 7% of men in the population [2, 7]. As a rule, laboratory and instrumental signs of male infertility are semen disorders that are quantitative and qualitative [2]. Male infertility is a polyetiological disease [1], which may be due to genetic factors. Genetic abnormalities are available azoospermia in 30-50% of patients [5, 6, 8].

It is known that there are several genetic causes of male infertility: chromosomal disruptions (Robertson translocations, structural changes in the karyotype, inversions); abnormal number of chromosomes (chromosomal disomy, development of Klinefelter syndrome); the appearance of fallen chromosomal sites (deletions) in the AZF locus of the Y chromosome; microdeletions and point mutations in the androgen receptor (AR) gene; abnormalities in the structure of sex chromosomes (local mutations) [3, 4].

Aim.

Identify the main genetic causes of azoospermia.

METHODS

The study included 92 male patients. All patients were aged 36±9 years. The study was carried out in accordance with the standards of good Clinical Practice (Good Clinical Practice) and the principles of the Helsinki Declaration. Inclusion criteria: absence of sperm in sperm (azoospermia), absence of pregnancy in the spouse for more than 1 year, consent to the processing of personal data. Exclusion criteria: female infertility factor, severe endocrine disorders, varicocele, cryptorchidism, sexually transmitted diseases. All patients underwent genetic examination — karyotyping, multiplex PCR with simultaneous amplification of several DNA fragments. Semen analysis was performed in accordance with WHO recommendations (2010). We determined the level of gonadotropins (follicle stimulating hormone (FSH) and luteinizing hormone (LH)) and testicular volume in all patients with genetic disorders. The examination of patients was performed using the test system Becman Coulter, Diagnostic System Laboratories (USA)

The statistical analysis was performed using spreadsheets "EXCEL" and "STATISTICA 6.0". The significance of differences between quantitative indicators was assessed using the Mann–Whitney test. Differences were considered significant at $p < 0.05$.

RESULTS

According to spermograms, azoospermia was recorded in all patients. We have not identified the genetic causes of the lack of germ cells in the sperm of 57(62%) men, and regarded these cases as idiopathic male infertility. The remaining 35 (38%) men had various genetic disorders. Of these, the majority were men with Klinefelter syndrome — 21 (60%). Microdeletions in the AZF regions of the Y chromosome were in 11 (31.4%) patients. The results of the genetic and clinical analysis (n = 36) of men with azoospermia are presented in Table 1. The average FSH level in men with genetically determined azoospermia of patients was 20.9±0.8 mIU/ml (Table 1). The average testicular volume was 10.25±1.2 ml. Those, these were patients with primary hypergonadotropic hypogonadism.

We recorded the highest fluctuations in FSH (21.2+1.4 IU/ml) in patients with Kleinfelter syndrome, and figure 1 shows a negative relationship between FSH levels and testicular volume in these patients.

Table 1. *The distribution of patients with azoospermia with a registered genetic pathology (n = 35)*

Results of genetic analysis		Number of patients n(%)	testicular volume (ml)	FSH level (IU/ml)
Kleinfelter syndrome (n=21)	47XXY	11 (31,4%)	8,2+0,8*	24,2+2,8*
	47XYY	4(11,4%)	10,4+0,6*	21,5+1,9*
	46XY(mosaicism)	6 (17,1%)	12+1,3*	18,1+2,1*
deletions in AZF regions (n=11)	AZFa	2(5,7%)	6,2+1,2*	24,8+2,4*
	AZFb	6(17,1)	7,0+1,4*	23,0+2,9*
	AZFc	3(8,5%)	11,0+0,9*	19,2+2,7
CFTR (Cystic fibrosis transmembrane conductance regulator)		2 (5,7%)	18,2+2,2	14,1+2,5*
AR (mutations in the androgen receptor gene)		1(2,8%)	9,0+1,4*	22,8+3,0*

** at p <0.05 when comparing testicular volume and FSH level.*

Fig. 1. *The dependence of FSH level and testicular volume in the Klinefelter syndrome*

DISCUSSION

Diagnosis of genetic abnormalities is extremely important in the examination of patients with male infertility in order to assess the success of assisted reproductive technologies (ART) [2, 7]. Syndromic genetic mutations are often the cause of azoospermia. The syndromic forms of pathospermia have a characteristic phenotype, so the recognition of this disease is not difficult even at the stage of primary diagnosis of male infertility. However, with microstructural genetic rearrangements, for example, with Kleinfelter syndrome (mosaic form), primary diagnosis is difficult because the phenotypic visualization of the patient is complicated [4]. Therefore, medical and genetic consultation in azoospermia should be performed necessarily to each patient.

Microdeletions of the Y chromosome are the cause of azoospermia in 15% of cases [7]. Identification of sub-regions of microdels of AZF region of the Y chromosome allows to evaluate personal prospects of treatment of male infertility, as microdeletions of AZFa, AZFb or AZFa/b are associated with the worst prognosis of testicular sperm production for ART programs [4].

In our study, most of the genetic abnormalities associated with azoospermia were represented by Kleinfelter's syndrome — 21(22.8%). Deletions in the region of the AZF of the Y-chromosome gene were detected in 11(11.9%) people. We believe that the indications for genetic research in male infertility are: azoospermia; in the presence of several unsuccessful attempts of art; in the case of habitual miscarriage of the fetus in the first trimester of pregnancy; in the presence of family relatives with congenital defects.

CONCLUSIONS

Genetic male infertility is associated with mutation of chromosomes, change in their number and violation of the structure. In men with severe infertility (azoospermia) genetic abnormalities occur in every third case.

Patients who are suspected of having genetic abnormalities should receive a comprehensive genetic examination before selecting assisted reproductive techniques that can reduce the potential risk of transmission of genetic aberrations to offspring. Given that there is a high percentage of men with idiopathic azoospermia, for this category of patients it is necessary to conduct additional genomic research (exoma sequencing and chromosomal micromatric analysis).

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HISTOMORPHOLOGICAL CHANGES IN THE KIDNEYS IN CASE OF CLOZAPINE POISONING

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INTRODUCTION

Poisoning is one of the most important issues of forensic medicine. Clozapine is an example of so called *atypical* neuroleptics [1]. The therapeutic threshold of this drug is comparatively narrow. A single therapeutic dose of this drug is 50–200 mg, the highest daily dose is 900 mg., whereas a fatal dose for an adult is about 2 g [2]. Clozapine is widely used in clinical practice to treat acute and chronic forms of schizophrenia, psychosis, manic conditions, bipolar disorders, aggressiveness and others [3]. More than one million people in more than 60 countries of the world undergo treatment with clozapine annually. All the facts mentioned above determine a high risk of clozapine poisoning. The number of criminal clozapine intoxications in Russia also remains high. Such poisonings are characterized by severe symptoms and high mortality.

The objectives of the study:
to estimate in the experiments on laboratory rats histomorphological changes in the kidneys in acute clozapine poisonings 3 and 24 hours after the intoxication.

MATERIALS AND METHODS

We performed a comparative study of histological sections of the kidneys of outbreed male rats weighing 290–350 g. Group 1 and 2 included 5 rats treated with clozapine oral dose (150 mg/kg) and euthanized 3 and 24 hours after drug administration, respectively. Control group included 5 intact rats.
The concentration of clozapine and its main metabolites was measured using high performance liquid chromatography method with mass spectrograph detection (HPLC-MS/MS) method.

RESULTS

No pathological changes were detected in the group of comparison. 3 hours after clozapine administration a slight expansion of the lumen of the capsules was detected in the glomeruli of the kidneys. The nuclei of some epithelial cells of the convoluted tubule of kidneys were not stained. There were some visible small vacuoles in the cytoplasm. Venous plethora was observed. The tubules of the medullary area were moderately dilated. The nuclei of epithelial cells of the tubules of the medullary area were round, mainly normochromic. There were cells with hyperchromic and hypochromic nuclei. There were small hemorrhages in the cortical layer.

Some protein was present in the tubules. 24 hours after the clozapine administration the nuclei of the cells were not stained. There was protein in the lumen of the tubules. On the border of the medullary area and medullary layer the vessels were moderately full-blooded, focal hemorrhages are observed. Some tubules in the brain layer are expanded. In case of treatment with clozapine the clozapine and the norclozapine concentration in kidney homogenate was 2,8 times higher and 5 times lower as compared with 3 hours, respectively. The concentration of clozapine-N-oxide slightly decreased.

CONCLUSION

In case of clozapine poisoning, a complex of severe morphological changes in the kidneys was observed. These signs along with the results of histological studies of other internal organs and chemical studies and can be used to evaluate the severity of the intoxication and the exact time of the poisonings.

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VALIDATION OF SIMULTANEOUS ASSAY OF CLOZAPINE AND ITS MAIN METABOLITES IN BLOOD SERUM USING HPLC-MS/MS METHOD

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INTRODUCTION

Clozapine is an atypical antipsychotic drug. It is frequently used in clinical practice. The number of criminal clozapine poisonings remains high. Clozapine is metabolized in the liver. Its main metabolites are norclozapine and clozapine-N-oxide [1]. According to the literature these metabolites have their own toxic effect [2]. However there is no validated method of simultaneous assay of the substances.

The aim of the study is to carry out the validation of simultaneous assay of clozapine and its main metabolites — norclozapine and clozapine-N-oxide — in blood serum using HPLC-MS/MS method. We represented calibration curves for clozapine and its main metabolites. We assessed the following parameters: selectivity, precision, accuracy, matrix effect, lower limit of quantification [3].

MATERIALS AND METHODS

The validation was carried out using chromatograph Agilent Technologies 430 Triple Quad LC/MS (Germany). We used PC programme Agilent Mass Hunter Workstation for series tripple Quadrapole vers. B06.00 build 6.0.6.25.4sp4 to represent chromatograms and Agilent Mass Hunter Quantitive Analysis vers. B 07.00 build 7.0.457.0 to proceed them.

RESULTS

Calibration curve. We used six levels of concentration for clozapine. The range of concentrations was from 0,125 to 5 μ/ml. Relative standard deviation (RSD) for used clozapine levels did not exceed 15%, accuracy was between 88,15% and 109,51%. We used seven levels of concentration for norclozapine. The range of concentration was from 0,0075 до 7,5 μ/ml. Relative standard deviation (RSD) for used norclozapine levels did not exceed 15%, accuracy was between 80,13% (concentration level 1) and 106,51%. We used five levels of concentration for clozapine-N-oxide. The range of concentration was 0,03–3,00 μ/ml. Relative standard deviation (RSD) for used levels of clozapine-N-oxide did not exceed 15%, accuracy was between 81,20% and 98,30%.

Precision and accuracy. Precision and accuracy for QC samples should not exceed 15% except the one for the LLOQ which should not exceed 20%. We assessed the precision and accuracy 3 times in 4 QC samples QC_1, QC_2, QC _3, QC_4 at the expected range of concentrations. All the figures we obtained met the criteria.

Lower limit of quantification. The lower limit of quantification (LLOQ) is the lowest concentration of analyte in a sample which can be quantified reliably, with an acceptable accuracy and precision. The LLOQ is considered being the lowest calibration standard. The lower limit of quantification we obtained for clozapine was 0,05 μ/ml. The *signal/noice ratio* (S/N) was 0,12. The lower limit of quantification we obtained for norclozapine was 0,0025 μ/ml; S/N was 0,14. The lower limit of quantification we obtained for clozapine-N-oxide was 0,001 μ/ml; S/N was 0,10.

Recovery. The recovery rate for clozapine was measured at concentrations of 5 μ/ml and 0,25 μ/ml, the recovery rate for norclozapine was measured at concentrations of 0,5 μ/ml and 0,1 μ/ml, clozapine-N-oxide — 1 μ/ml and 0,03 μ/ml. The recovery rate for each analyte and inner standard (IS) — amitriptyline — was estimated separately. The recovery rate for clozapine was 86,6%, the recovery rate for IS exceeded 99%, CV<15%. The recovery rate for norclozapine was 63% the recovery rate for IS exceeded 93%, CV<15%. The recovery rate for clozapine-N-oxide was 84% the recovery rate for IS exceeded 97%, CV<15%.

CONCLUSION

The validated method can be used for simultaneous assay of clozapine, norclozapine and clozapine-N-oxide in blood serum.

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A CASE OF METHANE POISONING

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PATIENTS AND METHODS

A case of acute methane intoxication has been studied.

The corpse of Mr. R (38 years old) without signs of violent death was found in a manhole.

RESULTS

During external examination extensive purple lividity located on the back surface of the head, neck, on the upper third of the chest, trunk, upper and lower limbs was observed. There were numerous petechial hemorrhages on the cheeks and chin. There were also multiple petechial hemorrhages in the connective membranes of the eyes.

Autopsy was performed. The myocardium on the section was greyish-brown, flabby and dull. There were areas of uneven blood-filling. The walls of the coronary arteries were thickened. The walls of the interventricular branch of the left coronary artery was circularly thickened, the lumen in the middle third was narrowed to 10% due to the presence of fibrous plaques.

A differential diagnostics between cardiac death and methane intoxication was needed. The rate of death was estimated according to the scale proposed by Dmitriy V. Bogomolov and Vladimir A. Putintsev [1, 2].

In this case, the rate of death can be described as fulminant (duration up to 30 minutes).

This was confirmed by the following signs: extremely mild severity of brain edema, detected macroscopically (significance index 0,29), fresh hemorrhages in the parenchyma of the lungs and brain (significance index 0,16), plethora of capillaries of internal organs, particularly into lungs and kidney cortex (significance index 0,19), the absence of the so-called signs of shock hemodynamics (significance index 0,24), absence of disseminated intravascular coagulation, or DIC syndrome (complete absence of microthrombi or their presence only within the body) and respiratory distress syndrome (index of importance to 0,19). Total significance index: 1,07 [1, 2].

Histological study, including histological study of the heart and the lungs. was performed. The results of histological study of the myocardium were as follows: "... secondary cardiomyopathy with diffuse goitriferous cardiosclerosis, focal stromal lipomatosis with acute degenerative lesions of the myocardium. Atherofibrosis of coronary arteries". There were no areas of fragmentation of cardiomiocytes, which is typical for cardiovascular death.

The results of histological examination of the lungs were as follows: "...areas of emphysema". Chemical analysis (gas chromatography) was performed. Methane was found in the blood and internal organs.

CONCLUSION

The forensic medical diagnosis was "methane intoxication", which was confirmed by the results of histological and forensic chemical study. Methane was revealed.

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OPTIMIZATION OF DENTAL IMPLANTATION COMBINED WITH CLOSED SINUS LIFT IN PATIENTS WITH LOW MAXILLARY SINUS FLOOR

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ABSTRACT — The experiment carried out on autopsied specimens, helped develop a model of minimally invasive surgical intervention in the upper jaw. Using the developed and tested dental implant allowed its primary fixation, and also, without using complex devices, it allowed introducing osteogenic material under the Schneider membrane dome, evenly distributing it around the implant. The development and implementation of the proposed implants that can ensure sufficient primary stabilization with immediate sinus lift will allow maximum use of the patient's own bone, which was preserved to a minimum extent.

KEYWORDS — dental implantation, sinus lift, osteogenic materials, dental implants

INTRODUCTION

One of the issues that dental surgeons have to face when it comes to dental implantation in the distal upper jaw areas is insufficient bone quantity and quality [20–31]. The recent years have witnessed wide implementation of a method that allows increasing the volume of bone tissue at the alveolar bone in the maxillary sinus bottom area, the sinus lift surgery [1–3, 5, 7, 8, 10–12, 14, 15, 17]. However, dentists often experience failures while performing such surgeries, including damaging the maxillary sinus mucous membrane; unsuccessful choice of osteoplastic material for the development of sufficient volume; infection in the sinus followed with serous or purulent sinusitis; migration of implants into the sinus cavity; oroantral fistula. Resorption of the introduced material or its replacement with connective tissue without bone tissue development can often be observed [1, 4, 7–9, 11, 19].

Recent publications offer various modifications of the surgery aimed at eliminating such complications. Controlled detachment and elevation of the maxillary sinus mucous membrane followed by the introduction of the required amount of augment with minimal operative injury can be reached through using various methods of "closed" ("soft", "balloon", etc.) sinus lift. However, all of them are associated with bone injury, and most of the protocol is performed "blindly" [3, 6, 8, 10, 12, 13, 15–18]. An implant installed in a single step, due to poor primary stabilization, can get mobile and start migrating, which will increase the rehabilitation time. There is no guarantee of the augmentation optimal distribution, as well as there are no clear indications for carrying out this or any other type of intervention, depending on the anatomical and clinical context [3, 9, 12, 13, 19].

On the other hand, closed surgical intervention (without making an opening on the maxillary sinus anterior wall) which is definitely less traumatic and allows narrowing the surgical field to a minimum, still implies performing this operation blindly, while inserting the graft into the maxillary sinus, in turn, presents another issue, both in view of the plastic specifics and the introduction method, which complicates the surgery [3, 10, 11, 14, 16, 17, 32–40]. The methods and models of implants we propose may help solve issues that dental surgeons face when using methods of closed sinus lift, as well as expand indications for single-stage implant placement, which helps to reduce the time needed for rehabilitation. Therefore, dental implantation in the upper jaw in case of significant atrophy of the alveolar process has not been fully studied yet, while the currently proposed methods of closed sinus lift with single-stage implantation are not always sufficient to solve the said issues.

Aim of study:
development and experimental approval for closed sinus lift with single-stage implantation in patients with a low maxillary sinus floor in order to increase the efficiency and to reduce the duration of dental treatment.

MATERIALS AND METHODS

The team of authors developed dental implants models (Patents RU 2395249, RU 155741), and a dental implantation method in case of significant atrophy of the alveolar process in the upper jaw (Patent RU 26000150. 2016); besides, for clinical purposes an intraosseous self-tapping construction was designed, which simplifies the surgery protocol (Patent RU 182012. 2018).

For bioethical considerations, prior to proposing a method for clinical use, it was important to prove experimentally that the design would ensure primary stability for the implant, and that it would also perform successfully the role of an osteoplastic material conductor with the development of a uniformly filled dome made up by the Schneider membrane and the maxillary sinus bottom.

The dental implant we developed contains an intraosseous element shaped like a hollow cylinder that has a thread on the outside for fixation in the bone, and an internal thread to connect with the internal element thread. The inner element is designed as a cylindrical rod with a thread on the outer surface and a hemispherical base; it has an axial through hole for the cannula, whereas the hole is connected to several evenly spaced holes in the hemispherical base of the inner element to pass the osteopathic gel into the sinus cavity and subsequent bone tissue germination into the holes, while the inner element has a measuring scale or marks inside its mouth. The inner element of the proposed design easily perforates the maxillary sinus bottom and exfoliates the mucous membrane. The locking element prevents the sinus perforation by the inner element. The invention allowed simplifying and reducing the invasiveness of dental implantation with significant atrophy of the maxillary alveolar process, increasing the bone tissue by 5–7 mm, enhancing the implant attachment reliability in the maxillary bone tissue.

To develop the installation technique of such implants, we carried out an experiment at the Department of Forensic Medicine, Samara State Medical University. We divided the whole operation into two stages: at the first stage we set a goal — to obtain primary fixation of the intraosseous element while not perforating the bottom of the maxillary sinus (Fig. 1).

The second stage implied creating sufficient space to introduce the osteogenic material bounded by the mucous membrane of the maxillary sinus and its bottom (Fig. 2).

Further, the location of the implant was identified and marked with a tapered drill to place the main hole. The major drill formed a hole, the stop (determining the distance to the sinus bottom) located on the drill did not allow the bottom to be perforated through to the sinus. An implant driver was used to screw-drive the intraosseous element all the way (Fig. 3).

For visual control, a "window" was made on the vestibular wall of the sinus, which allowed controlling the experiment (Fig. 4).

Then, using a special implanter, following the intraosseous element's inner thread, the inner element was screwed in up to the stop. Further, the movement went on until the typical feeling of the bone floor perforation in the maxillary sinus. Slow introduction of the inner element raised the mucous membrane of the sinus cavity bottom, while the degree of risk was assessed after each rise through the localization of the inner element's intraoral part (Fig. 4). These measures resulted in a Schneider membrane dome following (Fig. 2). Next, the implant driver was released, and using a special cannula, 0.3–0.5 ml of an osteogenic simulator (for clarity, we prepared an osteoplastic material simulator — an osteogenic Polistom gel, stained with a caries detector) was pressed in. Therefore, the inner element of the designed dental implant was installed before creating a 5–7 mm high dome filled with an osteoplastic material simulator (Fig. 5).

Subsequently, through the "window" we visualized the location of the osteogenic gel, which was evenly distributed in all areas, and filled the entire submembrane space (Fig.6).

RESULTS AND DISCUSSION

We carried out 8 experimental surgeries on both the right and left sides of the upper jaw at the chewing teeth area. In seven cases, primary fixation of the intraosseous element was achieved (in one case, the intraosseous element fell into the maxillary sinus). In all the cases, perforating the maxillary sinus bottom with an internal element and exfoliating the mucous membrane presented no issue. In the all cases, the gel easily penetrated into the cavity shaped by the Schneider membrane dome and the maxillary sinus bottom, and filled the said cavity; moreover, it enhanced its volume.

CONCLUSION

The experimental studies suggest that the proposed method of closed sinus lift with a single-stage implantation in patients with a low maxillary sinus bottom (less than 7–8 mm), helps expand the range of indications for dental implantation. The proposed implant models and operational benefit reduce significantly the risk of developing uncontrolled complications. The development and implementation of individual implants that can ensure primary stabilization with a single-stage sinus lift will allow maximum use of

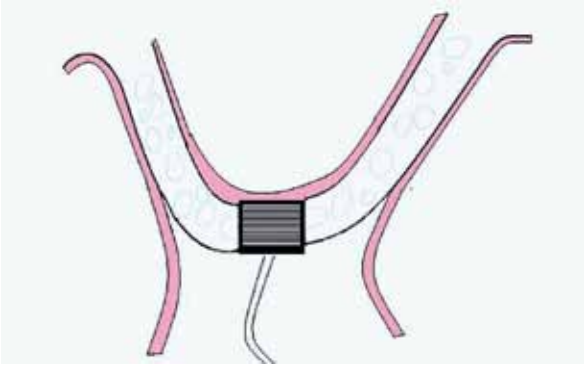

Fig. 1. *Installation of the intraosseous element of the proposed dental implant without perforation of the maxillary sinus bottom*

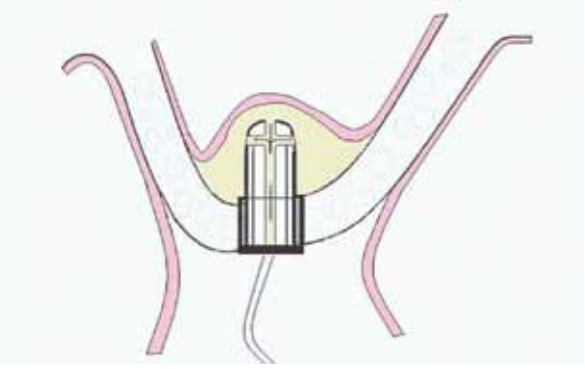

Fig. 2. *Installation diagram of the proposed implant's inner part and the path of movement for the osteopathic material*

Fig. 3. *Installing the intraosseous part of the implant*

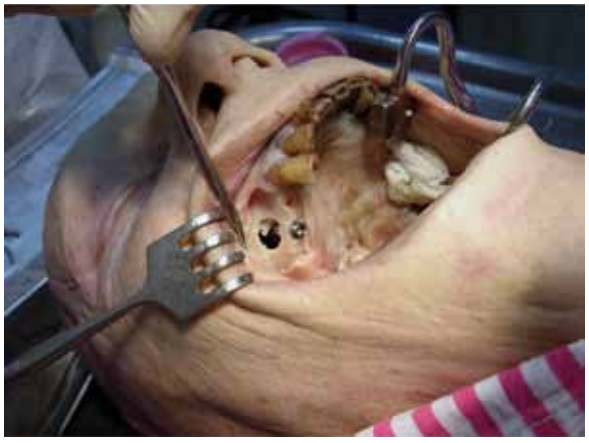

Fig. 4. *Visualization of the osteoplastic material pathway using a "window" on the maxillary sinus vestibular wall*

Fig. 5. *Visualization of the osteoplastic material simulator pathway*

Fig. 6. *The osteoplastic material fills the entire cavity shaped by the Schneider membrane dome and the sinus bottom*

the patient's own bone, which has been preserved, even though to a minimum extent.

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POTENTIAL INTRODUCTION OF CELL TECHNOLOGIES TO IMPROVE DENTAL IMPLANT SURFACE PREPARING

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ABSTRACT — An in vitro comparative analysis was carried out focusing on biological compatibility of an uncoated titanium dental implant surface, and hydroxyapatite spray-coated titanium implant, using osteoblasts cultures and dermal human fibroblasts. Cell identification implied morphological and biochemical methods as well as flow cytometry. The biological compatibility of the samples was studied through a direct contact under conditions identical to cell cultivation. The obtained outcomes suggest that hydroxyapatite applied through plasma spraying has a certain cytotoxic effect; therefore using bioceramics-coated implants in surgical dentistry will not contribute to osseointegration at the molecular and cellular levels.

KEYWORDS — dental implant; hydroxyapatite; cell and tissue technologies; medical titanium; cell cultivation; biocompatibility; tissue bioengineering

INTRODUCTION

Successful integration of implants and grafts into a tissue environment is the major requirement for restorative medicine. Employing cell and tissue technologies appears reasonable at the stages of biological association and functioning not only for the host tissues, yet also for the integrated foreign materials. Achieving long-term equilibrium in adaptive responses between the tissue and the implanted material (of synthetic, natural and biological origin) is the most important task that tissue engineering is facing. This issue is relevant in medicine, especially in areas like Dentistry and Maxillofacial Surgery, due to a great need for artificial bone-replacing materials, a need to restore not the anatomical features only, yet also the chewing function, using dental implants. These technologies are expected to increase significantly the effectiveness of traditional treatments [1–13].

The aim of osteoplasty, along with dental implant installation, is the integration of artificial materials with the tissue and long-term functioning of the entire set. The basic material used to manufacture dental implants is medical titanium, since oxide compounds on its surface contribute to the fixation and functioning of morphogenetic proteins and blood proteins involved in creating and restructuring the bone tissue [14–18].

The available published results of studies focusing on potential use of cell technologies to improve the direct and delayed dental implantation are sporadic and of fragmented nature. Experts note that optimizing osseointegration through dental implantation is extremely important for cases concerning facial aesthetics restoration and dental function where the front set of teeth is lacking, in people belonging to older age groups; in case of diabetes mellitus as well as in case of other somatic diseases that affect bone regeneration and require cell technologies [19–31].

Given the current stage of development in this new area and the state of the respective legislation, working on this issue in dentistry and microbiology can be carried out only under laboratory conditions and in experiment.

Respective literature offers numerous items by implant developers, who propose various ways to increase the materials' surface porosity aimed to improve osseointegration. Of these methods, not all ensure a positive effect on reparation, thus implying they will take a more detailed research not only from a mechanical or pharmacological point of view, yet also from the stance of living tissue response, i.e. biocompatibility. This makes finding the optimal option a harder process, which hampers introduction of innovation-based solution into practical healthcare [32–34, 39–41], whereas the ultimate goal of such solutions would be obtaining results that could ensure full reparative regeneration processes, thus bringing about mechanical integrity of the connection between the implant and the bone. At the moment, there is a stable opinion

stating that bone development on the implant surface implies two types of bone formation processes — contact osseogenesis, which is on the implant surface, and distant osseogenesis, i.e. from the maternal bone. These mechanisms feature their own specifics, but they share common cellular processes including proliferation and osseogenic differentiation of their own mesenchymal stem cells; the formation of osteoblastic cells that shape the bone, which is to undergo rearrangement later on.

Some experts believe that the contact and mechanical connection of the titanium surface — bone interface is of key importance to the morphology of the implant surface. Developing a strong connection with the bone tissue takes a certain biological activity from the implant surface structure, apart from its well-studied properties. The available scientific literature claim there are various options proposed to resolve the issues within the implant surface — living cell system. Thus study implied investigating the systems for implant surface preparing with hydroxyapatite, which is used widely in dentistry, yet remains questionable concerning its clinical implementation [32–37, 40].

Aim of study:
to develop and offer an in vitro-proven explanation for a technology of safe preparing of implants surface, which is to be done through studying the biocompatibility with human tissues, involving cell technologies.

MATERIALS AND METHODS

To test the biological compatibility and tropism for various types of cells, we obtained titanium samples from the Research Company Plasma Povolzhya (Saratov, Russia): Sample 1 — with neither coating nor sandblasting; Sample 2 — with titanium + hydroxyapatite (HA) coating. Osteoblast cultures and human dermal fibroblasts were used as the test systems. A total of 18 million dermal fibroblasts and 18 million fibroblast-like osseogenic cells were grown to be used in further research. At passage 4, cells were identified using morphological, biochemical methods as well as flow cytometry. The biological compatibility was studied through a direct contact in Petri plates under similar conditions for cell cultivation. The test system used cells of passages 4–8. The Petri plates with cultures with no samples were used as test systems; those were passaged and observed along with the experimental ones. At plating, the dosage in all cases was 20 thousand cells/cm^2 ($2 \cdot 10^4$). The interaction between the object and the test system remained under observation for 7 days. A total of 60 cups were examined. After the observation period, histological preparations of cell cultures were made. In unfixed monolayer cells, neutral fat was detected with sudan IV and hematoxylin. The stained preparations were studied and photographed using an automatic analytical system including an Olympus CX 21 microscope, an Olympus C-4000 ZOOM digital camera, and a system unit based on an Intel Pentium 4 processor. A total of 200 preparations were studied.

RESULTS AND DISCUSSION

Biocompatibility examination for Sample 1, titanium. A day later, both in the control and experimental plates, using an inverted microscope, a uniform monolayer was identified, where cells had structural features typical of dermal fibroblasts, and a growth pattern in close proximity of the sample (Figure 1a). In the remote area, no change in the culture growth was observed either (Table 1).

After 4 days, the cells, both in the control and experimental cups, still failed to reach monolayer density (Figure 1b), while the cells still retained their morphological and functional features both close to the sample and in the remote area.

A complete monolayer developed both in the control and in the experiment on Day 7. When stained with hematoxylin and sudan IV, the experiment fibroblasts were observed to retain structural features typical of healthy cells. The cell cytoplasm was oxyphilic and appeared to be homogeneous. The nuclei were of correct shape with smooth membranes. Neutral fat in fibroblasts was not detected (Figure 2a). Biocompatibility examination for Sample 2, HA coated titanium. In this study, the material did not affect the fibroblasts adhesive capacity in the culture. Observing the native culture through its dynamics revealed that 2 hours after transfer most of the cells adhered to the culture plastic and spread on it. After one day, the fibroblasts were expanded, joined their processes to each other, and began to develop a monolayer both close the sample (Figure 2b) and in the remote area. Fibroblasts, in their morpho-functional features, did not differ from cells located near the sample (Table 2).

On Day 4, the monolayer density increased slightly, both near the sample and in the remote area (Figure 3a). Cells were located in different directions. On Day 7, the situation was not much different from that of Day 4. On Day 7 of the experiment, when staining fibroblasts with hematoxylin + sudan IV, an increase was observed in the number of processes that got intertwined. The cytoplasm of the cells was oxyphilic, homogeneous. The cytoplasm of single cells contained vacuoles. The nuclei were correct oval shape. Neutral fat was not detected (Figure 3b).

Given the above, the study involving the dermal fibroblasts culture showed no visible response to the

samples, which suggests that the samples in question are inert for this group of cells.

Observation of the native culture of osseogenic fibroblast-like cells (Series 2, Sample 1) revealed that 2 hours into the transfer, most of the cells stuck to the culture plastic, and after 1 day spread on it. The cells began to develop a fairly uniform monolayer both around the periphery of the cup and in close proximity to the sample (Figure 4a). Cells did not differ in structure and growth nature from the control cultures. 4 days into the experiment, complete overgrowth of the cup bottom was observed both near the sample and along the periphery, while in some areas the growth pattern of the monolayer changed — the cell layers were located in different directions (Figure 4b).

The cells in those layers retained the fibroblast-like shape and fit together closely (Table 3).

After 7 days, some areas featured certain thinning of the monolayer if compared to the previous study periods. This was more obvious in close proximity to the sample rather than in remote areas (Figure 5a). The monolayer histological preparations, when stained with hematoxylin and sudan IV through these stages, showed osseogenic cells near the sample, which were located randomly in various directions. The cells changed their shape from fusiform to rhomboid. The processes thickened with their outlines becoming clearly cut. The cytoplasm of the cells was oxyphilic and appeared to be homogeneous. The nuclei were of different size of correct oval shape, with smooth membranes; chromatin had the shape of fine grain, with diffuse location. There were double nucleus cells to be observed. No neutral fat was detected in the cytoplasm (Figure 5b).

When observing the osseogenic fibroblast-like cells culture (Series 2, Sample 2), we could identify that 2 hours into the transfer, a small number of cells stuck to the culture plastic spreading on it. On Day 1, osseogenic fibroblast-like cells began to develop an uneven monolayer, and got connected to one another with their processes (Figure 6a). On Day 4, the number of cells over the entire surface increased slightly; the cells were interconnected with their processes. The monolayer was uneven (Figure 6b).

On Day 7, the number of the cells increased slightly, both near the sample and in remote areas (Table 4).

Cells got interconnected with their shoots. The monolayer was uneven; the cells were located in different directions (Figure 7a). When stained with sudan IV and hematoxylin, the cells in close proximity to the sample were of different shape, with 2–5 processes. The cytoplasm of the cells was oxyphilic and appeared homogeneous; certain cells had vacuoles of various shape and size. The nuclei were of different size, oval- and dumbbell-shaped. Fine-grain chromatin was diffusely located in the nuclei. The nucleoli were of various shape and size, 1 to 4 in the nucleus (Figure 7b).

The outcomes suggest that the experiment with the osseogenic fibroblast-like cells culture responded to the samples through impeded growth. Cells changed their shape from fusiform to rhomboid. There were double nucleus cells observed as well as nuclei of different size, oval and dumbbell-shaped. Some cells had vacuoles of various shape and size. This is indicative of a toxic effect that Sample 2 had on this group of cells.

CONCLUSION

1. The in vitro outcomes revealed that the response manifested by osseogenic fibroblast-like cells to titanium samples with hydroxyapatite coating and sandblasting, as well as to samples with no bioceramic layer was different.

2. Implant surface sandblasting is technologically reasonable, evidence to that being the maximum cell adhesion and a stimulating effect on cell proliferation.

3. No diagnostically significant functional change in the culture of osseogenic fibroblast-like cells was observed after studying with different surface structures of dental implants; while functional cytological differences fell within the normal range. Significant changes in the morphological features of the osseogenic fibroblast-like cells culture mean that hydroxyapatite applied via plasma spraying has a certain cytotoxic effect, which suggests that using bioceramic implants in surgical dentistry would not contribute to osseointegration at the molecular and cellular levels.

4. A comprehensive study of cell behavior and their interaction with tissue microenvironment will certainly lead to an understanding of regeneration activation; comprehensive growth regulation; cell death and differentiation, and in case advanced technologies become available, it would allow creating easy-to-use cell preparations or tissue-engineered structures to restore damaged tissues.

5. An objective study of tissue engineering regeneration capacity, which would allow restoring bone defects in the maxillofacial area, would take consistent preclinical and clinical trials controlled through laboratory and morphological research methods, which, in turn, might allow evaluating the severity and direction of regeneration process in its dynamics.

6. When preparing to introduce cell technologies, it is reasonable to establish the viability of the cells to be used, since a significant outcome can be achieved only with living cells, while using inactivated cells will have a weak stimulating effect.

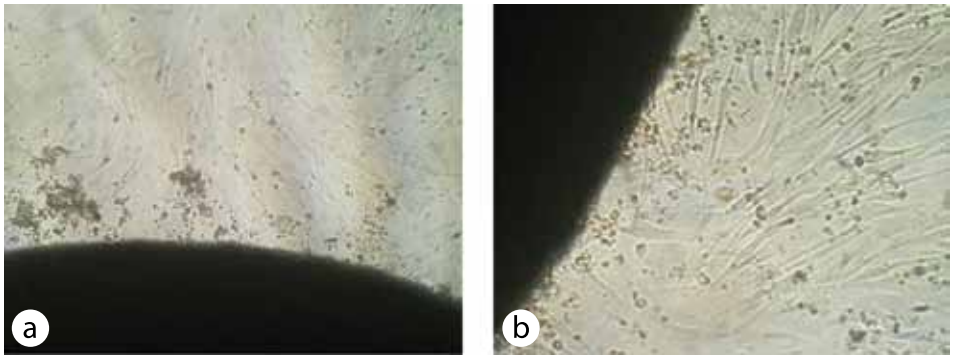

Fig. 1. *a) Sample number 1. Native culture of fibroblasts. 1 day after sowing. fibroblast culture near the placed sample, b) — 4 days of the experiment. Inverted microscope. Magnification 150*

Table 1. *Morphological features of dermal fibroblasts culture*

Control group	Sample # 1	Sample # 2
Spindle-shaped cells	+	+
Number of shoots: 2–4	+	+
Homogeneous cytoplasm	+	+
Clear cell boundaries	+	+
The nuclei are oval in shape, are usually located somewhat eccentric, contain 1–2 nucleoli	+	+
Good adhesion to plastic culture	+	+
Absence of vacuoles	-	+
Lack of neutral fat	-	-

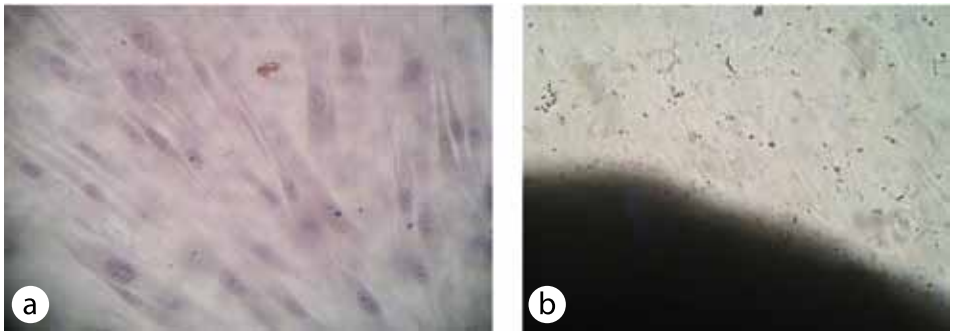

Fig. 2. *a) Sample number 1. Culture of fibroblasts near the placed sample, monolayer. 7 days of the experiment. Coloring sudan IV and hematoxylin. H. 200; b) Sample number 2. Native fibroblast culture in the sample area. 1 day experiment. Inverted microscope. Magnification 100*

Table 2. *Functional features of dermal fibroblasts culture*

Control group	Sample # 1	Sample # 2
After reseeding, most fibroblasts stick to the bottom of the culture dish and flatten it.	+	+
Cells form an incomplete uniform monolayer	+	+
Cells grow uniform monolayer	+	+
The cells reach a saturation density and enter the stationary phase	+	+

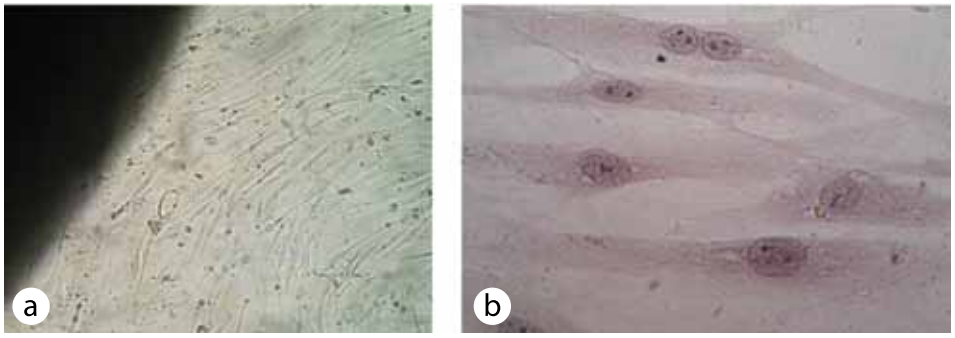

Fig. 3. *a) Sample No. 2. Native fibroblast culture near the sample. 4 day experiment. Inverted microscope. UV 150; b) Sample No. 2. 7 day experiment. Coloring sudan IV and hematoxylin. Magnification 200*

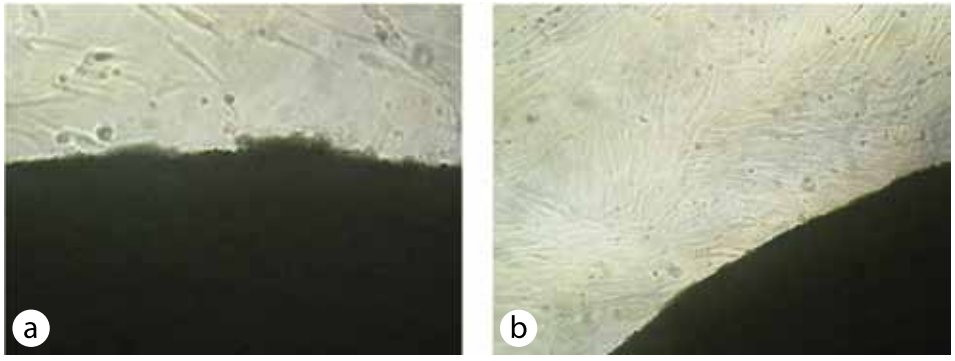

Fig. 4. *a) Sample number 1. Native culture of osteogenic human fibroblast-like cells, 1 day experiment. Cells near the sample. Magnification 200. Inverted microscope; b) Sample No. 1. Native culture of osteogenic human fibroblast-like cells, 4 day experiment. Cells near the sample. Inverted microscope. Magnification 100.*

Table 3. *Morphological features of osseogenic fibroblast-like cells culture*

Control group	Sample # 1	Sample # 2
Elongated cells with 2–5 processes	+	In the vicinity of the sample have a different shape
Homogeneous cytoplasm	+	+
Clear cell boundaries	+	+
The nuclei are oval, usually located in the central zone of the cell bodies, contain 1–2 nucleoli	Change their shape from fusiform to diamond shape. There are dual-core cells	Kernels of different sizes oval and dumbbell shaped
Absence of vacuoles	-	Some cells have vacuoles of various shapes and sizes
Neutral fat is missing	-	-

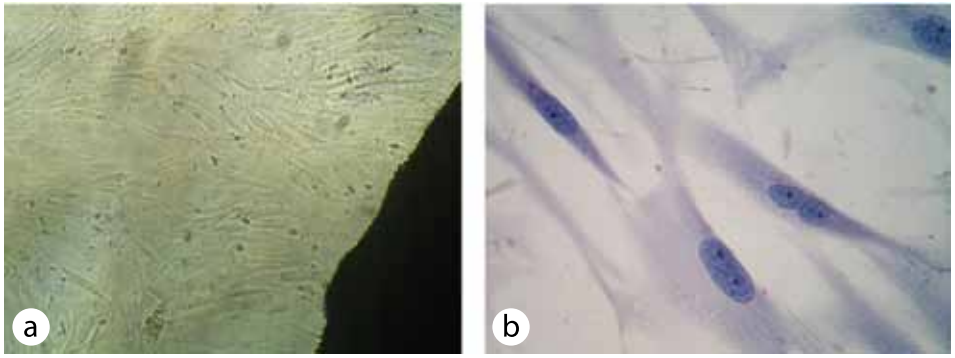

Fig. 5. *a) Sample number 1. Native culture of osteogenic human fibroblast-like cells, 7 days of experiment. Cells near the sample. Inverted microscope. Increase 100; b) Culture of osteogenic human fibroblast-like cells near sample No. 1. Monolayer, 7 experiment. Stained with hematoxylin and sudan IV. Magnification 400.*

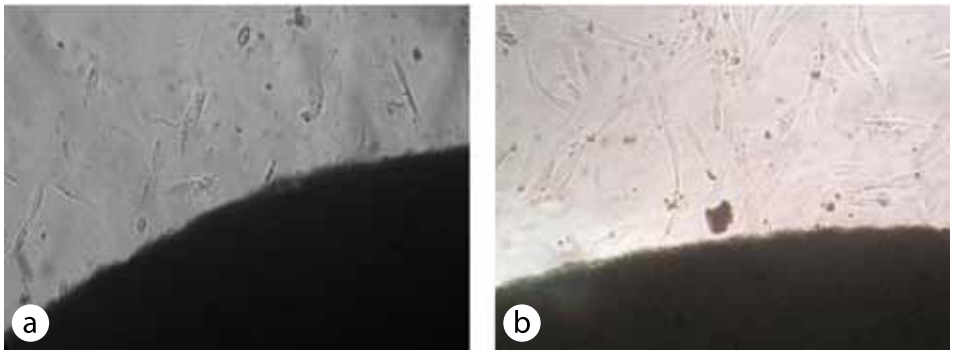

Fig. 6. *a) Sample No. 2. Native culture of osteogenic human fibroblast-like cells, 4 passage. 1 day experiment. Cells near the sample. Inverted microscope. An increase of 200; b) Sample No. 2. Native culture of osteogenic human fibroblast-like cells, 4 days of experiment. Cells near the sample. Inverted microscope. Magnification 200.*

Table 4. *Functional features of osseogenic fibroblast-like cells culture*

Control group	Sample # 1	Sample # 2
Most fibroblasts stick to the bottom of the culture dish and spread over it	+	+
Cells form an incomplete uniform monolayer	+	+
Cells grow uniform monolayer	+	+
The cells form a complete monolayer, mostly in one direction	Some monolayer discharge, more noticeably in the immediate vicinity of the sample	+
Well pronounced adhesion to culture plastic	+	+
The cells reach a saturation density and enter the stationary phase	+	+

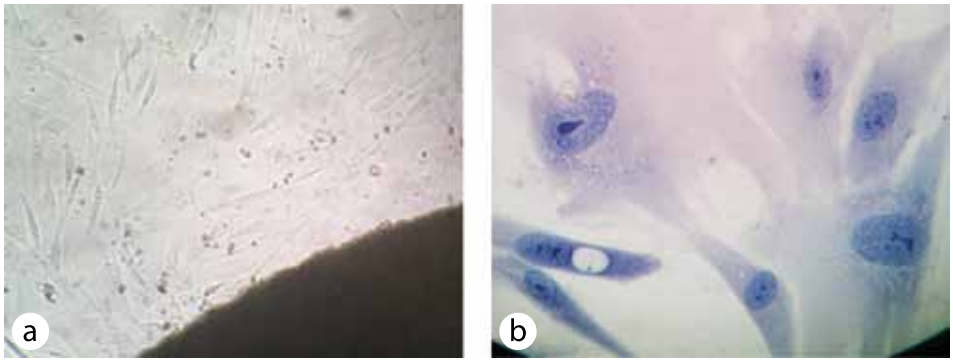

Fig. 7. *a) Sample No. 2. Native culture of osteogenic human fibroblast-like cells, 7th day of the experiment. Cells near the sample. Inverted microscope. An increase of 200; b) Culture of osteogenic human fibroblast cells. Cells near sample No. 2. Monolayer, 7 days of the experiment. Coloring sudan IV and hematoxylin. Magnification 400.*

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GINGIVAL MUCOSA PROLIFERATIVE ACTIVITY AND EPITHELIOCYTES APOPTOSIS INDICATORS IN PATIENTS WITH RAPIDLY PROGRESING PERIODONTITIS

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ABSTRACT — The purpose of the study implied identifying the nature of disturbances affecting proliferation and apoptosis in the epithelium of the gingival membrane in patients with rapidly progressing periodontitis (RPP). Studies have shown specific features of cellular homeostasis disorders manifested through a progressing epithelial proliferation lagging behind their apoptotic activity.

KEYWORDS — periodontitis; rapidly progressing periodontitis; proliferation; Ki-67; Bcl-2; apoptosis

INTRODUCTION

Practicing dentists come across RPP, which belongs to aggressive types of periodontitis with a continuous relapsing course. The RPP occurrence rate in Russia is 5–10% [1] and it has a tendency towards steady growth [2]. Unlike chronic periodontitis, where the microbial factor plays a key role, RPP is accompanied with a combined effect of three factors — the presence of specific periodontal pathogens; perverted immune response; genetic predisposition. Due to the complexity of the three-factors interaction, there is currently no single pathogenesis concept for RPP, which makes it difficult to diagnose and treat this nosology [3–5]. Nowadays, great importance in regulating periodontal tissues cell homeostasis belongs to the indicators Ki-67, Bcl-2 as well as the apoptosis index [6–9]. However, there is still no understanding of how these indicators change during RPP, including with comorbid conditions [10–18]. Immunohistochemical methods allow detecting more detail regarding the pathogenetic changes occurring through gingival epithelium cells renewal, their apoptotic elimination level, which will certainly expand the understanding of the pathogenetic processes underlying this disease, and will help improve diagnostics and treatment.

Aim of study:
to identify the nature of gingival cell proliferation and apoptosis disturbances in patients with RPP.

MATERIALS AND METHODS

To achieve the set goal, an examination was conducted involving 60 people. The main group included 20 patients suffering from RPP, 20 virtually healthy persons with intact periodontium (control group), and 20 patients with chronic generalized periodontitis (comparison group).

Inclusion criteria — persons of both sexes aged 18–45 years with no severe somatic pathologies. The participants were examined following a standard scheme including an index assessment of the oral cavity hygienic status (Green J.C., Vermillion J.R., 1964), the severity of inflammation processes in periodontal tissues (Muhlemann, in the modification by Cowell R. et al., 1975, Flesar T.J., 1980), PMA (Parma, 1960), PI (Russel AL, 1956), and an X-ray examination.

Immunohistochemical and morphological examination of periodontal tissues was performed on biopsy specimens, at curettage, removal of mobile teeth and extraction of teeth subject to orthodontic indications. For the periodontal diseases morphological diagnostics, a biopsy of the gingival mucosa marginal edge and gingival papillae, was performed. The obtained stuff was fixed in a 10% formalin solution for 24 hours, after which it was washed under running water, dehydrated in alcohols with a gradual increase in the concentration, and embedded in paraffin. To conduct a review microscopy, deparaffined sections were stained with hematoxylin-eosin. When conducting immunohistochemical reactions, monoclonal mouse antibodies to Ki-67 protein (MB-1, Ventana) and Bcl-2 ((124) M. Cell, 1:200 dilution) were used. The study was carried out using a Ventana BenchMark XT device and an Optiview DAB IHC Detection Kit system. The images of 10–12 visual fields were recorded with

a MIRAXDESK digital camera (ZEISS) built into a Leica DM 1000 microscope tube (magnification — 100× and 400×), and transferred to a computer. The number of expressing nuclei of the Ki-67 and Bcl-2 genes was recalculated as per 1m^2 using the Videtest-Morphology 4.0 morphometric software.

The cell proliferative activity was evaluated using the Ki-67 index, which was calculated by the formula below: I Ki-67= X / X_1 · 100%, where X_1 is the number of nuclei immunopositive to Ki-67, X is the total number of all the counted nuclei. A similar technique was employed when calculating the Bcl-2 index: I Bcl-2 = N / N_1 · 100%, where N is the number of nuclei immunopositive to Ki-67, N_1 is the total number of all the counted nuclei. Cell death through apoptosis was estimated through the apoptosis index, which was calculated using the following formula: Iapt (%) = Z/Z_1 · 100%, where Z is the number of nuclei in apoptosis, Z_1 is the total number of nuclei.

RESULTS AND DISCUSSION

Studies of intact periodontium showed that proliferation (I Ki-67 (10.24 ± 0.72%), I Bcl-2 (3.11 ± 0.08%)) dominate apoptosis (0.51 ± 0.07%), while having a low level of renewal and death of epithelial cells. In this case, low proliferation rates are balanced by low apoptosis activity.

In the comparison group, individuals with chronic periodontitis had an increased proliferative activity of gingival epithelial cells, which is evidence to the fact that the periodontal cells regenerative potential is high and their apoptotic activity remains moderate, while I Ki-67 is 20.31 ± 0.34%, IBcl-2 — 5.92 ± 0.04%, and Iapt — 0.61 ± 0.05%. In the group of persons suffering from RPP, we observed a breakdown of the periodontal tissues adjustment mechanisms, which was expressed in the inhibition of IKi-67 (2.02 ± 0.44%), IBcl-2 (2.14 ± 0.51%), against an increase in the apoptosis index by 3 times (1.53 ± 0.38%). We have identified a direct correlation between the values of the OHI-S hygiene index and the apoptosis index, both in case of RPP and in chronic periodontitis; however, the statistical strength of this relationship with RPP (r = 0.45) is weaker compared with chronic periodontitis (r = 0.91) (p <0.05); also, a direct correlation dependence of the PMA index with I apt (r = 0.720) and the reverse dependence with the proliferation index of Ki-67 (r = -0.501) (p <0, 05) was detected.

CONCLUSION

The study has shown that the development and progress of chronic periodontitis and RPP come accompanied by impaired cellular homeostasis. During the initial stages of inflammation in periodontal tissues, an increase in the cell proliferative activity was observed against virtually unchanged apoptosis index, which points at activated regeneration in the body; however, along with an increase in the tissues inflammation severity, there is a decrease in the epithelial cells proliferative potential as well as activation in their death manifested as apoptosis. In case of RPP, there is a breakdown of the periodontal tissues adjustment mechanisms, which reveals itself as inhibition of Ki-67 expression and an increase in the apoptosis index, which contributes to the progression of periodontal lesions inflammatory and destructive lesions.

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CYTOKINE PROFILE OF PERIODONTAL POCKET CONTENTS IN ESTIMATING THE SEVERITY AND EFFICIENCY OF TREATMENT OFFERED TO PATIENTS WITH REFRACTORY PERIODONTITIS

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ABSTRACT — Recent years have witnessed an increase in periodontitis prevalence, which is hard to cure while the treatment is administered properly, and has a persistently relapsing course. In this article, the enzyme-linked immunoassay with identification of the periodontal pockets exudate cytokine profile, allows us to carry out a better evaluation of the severity and effectiveness of treatment for patients with refractory periodontitis.

KEYWORDS — periodontal issues; refractory periodontitis

INTRODUCTION

Refractory (resistant) periodontitis develops despite treatment, has a complex course and reveals a huge number of complications [3, 6]. At the same time, the loss of supporting tissues goes on in several areas of periodontium [4, 7, 9, 11, 12]. The affected areas are infected with periodontal pathogens [8]. During that, the pathological process is accompanied by the development of dysbiosis and an imbalance of pro-inflammatory and anti-inflammatory cytokines in the periodontal pockets exudate, the severity of which features a direct dependence on the severity of inflammatory & destructive processes that are underway in periodontal tissues [5, 10]. This pathological process may get enhanced in case of comorbid conditions [13–22].

Aim of study: to identify the clinical efficiency of the cytokine profile in the pro-inflammatory cytokines IL-1β, TNF-α and the anti-inflammatory cytokine IL-10 in order to evaluate the severity and effectiveness of the treatment for refractory periodontitis.

MATERIAL AND METHODS

The examination involved 100 patients (53 females and 47 males). Group 1 included 40 patients with chronic generalized periodontitis, Group 2 — 40 patients with refractory periodontitis. The control group included 20 volunteers with intact periodontim. Refractory periodontitis was verified subject to the classification by G.F. Beloklitskaya (2007) [1, 2].

The criteria for setting the diagnosis of refractory periodontitis include a persistently relapsing course; loss of supporting tissues in several areas of the periodontium; massive loss of supporting tissues followed by teeth loss; a large number of exacerbations and complications.

All the patients underwent a comprehensive examination using standard clinical, laboratory and radiological methods. The immunological checkups included an assessment of the content of interleukin 1β, tumor necrosis factor α, and interleukin 10 in the gingival fluid, which was done through enzyme-linked immunoassay. The dynamics study for the immunological parameters of periodontal pockets exudate was performed both in the main group and in the comparison group. In order to obtain control values, a study of gingival fluid immunological parameters was performed in 20 healthy individuals with intact periodontium.

RESULTS AND DISCUSSION

Immunological studies showed a significant increase in the proinflammatory interleukin 1β concentration in the periodontal pockets fluid in both groups, while the patients with refractory periodontitis had an increase in IL-1β that was more prominent and reached 320.3 ± 20.6 pg/ml against 205.5 ± 19.5 pg/ml in the group with chronic periodontitis, whereas the control values were 110.2 ± 12.5 pg/ml. The increase in IL-1β is responsible for massive destruction of periodontal tissues due to stimulation of fibroblasts to the collagenase synthesis, and osteoblasts — to transformation into osteoclasts.

In the contents of periodontal pockets, the TNF-α indices in patients with refractory periodontitis exceeded significantly those in patients with chronic periodontitis (765.2 ± 34.2 pg/ml and 396.6 ± 47.5 pg/ml, respectively; control — 70.5 ± 10.7 pg/ml). Therefore, the higher the concentration of IL-1β and TNF-α, the more prominent the clinical image of the disease, since IL-1β and TNF-α are involved in the connective tissue destruction and bone resorption during periodontitis.

However, in patients with chronic generalized periodontitis, the content of IL-10 was reduced down to 5.3 ± 1.4 pg/ml, and in cases with refractory periodontitis — to 3.3 ± 2.1 pg/ml, at 14.1 ± 1.1 in control.

A comparison of the concentrations of pro- and anti-inflammatory cytokines revealed an imbalance in their ratio compared with the control values ratios. So, if in healthy donors the ratio of the IL-1β / IL-10 content in the gingival fluid was 7.9, then in chronic periodontitis this ratio enhanced due to an increase in IL-1β up to 47.7, and with refractory periodontitis — to 97.1

The content of the examined cytokine profile parameters (IL-1β, TNF-α, IL-10) in periodontal pockets varied depending on the periodontal tissues damage severity. We detected a direct correlation between the IL-1β and TNF-α concentration, and the values of the index describing the degree of inflammation in periodontal tissues (PMA), in case of chronic generalized periodontitis ($r = 0.61$, $r = 0.59$, respectively, with $p<0.05$). In case of refractory periodontitis, a negative correlation was observed between the concentration of the anti-inflammatory cytokine IL-10 and the PMA values ($r = -0.54$, $p<0.05$).

CONCLUSION

The immunological studies indicate that these processes in the oral cavity are accompanied by greater changes in local immunity in patients with refractory periodontitis, while secondary immune deficiency is developing, and these indicators (IL-1β, TNF-α and IL-10) can be used as reliable criteria for evaluating the severity of inflammatory periodontal diseases as well as the nature of their course.

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EFFECT OF JAW GROWTH TYPE ON DENTOFACIAL ANGLE IN ANALYZING LATERAL TELERADIOGRAPHIC IMAGES

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Specific features of the dentofacial morphology have always attracted various specialists — morphologists, dentists, forensic physicians [1, 2, 5, 10, 11, 12]. To date, lateral head teleradiographic images still offer a fairly accurate diagnostic basis for dentofacial pathologies, and are used in dental clinic for orthodontic and prosthetic treatment [3]. There are methods of computer diagnostics proposed, which allow cephalometric analysis of various images, such as teleradiographic images of lateral and direct projections, computer tomograms at different levels, allowing evaluation of various craniofacial structures as a whole, and dentofacial segments, in particular [4, 9]. Most coordinate points and planes of teleradiographic imaging have been discussed in works by both national and foreign experts, while such works are used to diagnose pathologies and identify the effect of comprehensive treatment [6, 7]. The emergence of new data concerning dentofacial features in view of the gnathic and dental indicators, will aim us at detecting a relationship between their parameters and the position of the skull planes, as well as reveals the relevance of the issue in question. Assessment of the facial area growth type attracts orthodontists, whose tasks include the treatment of occlusion anomalies and the prediction of treatment outcomes, as well as that of relapse [8]. When analyzing teleradiographic images, the major planes include the skull base plane, the orbital, spinal, occlusal, and mandibular planes, which extend horizontally (anterior-posterior direction). The known diagonal lines include lines N-Ba and N-Go, which are meaningful in identifying the face growth type. However, we have found no marks that would allow us to separate the facial and the cranial parts of the head, and detect the relationship of this plane with other lines of the head, which served the aim of the study.

Aim of study:
to identify specific features of the dentofacial angle in people with different types of jaw growth.

MATERIAL AND METHODS

A cephalometric study was carried out involving 151 persons falling in the age group of 21–35. To perform the teleradiographic image analysis, common points were marked: N (Nasion), C (Condylion), Ar (Articulare), T1, T2, Pg (Pogonion). We have proposed the facial area plane of the head or the cranial-facial line (CFL), which passed through the points N and C, and separated the facial section of the head from the cranial one. The mandibular plane (ML) had a traditional structure and passed through the most convex points of the mandible lower body edge (points Gn and T2). The intersection of these lines shaped the dentofacial angle, which we used for the cephalometry analysis in people with different types of facial growth (horizontal, vertical and neutral). The type of face growth was determined based on the size of the mandibular angle, which was shaped by tangent lines to the lower edge of the body and the mandible ramus bones. The angle from 119° to 123° corresponded to a neutral type of jaw growth. A decrease and an increase in the angle pointed at the horizontal and vertical types of growth, respectively.

RESULTS AND DISCUSSION

The cephalometric analysis showed that in people with a neutral type of the facial area had the mandibular angle at 120.73±1.18°. At the same time, the dentofacial angle formed by the intersection of the craniofacial and mandibular planes was 43.51±2.87°. In people with a horizontal face growth, the mandibular angle was significantly smaller ($p \leq 0.05$), 108.93±3.62° in the

group in general. Besides, a significant decrease in the dentofacial angle (down to 36.61±2.17°) was observed. The vertical type of face growth was associated to an increase in the angles in question up to 126.11±2.19° and 51.24±1.22°, respectively.

CONCLUSION

The above suggests that there is an additional criterion proposed for identifying the type of the facial area growth, namely, the dentofacial angle. Identifying orientation points for constructing the angle poses no issue, so that may prove a good tool for orthodontists seeking to predict the treatment outcomes.

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EFFICIENCY OF OSSEOINTEGRATION PROPERTIES MANIFESTATION IN DENTAL IMPLANTS WITH HYDROXYAPATITE PLASMA COATING

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Aim of study:
to identify the efficiency of osseointegration properties manifestation in dental implants with hydroxyapatite plasma coating.

Despite the progressing computerized radiological and ultrasound diagnostic methods, experimental studies have not lost their relevance yet and are still widely used in applied and clinical sense [8, 9]. Treatment of dental arches defects using intraosseous implants is a modern and effective method allowing rehabilitation of dental patients through different age periods, which is due to high prevalence of carious process [4]. Given the current progress in the dental practice, dental implants is rather a common way to restore dentition, which is of interest to specialists and is rather attractive for patients. Teeth are replaced in view of individual specifics per each dental system [3, 10, 11, 12]. Besides, special attention is paid to the shape and size of the dental arches [1,2,5]. Modern methods for examination of dental arches have been proposed, while the faults of the already available methods for biometric analysis have been taken into account [6, 7]. Reparative bone regeneration relies on the processes of osseoinduction and osseoconduction, which determine the healing of bone wounds by primary and secondary intension, and is regulated at the system level. Bone tissue, just like any material object, possesses certain strength and elasticity properties and is subject to morphological change, which requires morphological research.

MATERIALS AND METHODS

Three experimental animals (young mongrel dogs, 1.5–2 years old), had 6 smooth cylindrical implants with a bioceramic coating installed after the removal of the first molars.

The animals were taken out of the experiment after 3 and 30 days, taking into account the generally accepted requirements regarding working with laboratory animals. The respective bone fragments were cut out together with the installed implant. Both macro- and micro-status were evaluated. After decalcification, histological specimens stained with hematoxylin-eosin were made, after which studies were carried out with a light microscope.

RESULTS AND DISCUSSION

The experimental study showed that an inflammation phase was underway during the first three days after the implants were installed. At that time, various cells, including macrophages, were noted in the surgical area, with phagocytosis of the dead cells identified. The rough surface of hydroxyapatite, an apatite layer was detected, which did not differ from biological apatite. Clusters of preosteoblasts, which differentiated into osteoblasts, were observed, as well as their migration to the bone tissue defect and to the implant surface carrying hydroxyapatite particles. Osteoblasts on the hydroxyapatite surface developed osseous tissue.

On day 30 into the study, a layer of fibroblasts and osteoblasts was to be observed on the hydroxyapatite surface. The bone formation had occurred up until the entire bone defect was recovered.

One of the important properties of hydroxyapatite was its resorption. The study found that hydroxyapatite is not osteoinductive, yet does prove so when replacing defects. The development of bone tissue began from the defect edge and passed along the hydroxyapatite surface, while the development of a bridge between the bone tissue and hydroxyapatite was observed.

CONCLUSION

The above suggests that the complex studies outcomes prove the efficiency of osseointegration properties manifestation in dental implants with hydroxyapatite applied through the plasma coating method.

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CELL-POTENTIATED REGENERATIVE TECHNOLOGIES FOR RESTORING JAW BONE TISSUES IN CASE OF ODONTOGENIC INFLAMMATORY & DESTRUCTIVE PROCESS

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ABSTRACT — The study offers a view on the results of comparing conventional and advanced innovation-based cell-potentiated methods used to restore jaw bone tissue in case of odontogenic inflammatory & destructive process. The source of regenerative cells and stimuli was autologous stromal vascular fraction of adipose tissue (SVF-AT). The study involved 158 patients with moderate and severe periodontitis; 112 patients with odontogenic cystic formations. Using SVF-AT through directed regeneration of periodontal tissues (n = 95) improved significantly the clinical, functional and aesthetic outcomes of the treatment, ensuring a higher increase in clinical attachment (5.66 ± 0.02 vs 3.27 ± 0.03 mm, p <0.001) with a more complete and stable restoration of the support function, and a minimal residual recession of the gingival margin, if compared with the conventional treatment in the control group (n = 63). In maxillary defects osteoplasty, the removal of odontogenic cystic formations in the experimental group (58 interventions) was accompanied with complications less frequently (1.7% vs. 23.9%, p <0.001), while positive clinical outcomes with no recurring inflammatory & destructive processes were twice as common through the long-term observation period (96.6% versus 47.8%, p <0.001) against the control (67 interventions). The morphological assessment outcomes suggest significant inducing effect that SVF-AT works on reparative osteohistogenesis, evidence to that being multiple tissue remodeling sites with a higher density of microvessels and newly developed bone tissue in the biopsy obtained from the experimental group patients if compared to the control. Therefore, the newly developed cell-potentiated methods increase significantly the effectiveness of the treatment offered to patients with odontogenic jaw destruction.

KEYWORDS — stromal vascular fraction of adipose tissue, directed periodontal tissue regeneration, in situ bone tissue engineering, restorative dental surgery

INTRODUCTION

One of the promising areas for developing restorative dental surgery and maxillofacial surgery is the introduction of regenerative and tissue engineering technologies using stem or stromal cells. However, there are a number of objective reasons that impede the use of cell lines obtained outside the human body in practical healthcare, including certain biosafety issues. Stromal vascular fraction of adipose tissue (SVF-AT) is a heterogeneous set of native cells and is considered as a promising multifunctional regenerative resource for clinical use [1, 2]. The composition of SVF-AT includes multipotent mesenchymal stromal cells (MMSC-AT) (1.5% to 25% of the total number of nucleated cells), smooth muscle and endothelial vascular cells, macrophages and lymphocytes [3, 4]. A wide spectrum of angiogenic, anti-inflammatory, immunomodulatory cytokines and growth factors secreted by SVF-AT cells has been described [5]. Attempts are being made to replace cranial-maxillofacial bone defects with tissue-engineering structures, including the MMSC-AT culture and/or the newly isolated SVF-AT [6-9]. However, single and isolated observations presented in the respective literature do not allow evaluating the effectiveness and safety of using SVF-AT in maxillofacial surgery and dentistry.

Aim of study:
to evaluate the effectiveness of the newly introduced cell-potentiated ways to restore bone in dental odontogenic inflammatory & destructive diseases.

MATERIALS AND METHODS

The study implied observing 270 patients with different jaw bone tissue issues of various topography and volume, including 158 patients with moderate to severe periodontitis (Group 1, G1); 112 patients with odontogenic cystic formations (Group 2, G2). Depending on the SVF-AT use in each group, two representative comparison subgroups were identified – the control subgroup (CS) with conventional surgical treatment methods, and the experimental subgroup (ES) where the treatment involved the use of autologous SVF-AT. All the patients underwent examina-

tion, had no concomitant somatic diseases that might affect the treatment outcomes, and signed a voluntary informed consent to join the study. Table 1 offers a general view on the clinical material specifics.

In case of chronic periodontitis (G1), the access and treatment of periodontal pockets were performed through the standard technique for directed periodontal tissue regeneration (DPTR). The sanitized

Table 1. *General specifics of the clinical material*

Group and type of surgery	Age, min-max, Me	Distribution by gender, female / male	Control subgroup	Test Subgroup
Group 1. Directional regeneration of periodontal tissues	28-60, 42	83/75	63	95
Group 2. Cystectomy with osteoplasty of the maxillary defect	20-69, 52	44/68	67	58

There were no statistically significant differences identified in terms of the age & gender structure, the initial oral cavity status, the size and topography of the dentomaxillary bone defects between the compared subgroups ($p > 0.05$). The surgical intervention was preceded by a conservative-hygienic phase, including the oral cavity sanation, the mobile teeth immobilization, the mucogingival disorders elimination, preparing protective temporary orthopedic structures for the surgical treatment and rehabilitation period.

In the Plastic Surgery Department, syringe suction of 40–120 ml of subcutaneous adipose tissue from the hypogastric region of the anterior abdominal wall and lateral parts of the body was performed under local infiltration via tumescent anesthesia. The punctures were covered with aseptic cloths with a compression bandage applied. Within one hour, the patient remained in the hospital under observation. Lipoaspirate in syringes was washed with sterile physiological solution with a wide-spectrum antibiotic added, and delivered to the laboratory along with 10–20 ml of the patient's blood serum. The stromal vascular fraction was isolated in a laboratory meeting all the GMP standard requirements. 40–50 ml of the lipoaspirate were enhanced up to 60 ml with a physiological solution containing collagenase enzyme lyophilisate 50 mg (activity 180–290 units/mg) and placed in a sterile plastic bag. After exposure to a water bath for 20 minutes at 37° C, the suspension was distributed into test tubes and centrifuged for 20 minutes at a speed of 2750–3000 rpm. The upper layer of liquid lipids and floating adipocytes was aspirated, and the supernatant poured off. SVF-AT was collected from the tubes bottom and washed with autologous blood serum. Further on, SVF-AT was resuspended with autologous serum and the composition was analyzed using an automatic cell counter. When delivered from the laboratory to the clinic, the material was accompanied with a passport indicating the number of cells and their viability.

periodontal defects in the experimental subgroup (ESG1) were filled with SVF-AT, in the control subgroup (CSG1) a blood clot from the bone marrow spaces and the vital periodontal ligament were used for the same purpose. In both subgroups with deep and wide bone periodontal defects, a granulated osteoconductor was additionally introduced (Biosit-Elcor, St. Petersburg; Bio-Oss, Switzerland; Bone Ceramic, Switzerland). Isolation of the regeneration site was carried out with a biocompatible polytetrafluoroethylene barrier membrane (Ecoflon, St. Petersburg). A muco-periosteal flap was applied to the site with the wound sutured carefully. The postoperative care included antiseptic treatment of the oral cavity, and application of antibacterial gel. The stitches were removed on Day 12. The time for the membranes to remain in the tissues — 2 to 8 weeks.

In case of jaws cystic lesions (G2), cystectomy was performed employing a standard procedure. In the experimental subgroup (ESG2), bone defects of small size (up to 20 mm in diameter) were filled with SVF-AT only. For plastic treatment of mid-sized bone defects (20–30 mm), SVF-AT was used with crushed bone added, which was taken from the patient through the surgery, from intraoral donor sites. To eliminate larger bone defects (exceeding 30 mm), including damage to one or both of the cortical jaw plates, granulated osteoconductor was introduced into the graft in addition to SVF-AT and bone chips (Bio-Oss, Switzerland). The resorbed collagen membrane (Bio-Gide, Switzerland) was placed under the muco-periosteal flap prior to the wound closure. In the control subgroup (CSG2) with small and mid-sized bone defects, treatment was performed either under a blood clot or with an osteo-substituting material combined with a barrier membrane; for large sizes of cystic formations, cystotomy was the choice.

The study included modern methods of clinical, radiological, and instrumental diagnostics, as well

as cytological and morphological verification of the transplanted material and regenerated tissues. Control terms were set at 6, 12 and 24 months. To evaluate the treatment effectiveness, three categories of indicators were analyzed, including 1) the incidence of complications and positive outcomes; 2) the severity of the clinical symptoms; 3) the results obtained through instrumental, radiological and laboratory tests.

The study materials underwent statistical processing using the methods of parametric and non-parametric analysis based on the results of testing the compared sets for normal distribution. In the case of a confirmed normal distribution, the quantitative indicators were presented as arithmetic means (M) and standard errors (m). The quantitative indicators, whose distribution differed from the normal, were described through the values of the median (Me) and quartiles [Q1; Q3]. The differences were considered statistically significant at $p < 0.05$.

RESULTS

The results of cytological tests indicate the reliability of the adipose tissue treatment protocol employed for obtaining SVF-AT. The cells were viable, demonstrated the immunophenotype of multipotent mesenchymal stromal cells (CD13+, CD34–, CD44+, CD90+, CD105+), actively reproduced in vitro and synthesized the extracellular matrix components. The number of viable nucleated cells in a SVF-AT used for a single surgery ranged from 25 to 120 million.

Using SVF-AT during DPTR improved significantly the clinical, functional and aesthetic results when treating patients with moderate to severe chronic periodontitis. The incidence of postoperative complications (infection and partial necrosis of the regenerate) was significantly lower in ESG1, compared with CSG1 — 2.1% and 19.1%, respectively ($p<0.05$). After a cell-potentiated surgery, accelerated regression of inflammatory symptoms was observed with long-term stability of clinical indicators for the oral cavity status, such as gingivitis index, bleeding index, and periodontal index. The increase in the periodontal attachment after 2 years was 5.66 ± 0.02 mm in ESG1, which is 73% above the similar CSG1 indicator (3.27 ± 0.03 mm) where treatment was performed subject to the standard DPTR protocol ($p<0.001$). Periotestometry indicated complete restoration of the periodontal support function in the ESG1 patients, while the majority of patients with CSG1 maintained pathological mobility. The average Periotest value 24 months after surgery was 3.8 ± 0.1 and 15.6 ± 0.3 points in the experimental and control subgroups, respectively ($p<0.001$). An increase in the X-ray contrast structures prevailed in the area of defects, the elimination of which was done with SVF-AT (Fig. 1).

The results of a comparative histomorphological study suggest significant inducing effect that SVF-AT has on the restoration of damaged tissues in the tooth supporting structures, which can be seen from the fact that the earlier biopsy material obtained from the ESG1 patients revealed the presence of marginal periodontal tissue soft structures featuring the specific short junction epithelium, new connective-tissue attachment, and the development of young alveoli bone structures. The average microvessels density on regenerating tissues sections in the experimental subgroup was almost 2 times as high as in the control one: 58.2 ± 10.2 and 30.1 ± 7.5 units per 1 mm^2, respectively ($p = 0.047$).

Using SVF-AT in osteoplasty of maxillary defects after the removal of odontogenic cystic formations allowed a significant reduction in postoperative complications rate (1.7% versus 23.9%, $p<0.001$), an increase in the frequency of positive clinical outcomes with no recurrence of inflammatory & destructive process in long-term observations (96.6% versus 47.8%, $p<0.001$), achieving earlier and complete stabilization of mobile teeth as compared with conventional treatment methods. The results of X-ray study methods differed significantly in the studied subgroups, the method using SVF-AT autotransplantation appearing as more promising. In ESG2, after 2 months already, spot X-ray images showed uniform filling of jaws large defects with dense structures featuring no light spots in the central area, which was often the case in CSG2. In the long-term follow-up, most cases had no original defects boundaries visible, with a uniform trabecular structure of the reconstructed bone visualized. Computed tomography helped observe a return to the normal jaws anatomy without alveolar arch permanent deformations, with cortex and spongy bone in the intercortical area (Fig. 2).

Note to be made that there were no radiological signs of resorption in the newly formed structures, any ankylosis or resorption of the teeth roots in the long term. In CSG2, judging by the radiological signs, complete restoration of the bone was to be observed in relation to the alveolar crest smaller defects. In defects exceeding 20 mm, the development of radiologically relevant bone structures was observed only along the periphery, while the initial borders of the defect often remained visible.

According to the histological examination results, in smaller defects (up to 10 mm), reparative osteogenesis was identical in the compared subgroups and ended with the development of a sound bone. The recovery of mid- and large-size bone defects depended

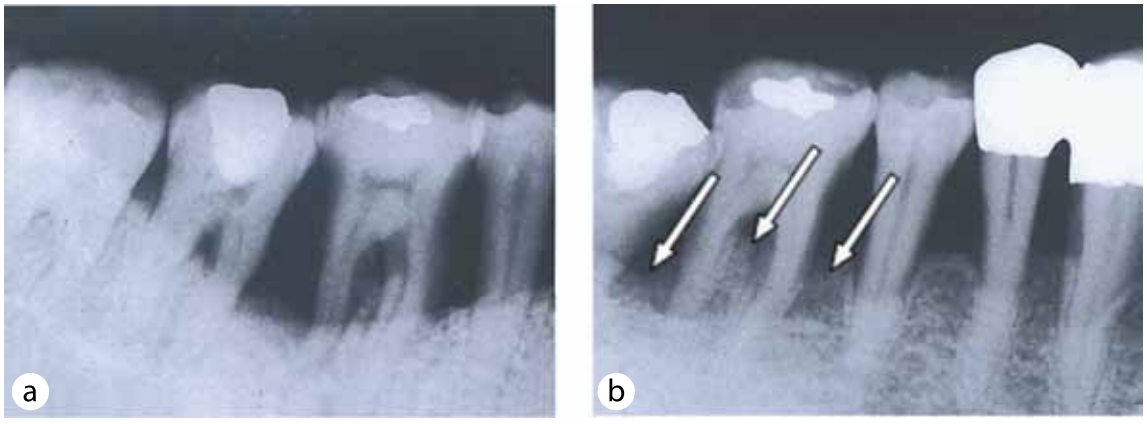

Fig. 1. *Radiographic outcome of the replacement for furcation defect of the lower molar (FIII) and supraalveolar periodontal defects of the adjacent teeth: a) before treatment; b) after the DPTR with SVF-AT transplantation, a significant increase in the interdental space structures (shown by arrows)*

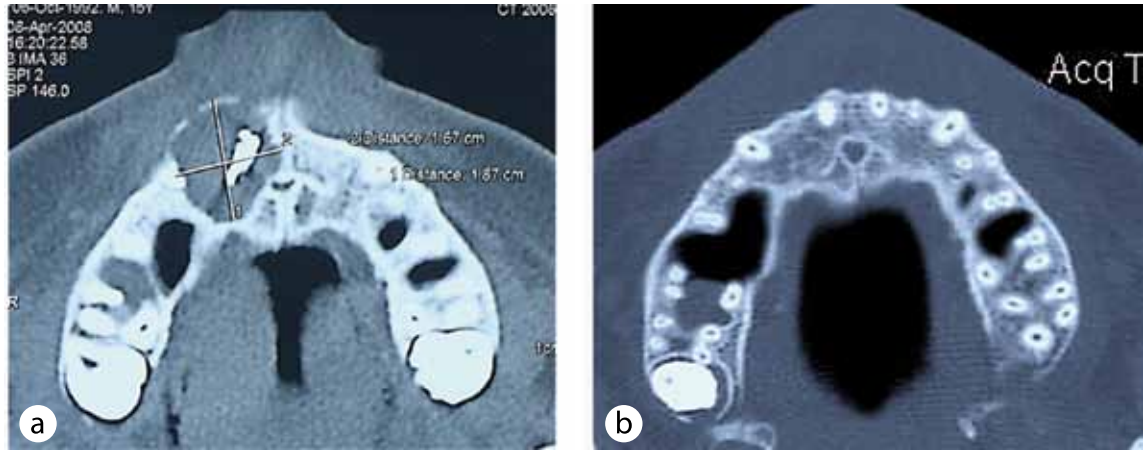

Fig. 2. *CT scan of the upper jaw, patient T. 16 years old: a) a large radicular cyst of the 1st jaw segment; b) 2 years after cystectomy and osteoplasty with transplantation of SVF-AT with bone chips into the bone defect: the defect is eliminated; the cortical plate restored; the jaw features normal X-ray anatomy*

on the treatment offered. After transplantation of SVF-AT into the bone defect, the regenerate was soon after presented with reticulofibrous bone tissue, which filled the defect evenly regardless of its size; later on regenerate *maturation* to the lamellar bone of osteonic structure was observed (Fig. 3).

The recovery of larger bone defects in the absence of an additional source of regenerative cells and stimuli (such as SVF-AT) was incomplete. In the nearest term, the histological samples from CSG2 were observed to have a polymorphic regenerate of loose fibrous connective tissue with tough fibrous bone and chondroid tissue sites. In the longer term, the regenerate was delimited, the marginal area of the bone cavity featured a mature bone, while the center was found to have moderately vascularized fibrous connective tissue.

DISCUSSION

Currently, the issue of safety is the cornerstone for cellular technology introduction. We did not observe any specific complications through the entire study, which indicates the absence of extra clinical risks in the implementation of SVF-AT autograft-potentiated treatment and rehabilitation methods for patients with inflammatory osteo-destructive dental issues. The technologies employed through this work are based on the use of newly isolated autologous minimally manipulated cellular material, which eliminates com-

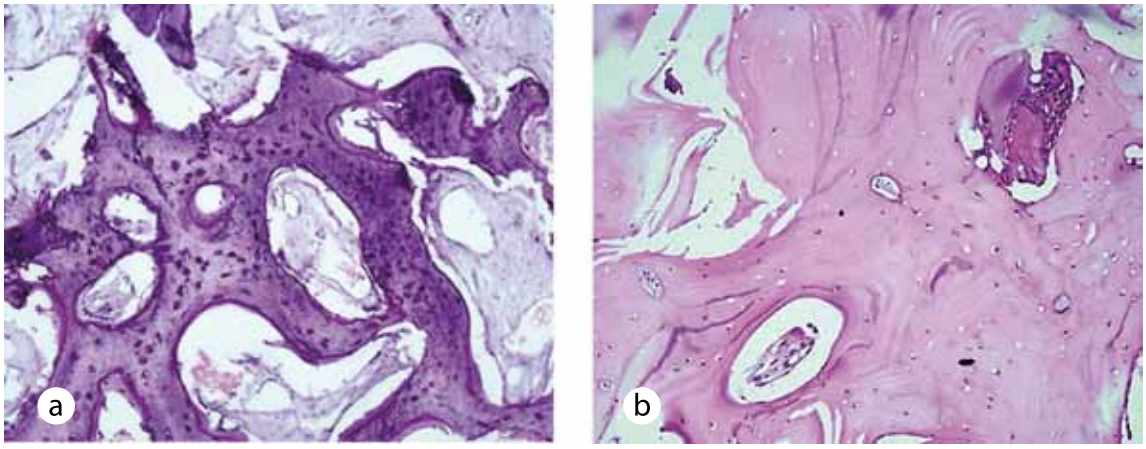

Fig. 3. *Histomorphology of trepanbiopsy from the post-cystectomy jaw defect area at different periods after osteoplasty using SVF-AT: a) 4 months — primary trabeculae of reticulofibrous bone tissue; b) 18 months — mature mineralized bone osteonic structure. Stained with hematoxylin and eosin. Magn.: 480× (a), 280× (b)*

pletely the disadvantages associated with the use of cell lines obtained ex vivo.

The results of the new treatments developed using SVF-AT exceed the results of the generally accepted approaches, evidence to that being the statistically significant differences in the total average values and relative indicators within the compared subgroups. It would be of interest to compare the registered (at the end of the 6-month follow-up period, common reference point) increase in periodontal (clinical) attachment (5.53 ± 0.02 mm) with the respective data available in literature. In their study, Y. Yamada et al. (2006) isolated mesenchymal stem cells from the bone marrow of the patients' iliac crest, then propagated them in a culture, combined with platelet-enriched plasma, and injected into periodontal defects. After 6 months, the increase in attachment was 4 mm [10]. R. Dhote et al. (2015) used a tissue-engineered construction of mesenchymal stem cells cultured on beta tricalcium phosphate combined with recombinant platelet growth factor. The patients were 14 people without systemic diseases, with an initial depth of periodontal pockets exceeding 5 mm. After 6 months, the increase in attachment was 3.91 ± 1.37 mm [11]. In case of periodontal regeneration with autologous bone marrow mononuclear cells on a gelatinous polymer carrier, Indian experts reached an attachment increase of 6 mm [12]. Our clinical results, in terms of the gained success, meet the results presented above by other researchers who have applied advanced cell transplantation and tissue engineering technologies, yet they feature certain advantage as they can be achieved in a safer, affordable and easily reproducible way.

The clinical outcomes demonstrate the safety and efficiency of the cell-potentiated approach in treating cystic issues in jaws. It is a known fact that when replacing long-existing defects with altered bone walls trophism, bioresorbable materials undergo organotypic rearrangement much slower, which requires special conditions that would improve blood supply to the defect area [13–18]. One of the reasons behind incomplete osteo-substitution of larger defects in the control subgroup was the lack of blood supply to the central parts of the graft due to remote location from the microcirculation stream along the defect periphery, as well as insufficient centripetal neovascularization rate. The regenerate histomorphology showed that under the same conditions, yet with SVF-AT, the osteoreparation outcomes changed radically. The reason for this, as we see it, was the in situ implementation of a number of positive SVF-AT properties, described in the literature for its cellular components and confirmed by the outcomes of their own experimental and cytological studies specifically for the fraction isolated according to a special protocol, i.e. 1) the capacity to stimulate osteogenesis, angiogenesis and neovascularization; 2) the capacity to provide mechanical stability in the wound due to the binding properties of the fibrous component, as well as to develop a functional matrix accessible for cellular interactions; 3) the capacity to differentiate and productively join the development of an organotypic regenerate [19–23].

CONCLUSION

Using the proposed cell-potentiated methods for the restoration of the jaws bone tissue allows a signifi-

cant increase in the efficiency of treatment offered to patients with osteodestructive dental diseases.

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INNOVATION-BASED APPROACH IN RECONSTRUCTION OF REDUCED JAW ALVEOLAR RIDGE BONE USING CELL REGENERATION TECHNOLOGIES

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ABSTRACT — Aim. This study was performed to assess the size, structure and functional features of the reconstructed alveolar ridge using the autologous stromal and vascular fraction of adipose tissue (SVF-AT) up to 10 years.
Materials and methods. This study evaluates 141 patients (61 males, 80 females) aged range 45 to 78 years (Moda 57 years) with jaw alveolar ridge regression. 112 osteoplastic surgical procedures in the test (TG) were performed using SVF-AT with subsequent placement of 297 dental implants in the reconstructed ridge; in the control (CG) — 117 osteoplastic surgical procedures were performed using generally approach with subsequent placement of 323 dental implants. The alveolar ridge size and reconstructed bone features was evaluated in terms of up to 10 years. Histologic and histomorphometric study of 27 reconstructed bonecor-biopsies was conducted. The data were statistically analyzed.
Results. The results of this comparative study confirm the advantages of the proposed cell-potentiated approach over the current generally accepted methods for the reconstruction of the alveolar jaw ridge. The use of SVF-AT with osteoplastic material allows to achieve new and sufficient bone growth with an insignificant risk of complications and reoperations (8% and 21% of cases in TG and CG, respectively, $p = 0{,}231$), optimal morphological properties of the regenerate ($40{,}14 \pm 3{,}36$ and $24{,}23 \pm 2{,}63$% of bone tissue in TG and KG respectively, $p = 0{,}001$), that provides reliable fixture integration in the reconstructed alveolar ridge and high efficiency of the implant-supported restorations (97% and 88% in TG and KG, respectively, up to 10 years, $p < 0{,}001$).
Conclusion. The proposed innovative approach can be recommended as a basis for a valid surgical protocol with a pronounced of the jaw bones regression transformation. This will allow more successful and predictable use the implant-supported prosthetic restorations in reconstructed ridges in this category of patients.

KEYWORDS — regression bone transformation, stromal and vascular fraction of adipose tissue, reconstruction of the alveolar ridge, dental implantation

INTRODUCTION

Given the current stage of research and technology progress, dental implants installation is the best option to support dentures with partial or complete loss of teeth. However, it requires a certain amount of supporting tissues, which is often lacking due to the regressed transformation of the alveolar ridge bone, which is an inevitable effect of missing teeth in the natural bite along with disappearing endoosseous mechanical stimulus [1]. The progressing over time bone atrophy leads to osseogenic failure accompanied with low activity in osseoinductive factors of the systemic and/or local level, small number of cambial cells, reduced density of hemomicrocirculatory bed functioning vessels [2]. A significant limitation to the three-dimensional increase in the lost structures over a considerable length of the alveolar ridge bone is the lack of vertical walls, which requires additional use of osteoplastic materials featuring frame functions.

Autologous bone transplant is known to be the gold standard for the recovery of maxillofacial area defects [3]. This procedure, however, has certain drawbacks — an additional area of operational trauma in the oral cavity or extraoral localization; a limited amount of graft; a two-stage operation protocol with an increase in the intervention time and morbidity. Patients often suffer from neurosensory disorders in the donor area. The probability of bone autograft resorption is high [3, 4]. The results can be improved through combinations of auto-bone with various animal- or synthetic-origin bone substitutes that do not possess osteoinductive and angiogenic potential; they can be used to replace minor defects yet prove ineffective in case of larger or lengthy issues [5, 6]. Besides, the remaining non-resorbed bone substitute granules impair significantly the quality of the regenerate.

Given the above, there is a significant clinical need for full-function osteoplastic materials that can be used to eliminate the jaw bones regression transformation followed with dental implantation.

Aim of study

To evaluate the size, the structure and capacity for chewing load in the bone reconstructed with autog-

enous stromal-vascular fraction of adipose tissue (SVF-AT) through a long-term study (up to 10 years).

MATERIALS AND METHODS

The study involved 141 patients (61 males and 80 females) aged 45–78 (median age — 57) with regressed transformation of the alveolar ridge bone of various degree and duration. All the patients needed an increase in the bone tissue prior to dental implantation. The study did not include patients with uncontrolled general somatic diseases.

The treatment and further clinical observation included the period of 2006–2018. All the patients signed a voluntary informed consent to undergo a comprehensive rehabilitation of the dental system, including the use of the SVF-AT. A positive conclusion was obtained from the Ethical committee of the Stavropol State Medical Academy (Protocol # 6 of December 17, 2010). The observation went on until all the stages of dental prosthetics were completed including the evaluation of the reconstructed alveolar ridge bone parameters, as well as the status of the artificial supports in the reconstructed ridge bone through later observation periods — up to 10 years.

In the test group (TG, 68 patients, 112 surgeries) vitalized SVF-AT heterogeneous osteoplastic material was used for osteoplastics; in the control group (CG, 73 patients, 117 surgeries) the same material was used yet with no cellular component. The studied groups had a comparable distribution by gender and age ($p>0.05$). The preoperative computed tomography results showed that the average residual height of the alveolar ridge in the proposed implantation area was 5.04 ± 0.16 mm and 5.49 ± 0.17 mm; the residual width was 3.13 ± 0.06 mm and 3.46 ± 0.01 mm in the TG and CG, respectively. Table 1 shows the distribution of the clinical material based on the type of surgical intervention, on the number of patients, and on the installed osseointegrable dental implants in the groups in question.

Prior to the reconstructive surgery, the patients underwent teeth sanitation, removal of insolvent orthopedic constructions, professional oral hygiene with monitoring of the patient's sustained skills in individual dental and gingival control, periodontal inflammatory and destructive disease treatment, preliminary correction of prosthetics, and received temporary orthopedic structures not relying on the bone augmentation areas.

The planning stage involved diagnostic wax modeling of the final orthopedic work in an articulator; identified the required locus of surgical interventions; made navigation patterns for placing artificial supports in the right spots and with correct angulation.

Lipoaspiration was performed in the plastic surgery department. The isolation of SVF-AT was carried out in a research institute laboratory (Prof. Ochapovsky Krasnodar Regional Hospital #1; Krasnodar, Russia) following the method into which we introduced certain improvements [7, 8].

Autogenous SVF-AT separation Protocol

Autogenous blood serum (ABS; volume – 10 ml) was prepared following the standard procedure. Local infiltration with tumescent anesthesia, Sol. Lidocaini 0.3%, 500 ml, with adrenaline (1: 500000) through punctures with a #11 scalpel using a 12 G cannula (Khuori Harvesting Cannula), a 50 ml LuerLock syringe and a locking device (ByronMedical) were used to aspirate 40–50 ml of subcutaneous fat tissue from the anterior abdominal wall. The punctures were covered with aseptic drapes with a compression bandage applied. For one hour, the patient stayed in the hospital under observation. The lipoaspirate in syringes was washed with sterile saline with a wide activity spectrum antibiotic and further delivered to the laboratory in a thermal container. The volume of the lipoaspirate was brought to 60 ml with physiological saline containing lyophilized enzyme collagenase (50 mg), to be further put in a sterile plastic bag. After a 20-minute exposure at (37 C) (ELMI thermostat, Laboratory Equipment) the suspension was distributed in test tubes with its volume taken up to 10 ml with physiological saline, and centrifuged for 20 min (CLMN-P10-02 centrifuge). The upper layer of liquid lipids and flotizing adipocytes was aspirated, and the supernatant drained. The SVF-AT was collected from the tubes bottom, while the washing cycle was performed by resuspending in the ABS and centrifuging. Next, SVF-AT was resuspended in 5 ml of ABS and kept like that (until it was used) at a temperature of +4°C. When delivered to the clinic, the material was supplied with a cell counting protocol indicating the viability.

Cell viability was assessed using the vital trypan blue stain, whereas the count was performed on a Countess cell counter (Invitrogen, USA). After SVF-AT was isolated, a sample was taken for immunophenotyping. Since SVF was represented with different types of cells, while mesenchymal stromal cells were one of the main types, we decided to cultivate the cells until the first passage with further identification of the immunophenotype of adherent cells using the immunofluorescent method, which would allow confirming the quality of the stromal vascular fraction. The following monoclonal antibodies were used: CD13 (Serotec), CD31 (BD, Pharmingen), CD34 (BD, Pharmingen), CD44 (Abcam), CD90 (Calbiochem), CD105 (Serotec), Pro-collagen Type I (Taka-

Table 1. *Characteristics of clinical material*

Type of surgery	Number of operations, localization, study group	Number of patients	Number of dental implants installed
Sinus lifting open	107 TG – 55 CG – 52	67 TG – 35 CG – 32	301 TG – 154 CG – 147
The operation of horizontal, vertical and three-dimensional augmentation of the alveolar ridge	122 UJ TG – 26 UJ CG – 28 LJ TG – 31 LJ CG – 37	74 UJ TG – 15 UJ CG – 20 LJ TG – 18 LJ CG – 21	319 UJ TG – 80 UJ CG – 96 LJ TG – 68 LJ CG – 75
Total	229 TG – 112 CG – 117	141 TG – 68 CG – 73	620 TG – 297 CG – 323

Note. *TG – test group; CG – control group; UJ – upper jaw; LJ – lower jaw.*

ra), Fibronectin (Abcam), SMA (Sigma), Desmin (Sigma) and C-kit (BD, Pharmingen). To identify the nuclei, the cells were stained with fluorescent DAPI (Dako). The preparations were placed into a medium for fluorescent preparations (AquaPoly/Mount; Polysciences, Inc). Staining visualization and image analysis were performed using an Axiovert 200M fluorescence microscope (Zeiss, Germany) equipped with a digital camera (20× and 40× obj.).

Methodology for preparing vitalized (activated) SVF-AT osteoplastic material.

Granulated and (or) bioresorbable osteo-substituting material presented as a block was introduced into a tube with the SVF-AT, resuspended in 10 ml of autogenous blood serum. The following proportions were observed: 1 part of the concentrated fraction (the sediment volume on the bottom of the tube after centrifugation) to 2–4 parts of the osteo-substituting material. Within the 15–30 minute exposure with regular shaking of the tube, the SVF-AT got adsorbed on the surface and inside the pores of the cancellous bone substitute, and the serum became transparent. Immediately before use, the block-type osteo-substituting material was removed. The granulated material was removed from the tube bottom after centrifugation at a speed of 1000 rpm for 4 minutes and the supernatant was drained. A morphological study of 5 samples of vitalized SVF-AT granulated osteoplastic material included making monolayer cytological preparations (Cytospin-4 cytocentrifuge; Shandon, United Kingdom), their fixation in May-Grunwald solution, staining subject to the Romanovsky method. The examination and photography of the preparations was carried out using an Axiostar microscope (Zeiss, Germany) (magnification ×100 and ×200).

In case of three-dimension regression transformation of the jaws alveolar ridge, autogenous cortical blocks up to 1.5 mm thick, taken from the area of the external oblique mandible line, or xenogenous cortical blocks and bone plates (200, 400 and 600 micron thick) were used as a frame (Lamina, OsteoBiol; OsteoplantFlexCortical , BioTech, Italy), fixed with screws to the cortical plate of the recipient bed. The gaps were filled in the TG with a mixture of SVF-AT with osteoconductor granules (BioGen, BioTech, Italy) and autogenous cancellous bone taken by trepan from the donor retromolar area, maxillary tubercles or a toothless alveolar ridge, crushed in a bone mill. In the control group, the same osteoconductors were used, yet with no cellular material. A membrane (Heart, BioTech, Italy; Evolution, OsteoBiol, Italy; Bio-Gide, Geistlich, Switzerland; e-PTFE, GoreTex, USA; Ecoflon, St. Petersburg, Russia) was placed under a soft tissue flap, and then the wound was sutured without tension by mattress and cross stitches. Prior to suturing the donor area the soft tissues at the bone wound were covered with collagen (Osteoplast, Vitaform, Russia).

To increase the size of the subantral area at an open sinus elevation, ordinary (in CG) or vitalized SVF-AT (in TG) granules of osteoconductive material were placed under the cranially displaced Schneider membrane, after which the wound was sutured with 4-0 Teflon without tension.

Postoperative instructions included liquid and soft food, oral cavity antiseptic treatment with a 0.05% chlorhexidine solution until the stitches were removed, brushing the teeth twice a day with a sonic brush, except the surgery site. Non-steroidal anti-inflammatory drugs were administered. Preventive antibiotic therapy was performed. Painkillers were prescribed as needed. Examinations and dressings were performed on the

day following the surgery, and then once every 3 days. The stitches were removed 2 weeks after the surgery.

6–8 months later, a second cone-beam computed tomography was performed to evaluate the reconstructed bone status, after which 620 osseointegrable dental implants (DI) were installed followed by preparing non-removable or hybrid orthopedic structures. Artificial supports with different immersion degree into tissues were used — 346 implants were installed into the gum level; while 274 titanium alloy, Grade 4 implants were installed into the bone level (or subcrestal). The healing caps were installed following the standard load protocol — 2–3 months after the implants installation.

Histological examination: 27 trepan biopsies from the reconstructed bone (14 from TG and 13 from CG) obtained during the development of dental implant wells were fixed in 10% neutral formalin, washed, decalcified in trilon-B, and further, according to the standard technique, 5 μm sections were prepared with hematoxylin and eosin staining. The study was performed with a light-optical microscope at standard magnifications (7×, 10× oc.; 10×, 40×, 60×, 90× obj.). Not less than in four sections of each sample, the relative area of vital mineralized tissue, non-vital mineralized tissue and non-mineralized tissue, were identified and calculated. The tissue structures area analysis in digital micro-images was performed with the 3D-doctor program (Able Software Corp., USA).

The data obtained through the study underwent statistical processing using the methods of parametric and non-parametric analysis following the compared sets testing results for distribution normality. The statistical analysis was performed using IBM SPSS Statistics 23 software.

RESULTS AND DISCUSSION

Patients easily went through the lipoaspiration procedure, with no complaints and complications registered. The number of viable nucleated cells in the portions of SVF-AT used for each individual patient ranged from 25 to 60 mln. The immunofluorescence data revealed that at the end of the first passage, 95–100% of the SVF-AT culture cells expressed mesenchymal stromal cell (MSC) markers: CD13, CD44, CD90, CD105. A small number of cells (15–25%) in the samples expressed CD31 (endothelial cell marker), C-kit (receptor for stem cell factor SCF and some other progenitor cells), desmin and smooth muscle actin (muscle cell markers). No CD34-positive cells were detected in the culture (hematopoietic stem cell marker).

Cultured cells are synthetically active in relation to the extracellular matrix components, such as fibronectin and collagen I (the cells revealed expression of Pro-collagen I, the precursor of type I collagen). The identified phenotype corresponds to the known features of MSC [9, 10].

Fig. 1 shows a micro-image of an SVF-AT vitalized osteoplastic material smear.

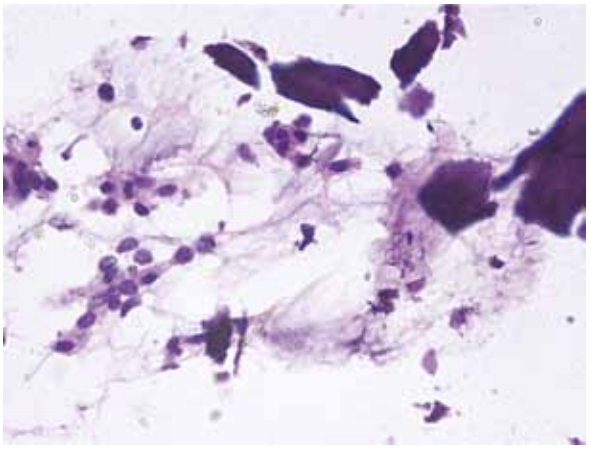

Fig. 1. *Cell smear of test group osteoplastic material: microfragments of xenogenic bone matrix Bio-Oss are surrounded with a cell-rich fibrous substance — autogenic SVF-FT. Romanovskystaining. Magnification ×200*

In most cases, the postoperative period of intraoral intervention entailed no complication and was accompanied with mild general discomfort and moderate local pain. The number of cases and the types of complications in the study groups can be seen from Table 2.

An analysis of the sinus elevation complications frequency in the studied groups revealed no statistically significant difference ($p > 0.05$). In cases where augmentation was performed on significantly reduced areas of the alveolar ridge with a relative deficit of epithelial soft tissues, some cases of seam divergence and exposure of osteoplastic material were observed. This condition poses a significant risk of the graft loss. Nary Filho et al. (2014) performed an analysis of autologous bone blocks exposed to the oral cavity after the alveolar ridge reconstruction, and detected bacterial colonization, similar in microflora species and their distribution to chronic jaw purulent osteomyelitis [11]. Our study confirms a higher resistance level of the graft containing SVF-AT to infection, even if it comes to a direct contact with the oral environment. A statistical analysis using the relative risk index (RR) shows that after the seams diverged with osteoplastic material exposure, the

Table 2. *Characteristics of early complications*

Type of surgery	Type of complication	Test group		Control group		p
		abs.	%	abs.	%	
Sinus lifting open	Acute maxillary sinusitis	0	0	1	1,9	0,486
	Encapsulation of osteoplastic material	0	0	2	3,8	0,234
	Total	0	0	3	5,7	0,111
The operation of horizontal, vertical and three-dimensional augmentation of the alveolar ridge	Early suture failure with secondary healing	8	14,0	4	6,2	0,231
	Early failure of sutures with infection and loss of the graft	1	1,8	14	21,5	<0,001*
	Total	9	15,8	18	27,7	0,131
Total		9	8,0	21	18,0	0,027*

** — statistical significance of differences in p<0,05*

probability of further infection and loss of graft was 7 times lower in the TG than in the CG (RR 0.143; 95% CI 0.022–0.922; p<0.05). The potential mechanisms of this outcome include a direct antibacterial effect of mesenchymal stromal cells that are part of SVF-AT [12, 13, 14], immunomodulation with an increase in the phagocytic M2-macrophages proportion [15, 16, 17], and accelerated graft reperfusion [18, 19, 20].

Table 3 shows the measurements results obtained from the study groups for the alveolar ridge height and width before the surgery and 6–8 months after it (prior to dental implantation).

available bone, including the reconstructed area, in TG exceeded a similar value in the CG by 20.3% (p <0.001); the width — by 7.6% (p <0.001) (see example in Fig. 2).

Note to be made that the estimated indicators depend on the volume set for the future reconstructed bone due to the size of the cortical frameworks.

In both groups' samples obtained 6 months after the osteoplasty, staining with hematoxylin and eosin allowed identifying mineralized bone, osteoid and residual particles of osteoplastic material (Fig. 2). However, the ratio of the main regenerate tissue components, their morphological features and distribution

Table 3. *Measurements of alveolar ridge, (mm), (M±m)*

Parameter	Deadline	Test group		Control group		p
		M±m	n	M±m	n	
Height	Before surgery	5,04±0,16	112	5,49±0,17	117	0,055
	After operation	9,37±0,15	112	7,79±0,14	117	<0,001*
	p1	<0,001*		<0,001*		
Width	Before surgery	3,13±0,06	112	3,46±0,01	117	<0,001*
	After operation	8,21±0,16	112	7,63±0,05	117	<0,001*
	p1	<0,001*		<0,001*		

*p — significance of differences between study groups; p1 — significance of differences before and after surgery; * — statistical significance of differences (p<0,001)*

As follows from the table, after augmentation osteoplasty, both groups revealed a statistically significant increase in the respective parameters of the reconstructed area (p <0.001); a sufficient amount of supporting bone was obtained for dental implantation. When comparing the groups, the height of the

indicated a more active and productive osteogenic process in the TG if compared with the CG. Table 4 offers a view on the results of histomorphometric analysis of trepan biopsy specimens of the groups.

As follows from the table, the relative area of the vital mineralized, i.e. regenerated, bone tissue

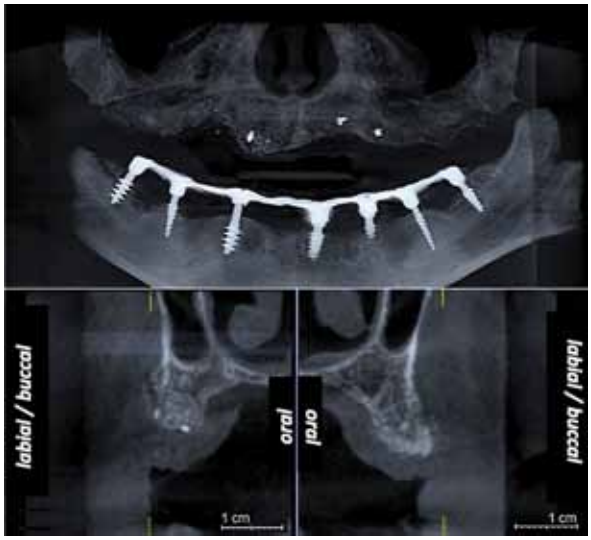

Fig. 2. *CT of the patient B-va following maxilla alveolar ridge CD-reconstruction in 6 months: in the test group (second sector) there is a double and uniform increase in the mineralized bone volume, a homogeneous trabecular structure, the recipient zone border are not visualized; in the control group (the first sector) , due to premature removal of fixing screws and partial graft loss, there was no formation of the expected volume of supporting tissues, the formed structures heterogeneity is determined due to the presence of unresorbed granules osteoplastic material, the recipient zone border of the reduced alveolar ridge are clearly visualized*

earlier placement of artificial supports following the osteoplasty using SVF-AT.

2–3 months after the installation of the intraosseous part, an initial assessment of the implant stability was carried out. In the TG, there were no cases of artificial supports non-survival registered. In the CG, osteointegration was missing in 5 dental implants (in 3 patients) including 2 implants in the upper jaw and 3 in the lower jaw; 3 out of 5 unstable implants were installed subcrestally, 2 — to the gum level. These patients underwent reimplantation after preliminary surgical interventions. The dependence between the frequency of intraosseous implant primary non-integration and the osteoplasty method was statistically significant (p = 0.032). Given the fact that the implants were identical, just like the technology for their installation and the postoperative loading conditions, the differences can be explained by optimal biological specifics of the bone tissue restored by the plastic material including SVF-AT.

SVF-AT, as part of the graft composition, has been found to contribute to the development of a better periimplant soft tissue profile with an attached keratinized gum of 2 mm or more. Due to the lack of a similar effect in the CG, at the stage of gum healing caps installation, an extra surgical intervention was often performed — apical reposition of the flaps

Table 4. *Histomorphometric evaluation results (area of tissue structures, in %)*

Bone Regenerate Components	Test group	Control group	p
Vital mineralized tissue	40,14±3,36	24,23±2,63	0,001*
Nevital Mineralized Tissue	13,31±1,59	24,98±1,97	<0,001*
Non-mineralized fabric	47,11±2,07	50,79±2,10	>0,05

** – statistical significance of differences (p<0,01)*

in the trepan biopsy specimens sections of the TG was 1.7 times larger than that of the CG (p <0.01). The relative area of non-vital mineralized tissue, reflecting the level of residual non-resorbed osteo-substituting material in the TG, on the contrary, was 1.9 times as small as that in the CG (p <0.01). There we observed a tendency towards a decrease in the number of non-mineralized tissues, including bone-marrow and fibrous tissues, in the TG samples if compared with the CG; however, the difference of 7.3% cannot be viewed as statistically significant (p>0.05) (Fig. 3).

The fact that the bone tissue content in the TG biopsy material was significantly higher than in the CG material allows us considering the possibility of

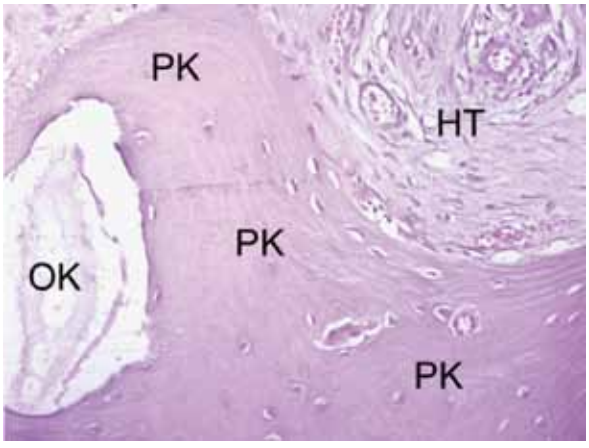

Fig. 3. *Histological section of reconstructed bone trepan-biopsy material of test group: vital mineralized tissue of regenerated bone (PK), nonvital mineralized tissue of residual osteoconductive bone substitutes (OK) and intertrabecular non-mineralized tissue (HT) are identified. 6 months after osteoplasty. Hematoxylin & eosinstaining. Magnification ×200*

to shape the required width of the attached gingiva around the artificial supports. It is a well-known fact, that keratinized gums around the implant, apart from the aesthetic value, is an important factor helping prevent gingival recession, periimplantitis and delayed disintegration [21, 22, 23]. SVF-AT contributes to an increase in the width of the attached/keratinized gingiva, increasing significantly the oral cavity vestibule size in the surgical area and reducing the risk of complications. A similar effect was described by Gjerde et al. (2018) after alveolar ridge augmentation with bioceramic scaffolds, seeded with a mesenchymal stromal cells culture isolated from the bone marrow [24]. The authors came to a conclusion that the cells have a positive effect not only on the biomaterial osteogenic remodeling yet also on the adjacent soft tissues, promoting their healing and regeneration [24]. The phenomenon in question can be explained by paracrine activity of the cells that are part of SVF-AT, and that synthesize a number of angiogenic and trophic factors [25, 26].

In the initial loading period, the indicator of peri-implant marginal bone loss (PMBL) reflects indirectly the response of the supporting structures in the proximal bone-implant contact area to chewing loads. As Table 5 shows, at any level of implant installation and through all control periods, bone loss was significantly lower in the TG if compared to the CG (p <0.001).

ment, and a significant proportion of regenerated bone tissue on histomorphograms.

Within a period of up to 5 years, the rate of successfully functioning dental implants in the TG was 98.3%, in the CG — 93.5%; within the observation period of up to 10 years — 97% and 88%, respectively. A statistical analysis confirmed the relationship between the augmentation osteoplasty method at the atrophied alveolar ridge and long-term outcome of prosthetics relaying on intraosseous implants at p<0.006. The relative risk calculation indicates that within a period of up to 5 years, the risk of losing a dental implant due to the supporting bone resorption was 11 times as low after augmentation osteoplasty using SVF-AT if compared with osteoplasty using ordinary osteoplastic materials (RR 0.099; 95% CI 0.013–0.761; p<0.05).

An example of a long-term outcome of the reconstruction of the atrophied alveolar part of the mandible and the subsequent dental implantation in a patient of the main group is presented in Fig. 4.

At later stages of observation, the sizes of the alveolar ridge area reconstructed with SVF-AT were more stable compared to the outcomes of reconstruction using conventional methods, which reveals indirectly the use of permanent bone tissue remodeling around the osteointegrated dental implants against the regular chewing load background. Within terms of up to 5 years, the horizontal size of the ridge in the TG

Table 5. *Peri-implant marginal bone loss, (mm)*

Implant installation level	Deadline after dental prosthetics	Bone loss, mm				p
		Test group		Control group		
		M±m	n	M±m	n	
Subcrestal	6 months	1,13±0,04	109	1,64±0,03	152	<0,001*
	12 months	1,47±0,08	109	2,88±0,09	152	<0,001*
p1		<0,001*		<0,001*		
To gum level	6 months	0,27±0,01	188	0,98±0,08	171	<0,001*
	12 months	0,33±0,03	188	1,69±0,08	171	<0,001*
p1		0,059		<0,001*		

*p — significance of differences between study groups; p1 — significance of differences of indicators in terms of 6 months and 12 months; * — statistical significance of differences (p<0,05)*

The differences between the groups by this indicator point at functional advantages of the supporting bone reconstructed with SVF-AT. Perhaps this is also due to the principle of structure and function interrelation, as well as to the described above macro- and microstructural features of the reconstructed alveolar ridge in TG patients, including the optimal size of the implant bearing bone, high-quality soft tissue environ-

was reduced statistically insignificantly (by an average of 0.37 mm (p = 0.103), which turned out to be 4 times as low compared to the control, where the width decreased by an average of 1.6 mm (in the CG the differences of indicators within terms of 6–8 months and up to 5 years were significant at p<0.001). The alveolar ridge height during these periods decreased statistically significantly in both groups (p<0.004),

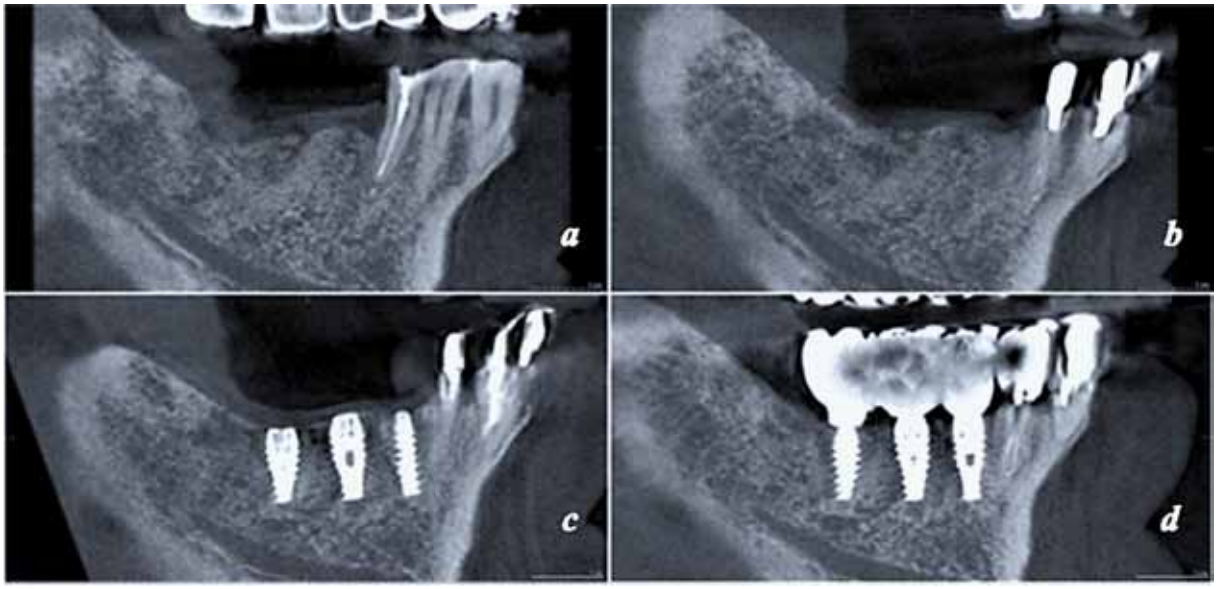

Figure 4. *Long-term clinical result of the replacement of the end defect of the alveolar crest and the restoration of the dentition of the fourth jaw segment. Computed tomography, the main research group: a) before treatment — uneven atrophy of the alveolar ridge after extraction of 45, 46 and 47 teeth; b) 6 months after the reconstruction — a sufficient amount of bone tissue was obtained to accommodate dental implants; c) 3 months after the installation of dental implants — the structure and density of the near-implant bone tissue indicate successful osseointegration; d) after 24 months of functioning of the denture structures — there is no near-implant marginal bone loss, there is no reduction in the height of the reconstructed alveolar crest, a high degree of bone-implant contact*

although the loss rate for the bone vertical dimensions in the CG was 2.8 times as high as in the TG: the average decrease was 1.67 mm and 0.59 mm in the CG and the TG respectively. Within the period of 5 to 10 years, the reduction rate for the reconstructed bone volume dropped significantly, averaging 0.08 mm in the height and 0.06 mm in the width in the TG, and 0.09 mm in the height and 0.2 mm in the width in the CG (the indicators changes for this period were not statistically significant, $p>0.05$).

CONCLUSION

The comparative study confirmed the advantage of using the proposed innovation-based approach rather than the currently common methods for reconstructing the jaw alveolar ridge. The use of autogenous stromal vascular fraction of adipose tissue (SVF-AT) as a source of regenerative cells and stimuli in the osteoplastic material composition increases the efficiency of preimplantation osteoplasty of the reduced alveolar ridge — it allows a sufficient increase in the bone size with minimal risk of complication development; proper morphological features in the reconstructed tissue; reliable osseointegration, and long-term functioning for the dental implants. This approach can be recommended as a basis for a valid surgical protocol in case of significant regression transformation of the jaw bones. This would allow a more successful and predictable use of the most advanced methods for rehabilitation of patients suffering from non-heritable edentulism using dental prosthetics on intraosseous supports.

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ASSESSMENT OF MOMETASONE FUROATE EFFECTIVENESS FOR PREVENTION OF INFLAMMATORY COMPLICATIONS AFTER SINUS LIFT SURGERY

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ABSTRACT — This article focuses on the functional changes in nasal breathing that occur after the sinus lift surgery, registered by anterior active rhinomanometry. Also, the authors of the study evaluated the effect of mometasone furoate on the postoperative period.

KEYWORDS — maxillary sinus, sinus lift surgery, nasal septa, mometasone furoate, anterior active rhinomanometry

INTRODUCTION

The use of implant-supported dental dentures is currently the standard in the functional and cosmetic rehabilitation of patients with missing teeth [1–7]. However, in many patients, implant placement is not possible without prior reconstructive surgery aimed at restoring bone volume in the alveolar process of the maxilla. To solve this problem, the most common osteoplastic surgery performed in the distal upper jaw is the sinus lift [13]. The lack of medical history, diagnosis of the pathology of the maxillary sinus and the structures of the osteomeatal complex at the preoperative stage is one of the main reasons for the development of the chronic inflammatory process after reconstructive interventions performed by the oral surgeon [14, 16, 17].

It was established that morphological changes in inflammation of the mucous membrane of the maxillary sinus are chronic. It is known that morphological changes due to inflammation of the mucous membrane of the maxillary sinus are chronic. The results of histological studies of features posttraumatic processes in the mucosa of the maxillary sinus in an experiment on animals have shown that a fracture of bone wall causes the development of alternative, exudative inflammation of the sinus mucous membrane, which is expressed in the formation of edema immediately after the injury, followed by its increase up to the seventh day. In the more remote periods, 4 weeks after injury, mucous membranes of the post-traumatic process with signs of metaplasia of the multi-row epithelium predominate with a stratified squamous cell and hyperfunction of the glandulas of the sinus [9–11].

In addition, the development of postoperative edema of the mucous membrane may contribute to the inhibition of the functions of mucociliary clearance and block the natural fistula of the maxillary sinus with the middle nasal passage [15]. Topical steroid drugs have proven themselves in the treatment of acute sinusitis, and in the prevention of the development of inflammatory complications and relapses after surgical interventions on the upper respiratory tract. The pronounced anti-inflammatory and anti-exudative effects of this group of drugs are an important condition for maintaining the functional integrity of the osteometal complex structures in the postoperative period [8, 12].

Aim

The aim of the study was to study the functional changes in nasal breathing in the postoperative period after sinus lift surgery and the impact of mometasone furoate.

MATERIAL AND METHODS

In the period from 2016 to 2019, 60 patients with partial and complete absence of teeth in the distal parts of the upper jaw were diagnosed and treated. Patients planned to manufacture implant supported dentures. Due to the insufficient vertical size of the bone tissue of the alveolar process in these areas and its low density, the patients were planned to undergo the sinus lift surgery using the lateral wall window method.

All patients underwent cone-beam computed tomography on the Vatech apparatus (South Korea) preoperatively, consultation of an ENT doctor. Based on preoperative diagnosis, abnormalities of the structures of the nasal cavity and osteomeatal complex on

the side corresponding to the planned operation were revealed in 30 patients. These included the curvature of the nasal septum (27 patients, 90%), pathology of the middle turbinate (14 patients, 46.7%), hypertrophy of the hooked process (5 patients, 16.7%), hypertrophy of the ethmoid vesicle (6 patients, 20%), Haller cells (1 patient, 3.3%), thickening of the nasal mucosa (19 patients, 63.3%) (Fig. 1).

Fig. 1. *Cone-beam computed tomography of a patient with severe deviation of the nasal septum to the left*

These patients formed a group I where a mometasone furoate (Nasonex) was added to the course of standard medical therapy prescribed after surgery by the ENT doctor to prevent possible obstruction of the natural fistula as a result of mucosal edema and the development of further complications. The dosage was 200 mcg per day (2 inhalations in each half of the nose 1 time per day). The drug was started 3 days before the operation to achieve a cumulative effect in the mucous membrane of the nasal cavity and maxillary sinus, and lasted until the 30th day postoperatively. Patients in the control group II (30 people) had no abnormalities in the anatomy, and they received standard therapy, including antibacterial drugs (Augmentin 875 mg — 1 tabular 1 hour before surgery, then 1 tab 2 times a day for 7 days), selective nonsteroidal anti-inflammatory drugs as painkillers (Nimesil — 1 pack with pain), antihistamines to reduce the effects of postoperative inflammatory reactions of traumatic origin (Erius — 1 tabl once a day for 3 days), nasal decongestants (Tizin Xylo — 1 inhalation 2 times a day for 7 days).

For the determining the functional integrity of the structures of the nasal cavity and evaluating the effectiveness of mometasone furoate, all patients underwent anterior active rhinomanometry using the ATMOS Rhino 300 apparatus.

The device allows determining the volume of the air flow and resistance separately for the right and left half of the nose, total flow (Fl.L+R) and total resistance (ResL+R), calculated by the device automatically by the formula R=ΔP/V. Free breathing is considered when Fl.L+R is more than 700 ccm/sec and ResL+R is less than 0.29 Pa. The values of the total volume flow and the total resistance for which occur violations of nasal breathing are presented in Table 1.

Table 1. *Degree of nasal obstruction*

Degree of nasal obstruction	Fl.L+R (ccm/sec)	ResL+R (Pa)
I (slight disturbances of nasal breathing)	699–500	0,29–0,39
II (moderate nasal breathing problems)	300–499	0,4–0,49
III (severe nasal breathing disorders)	Less than 299	0,5 and more

Anterior active rhinomanometry comprehensively and objectively using numbers and graphs allows you to assess the state of nasal breathing, determine which half of the nose breathes better and how much, whether the Fl.L+R figures are at or below the norm, whether volume flow increases in the right and left halves of the nose with increasing pressure or not, how high is the nasal resistance.

The quantitative variables were described by the following statisticians: the number of patients, the arithmetic mean value (M), the standard deviation from the arithmetic mean value (∂). Qualitative variables were described by absolute and relative frequencies (percentages). Differences were considered statistically significant at an error level of $p < 0.05$. The calculation was performed on a personal computer using the Numbers application.

RESULTS

All patients underwent a sinus lift using the lateral wall window method. Repeated anterior active rhinomanometry was performed on the 3rd and 30th days of the postoperative period prior to daily usage of nasal drugs. The research results are presented in Table 2.

Table 2. *The average values of the total flow (Fl.L+R, ccm/sec) and total resistance (ResL+R, Pa) in patients of the studied groups at different stages of treatment (n=60)*

	Preoperatively		3rd day post-op		30th day post-op	
	Fl.L+R	ResL+R	Fl.L+R	ResL+R	Fl.L+R	ResL+R
Group I	605,80±52,75	0,34±0,05	571,43±89,62	0,37±0,05	597,60±69,75	0,34±0,04
Group II	755,17±32,19	0,24±0,03	669,70±89,27	0,29±0,05	731,30±56,98	0,25±0,04

DISCUSSION

Analysis of the total volume flow (ccm/ses) and total resistance (Pa) in patients who were prescribed Nasonex in the postoperative period showed that on day 3, the reduction in Fl.L+R compared to the preoperative values was 5.67%, while the ResL+R increased by 9.52% (Fig. 2).

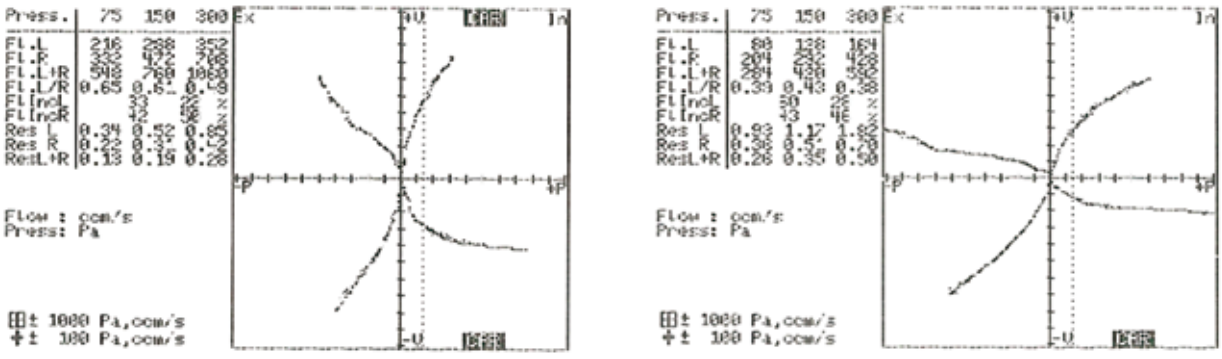

Fig. 2. *Research results of anterior active rhinomanometry*

In the group of patients who did not receive the mometasone furoate Fl.L+R, in the same period of time, decreased by 11.32%, while the ResL+R increased by 20.81%. These differences were significant ($p < 0.05$). On the 30th day of the postoperative period, the values were as follows: in group I patients, there was a decrease in Fl.L+R, compared to the preoperative, by 1.35%, an increase in ResL+R, by 1.28%; in group II, the difference in Fl.L+R was 3.16%, ResL+R — 5.73%. Graphs of changes in the values of the total volume flow and the total resistance at various stages of treatment are shown in Fig. 3.

CONCLUSION

The sinus lift surgery is associated with a large amount of injury caused by damage to the bony walls of the maxillary sinus and the separation of its mucous membrane. Edema that develops in the postoperative period can lead to obstruction of the natural fistula, especially in the presence of a concomitant pathology of the nasal cavity. The mometasone furoate, prescribed in the course of accompanying drug therapy, has a pronounced anti-exudative effect and has a positive effect on maintaining the functional integrity of the structures of the nasal cavity and osteomeatal complex. This allows to reduce the number of inflammatory complications resulting from impaired drainage and sinus aeration in the postoperative period.

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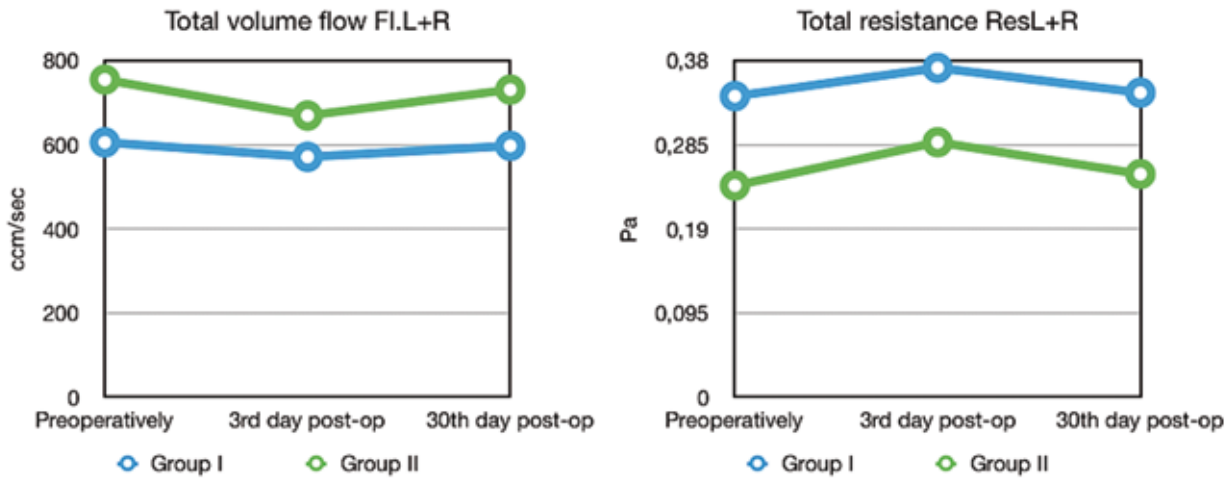

Fig. 3. *Graphs of changes in the total volume flow (ml / s) and total resistance (Pa)*

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CLINICAL AND LABORATORY EVALUATION OF THE EFFECTIVENESS OF THE APPLICATION OF NONSTEROID ANTI-INFLAMMATORY PREPARATION OF THE COXIB GROUP

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INTRODUCTION

Most surgical dental procedures in the pre- and postoperative periods are accompanied by pain sensations of greater or lesser intensity, so pain control is one of the most urgent problems of dentistry [3]. The presence of pain significantly reduces the quality of life, ability to work and social activity of patients. Pain that occurs in the maxillofacial region is most often associated with the development of the inflammatory process, both infectious and traumatic origin. Effective pain therapy should include a clear understanding of the mechanisms of its development, as well as the integrated use of medication that affect the main links of pathogenesis.

There are several groups of drugs used for pain relief. The most common group of drugs for this purpose is a group of non-steroidal anti-inflammatory drugs [4, 5]. They have anti-inflammatory, analgesic, antiplatelet and antipyretic effects.

A common element of the mechanism of action of NSAIDs is the inhibition of the synthesis of prostaglandins (PG) from arachidonic acid by inhibiting the cyclooxygenase enzyme (COX) [2]. By the ability to predominantly inhibit COX-1 or COX-2, NSAIDs of three groups are isolated: non-selective, inhibiting the activity of both enzymes; NSAIDs, predominantly suppressing COX-2 and having a weaker effect on COX-1; selective for COX-2, which almost do not have effect on COX-1. According to a number of authors, among all appointments in surgical practice, more than 70% fall to non-selective NSAIDs (ketoprofen, ketorolac, ibuprofen) that have activity against both cyclooxygenase-1 and cyclooxygenase-2.

The main negative property of all NSAIDs is the high risk of the development of undesirable reactions from the gastrointestinal tract (GIT) [17]. At present, a specific syndrome, NSAID-gastroduodenopathy, has even been isolated [5]. The expansion of the range of drugs for the treatment of pain syndrome facilitates the choice of drugs based on the point of application and the mechanism of their action. A pressing issue is the objective assessment of the degree of inflammatory response and assessment of the dynamics of anti-inflammatory therapy conducted, as well as the introduction of new, modern drugs into surgical dentistry [1, 9]. To reduce the effects of NSAIDs on the mucous membrane of the gastrointestinal tract, a new highly selective drug Etoricoxib (Arcoxia) was developed. Arcoxia is a selective inhibitor of COX-2 with pronounced analgesic and anti-inflammatory action, has a rapid onset of action and prolonged relief of pain [10, 19]. The inflammatory response is a universal protective and adaptive response to exposure to exogenous and endogenous damaging factors, including in the surgical area [14, 18].

Biologically active substances such as histamine, interleukins (IL), products of arachidonic acid metabolism provide stimulation of the inflammatory reaction. The basis of the pathogenesis of tissue damage is the launch of the cytokine cascade, which includes, on the one hand, pro-inflammatory cytokines, and on the other, anti-inflammatory mediators. Hyperproduction of cytokines leads to the development of a systemic inflammatory response and may cause the development of a number of pathological conditions, in particular, septic shock. A balance of pro- and anti-inflammatory cytokine production is a prerequisite for maintaining perioperative immune homeostasis. Some studies have also shown that in the development and maintenance of chronic inflammation in periodontal tissues, increased production of matrix metalloproteinases (MMPs) is important — groups of enzymes that are produced by different cells and microorganisms of

the oral cavity, participate in the mechanisms of the immune response, break down most- proteins of the extracellular matrix and the basement membrane [6, 7, 11-13, 15, 16]. Under the conditions of the disease, MMPs are involved in the degradation and processing of a variety of growth factors, cytokines and substances involved in apoptosis, cell adhesion and, together with other extracellular proteinases, can cause irreversible damage to tissues [20-26]. In this regard, the study of the level of MMP and related growth factors and proinflammatory cytokines could be an additional criterion for evaluating the effectiveness of NSAIDs, taking into account the point of application and the mechanism of their action.

The aim of the study is to substantiate the anti-inflammatory effect of Etoricoxib based on the study of the dynamics of IL-6, MMP-2, MMP-8 and vascular endothelium growth factor (VEGF) in the oral fluid of patients during surgical dental procedures.

MATERIALS AND RESEARCH METHODS

The study included 28 patients (12 men and 16 women) aged from 20 to 35 years who underwent examination and treatment at the Moscow State University of Medicine and Dentistry named after. A.I. Evdokimov of the Ministry of Health of Russia at the Department of Surgical Dentistry with a diagnosis of dental retention (K01.0), dystopia of the teeth (K07.3). Patients were divided into groups as follows:

The study group: 11 patients without somatic pathology, not undergoing treatment with the use of drugs, with no pathology on the part of periodontal tissues; they underwent planned surgical manipulations (removal of third molars) with the appointment of local antiseptic therapy and etoricoxib 90 mg according to the following scheme: 1 tablet per day 2 days before surgery, 1 tablet per day of surgery and subsequent days if there is pain 1 tablet per day.

Control group: 17 people — conditionally healthy patients with healthy oral cavity who did not undergo surgical interventions.

Before treatment, all patients underwent a comprehensive examination, which consisted of a survey, clarification of complaints, collecting a history of illness and anamnesis of life, finding out the presence of concomitant general somatic pathology and the fact of taking medications. Further, skin and visible mucous membranes, face configurations, and palpation of regional lymph nodes were evaluated, and then the oral cavity was examined. The degree of mouth opening, the state of the mucous membrane of the vestibule and the oral cavity itself was evaluated. All the teeth were examined. Particular attention was paid to the state of periodontal tissues. Patients with the presence of clinically severe pathology of periodontal tissues (gingivitis and acute periodontitis) were excluded from the study group. All patients had no contraindications for taking Etorikoksib, which was confirmed by examination by a general practitioner. All patients signed voluntary informed consent.

Clinical evaluation of the local inflammatory process was performed on days 1, 3, 5, and 7 after the operation, based on the degree of edema and hyperemia of the postoperative mucosa, as well as the subjective assessment of patient pain by an 11-point digital rating scale of pain intensity, which consists of 11 points from 0 *no pain* to 10 *pain that cannot be tolerated*. (Visual analogue scale of pain, VAS).

46 samples of unstimulated saliva were tested. In the study group, markers were determined according to the following scheme: first collection — 2 days before surgery, then patients were prescribed etoricoxib 90 mg, 1 tablet per day, second collection — on the day of surgery (before the manipulation), third collection — 7 days after the manipulation. Most patients received etoricoxib for 2 days after surgery. In the control group, a single collection of mixed saliva was performed.

Methods of collecting saliva: the collection was carried out in the morning, on an empty stomach in 1st and 3rd collection and after at least 2nd hours in the second collection, in graduated tubes. Before the procedure, the patient rinsed his mouth with distilled water for 30 seconds, followed by 5 minutes of rest. Then the patient swallowed all the accumulated saliva, after which the direct collection of material began for 15 minutes. At the end, the tube was tightly closed with a lid, placed in a container with ice and delivered to the laboratory for one and a half hours. In the laboratory, the tubes were centrifuged at a speed of 3000 rpm for 10 minutes at 4° C, after which the saliva was frozen and stored at -80° C until the test. Concentrations of MMP-2, MMP-8, IL-6 and VEGF were determined using Human MMP-2 (total), Human MMP-8 (total), Human IL-6 and Human VEGF (Quantikine®, R & D Systems, USA) in accordance with the manufacturer's instructions. Before starting the determination, saliva samples stored at -80° C were thawed at room temperature, centrifuged at 10,000 rpm for 2 minutes, and diluted 100 times with standard diluent solution included in the kit. The measurements were performed on a BEP 2000 Advance automatic immunoassay analyzer (Siemens Healthcare Diagnostics, Germany). The contents of MMP-8 and MMP-2 were expressed in nanograms (ng), IL-6 and VEGF — in picograms (pg)

per 1 ml of saliva. The obtained data was processed using the program "Statistica 7.0". When comparing indicators and analyzing their interrelationships, the non-parametric criteria of Mann-Whitney, Kruskel-Wallis, Wilokson pair test, Friedman ANOVA were used. Differences and correlations were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The initial content of MMP-2 and VEGF in the saliva of patients who then underwent surgery and who received Etoricoxib at a dose of 90 mg was significantly lower than in the control group (Table 1). The reduction in MMP-8 and IL-6 levels was not statistically significant.

Table 1. *Content of MMP-2, MMP-8, IL-6 and VEGF in mixed saliva of patients who received 90 mg of etoricoxib before and after surgery, and the control group*

Group		N	MMP-2	MMP-8	IL-6	VEGF
Control		17	17,0 14,5-18,6	143 96-278	7,7 3,8- 11,5	2899 1924-3338
Patients receiving 90 mg of Etoricoxib	Visit 1	11	12,01 10,1-13,3	110 66,2-194	5,4 1,6-9,4	16421 1352-2510
	Visit 2	10	13,9 11,4-17,0	133 40,8-278	13,4 3,5-17,8	14971 1147-1859
	Visit 3	8	14,21 12,5-15,7	111 68,4-142	23,61 13,9-67,1	18461 1454-2249

** — Indicators of median, lower and upper quartile are presented (25-75%); 1 p <001 with respect to control*

In the postoperative period, the levels of MMP-2, MMP-8 and VEGF did not significantly change, and the level of IL-6 significantly increased ($p = 0.011$ by Friedman ANOVA test) from 5.4 to 23.6 pg / ml by median.

As clearly shown in the graphs, in most patients, the level of MMP-2 (Fig. 1) increased immediately before the operation while receiving etoricoxib (visit 2) and returned to the initial level 7 days after it (visit 3). The level of MMP-8 changed in different directions (Fig. 2): it increased in 6 and decreased in 5 patients. VEGF level slightly decreased or remained almost unchanged (Fig. 3), whereas IL-6 increased significantly after taking etoricoxib before the operation and continued to increase a week after it in all examined patients (Fig. 4).

Pain syndrome was observed only in 2 out of 11 patients of the studied group. Significant differences in the initial levels of markers, as well as the dynamics of their changes on the background of the drug intake and surgical intervention in these patients, have not been found from the indicators of patients without a pronounced pain syndrome. Thus, during reception of the non-steroidal anti-inflammatory drug etoricoxib at a dose of 90 mg, all patients experience an increase in the level of the pro-inflammatory cytokine IL-6 in unstimulated saliva, which lasts a week after the surgery 5–6 days after stopping the drug. Changes in the levels associated with inflammatory processes in paradont MMP-8 and MMP-2, as well as the stimulator of neoangiogenesis VEGF has a multidirectional nature, not associated with the presence of pain and the effect of treatment.

In the studied group, edema and hyperemia of the mucous membrane of the postoperative region in 87% of cases were expressed on the second and third days after surgery, on the fifth day their decrease was noted at 92%, on the seventh day the absence of edema was 100%. 98% of patients in study group noted the absence of pronounced pain syndrome and the absence of the need to take NSAIDs after the intervention, 73% of patients in subgroup 2 of the study group stopped taking Etoricoxib 90 mg on the third day after the intervention, noting the absence of pain, which can indicate the presence of a cumulative effect when taking the drug.

Clinical results showed intense regression of signs of local inflammation, such as edema and hyperemia of the mucous membrane of the post-operating area, as well as a marked reduction in pain sensitivity in patients of study group, which indicates a high anti-inflammatory and analgesic activity of the drug etoricoksib 90 mg.

When studying the levels of proinflammatory cytokines, matrix metalloproteinases and vascular endothelial growth factor in the mixed saliva of patients, adequate dynamics of these factors was established when prescribing Etoricoxib 90 mg in patients during surgical dental surgery, but generally reflecting the pharmacokinetics and pharmacodynamics of Etoricoxib drug.

As a result of the study, a drug Etoricoxib 90 mg revealed pronounced analgesic and anti-inflammatory activity during outpatient surgical interventions. In all cases, patients noted the absence of pain in the postoperative period for 3 days (after 4 days of taking the drug).

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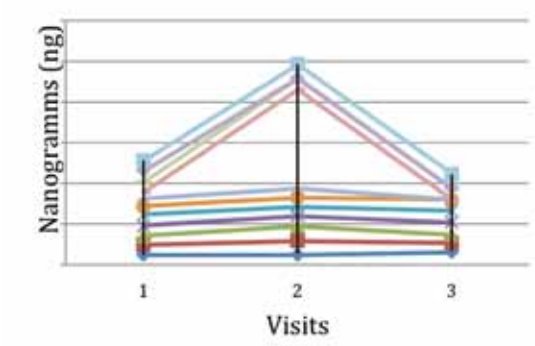

Fig. 1. *Dynamics of the content of MMP-2 in the saliva of individual patients*

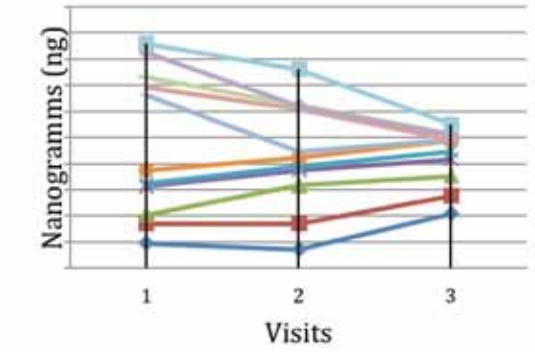

Fig. 2. *Dynamics of the content of MMP-8 in the saliva of individual patients*

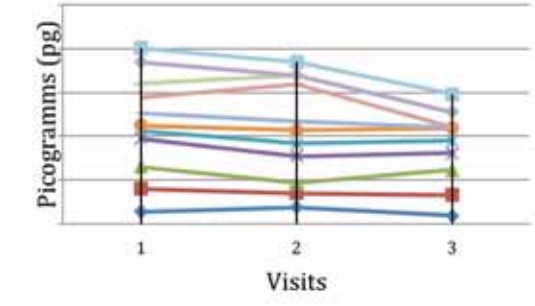

Fig.3. *Dynamics of the content of VEGF in the saliva of individual patients*

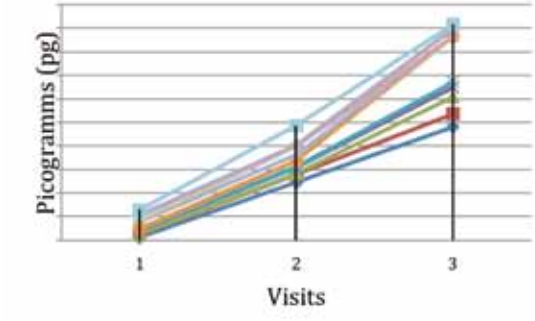

Fig. 4. *Dynamics of the content of IL-6 in the saliva of individual patients*

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SPECIFIC FEATURES OF CENTRAL POINT LOCATION BETWEEN INCISORS IN PEOPLE WITH PHYSIOLOGICAL OCCLUSIONS

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Specific features of human front teeth location have been presented in respective works, which serves as proof to the relevance of this study, both in applied and in clinical aspects [1, 6, 8]. The said parameters are taken into account when modeling teeth for academic purposes, when determining the gnathic and dental types of dental arches, when diagnosing occlusion anomalies, and when designing artificial dental arches in complete removable dentures [2, 3]. Specific features of teeth inclination in people with various types of dental arches have been presented and explained here for clinical use. There has been offered torque estimates corresponding to standard, low and high values [5]. Data on the teeth inclination with regard to the gender dimorphism have been presented as well [10]. In clinical orthodontics, when selecting prescription braces used in arch equipment method, special importance is attached to the teeth inclination in the vestibular-lingual direction, which is called the inclination angle, or the teeth torque [4]. Experts state that the value of the teeth inclination affects the position of the key teeth regarding the major anatomical marks [7, 9, 11, 12]. However, no accurate data on the inter-arch distance is to be found in the respective literature. Yet, this parameter is important when modeling artificial dental arches in people with lengthy dental defects and/or with complete secondary edentulism. All of the above explains not only the relevance of the issue in question, yet may allow solving specific clinical tasks when offering prosthetic treatment to patients, which was the purpose of the present study.

Aim:

to identify the interincisal point deviation from the anterior part of the alveolar arch in people with physiological occlusions.

MATERIALS AND METHODS

A survey was carried out involving 118 people with orthognathic occlusion.

3 groups were identified. Group 1 patients featured teeth retrusion while the torque values ranged among low values. This group included patients with mesognathic microdontic dental system and brachygnatism with normo-and microdontism. Group 2 featured average values for the teeth torque (mesognathism with normodontism, dolichognathism with macrodontism, and brachygnathism with microdontism). Group 3 patients had high torque, which was due to the front teeth protrusive position in people with mesognathism and a macrodontic dental system, as well as in case of dolichognathism with normo- and macrodontism. The types of dental arches were identified following the recommendations obtained from experts. The distance between the central points of the dental and alveolar arch was determined by the cone-beam computed tomography method. The obtained images were used to select the necessary tomogram sections, and then from the alveolar arch point in the medial incisors area, a perpendicular was drawn to the occlusal plane. Software was used to measure the distance from the constructive point to the vestibular edge of the upper jaw medial incisor, which corresponded to the interdental point location, or the dental arch central point. The statistical data was processed subject to generally accepted methods employing personal computer statistical analysis software.

RESULTS AND DISCUSSION

The measurements based on the cone-beam tomograms revealed that Group 1 patients with mesognathism and microdontism had an inter-arch distance between the dental arch interdental point and the alveolar arch central point at 4.0 ± 0.92 mm. The

dental arches were narrow. In case of brachygnathic microdontic dental arches, the distance between the dental arch interdental point and the alveolar arch central point was 3.87 ± 0.92 mm. The transverse parameters of the dental arches had average values. At the same time, in people with brachygnathic types of dental arches with normodontism, the distance between the examined central points of the dental and alveolar arches was 4.11 ± 0.84 mm. The arches were wide. Therefore, regardless of the dental arch width, the distance between the central points of the dental and alveolar arches with retrusive incisors in Group 1 was about 4 mm.

Standard values of the inclination angle (incisors torque) were observed in Group 2 patients with dolichognathic microdental type of dental arches. However, the dental arches were narrow. Nevertheless, the inter-arch distance was 7.0 ± 0.79 mm. The patients of this group with mesognathic normodental type of dental arches had a distance between them at 7.08 ± 0.85 mm, while the dental arches were of medium size. In case of the brachygnathic macrodontic type of dental arches, the distance between the central points of the dental and alveolar arches was 6.97 ± 0.91 mm, yet the dental arches were wide. Therefore, in patients of Group 2, regardless of the dental arches width, the distance between the central points of the dental and alveolar arches at the incisors neutral position was about 7 mm.

Group 3 patients featuring dolichognathic normodontic dental arches were found to have high values of torque. The distance between the arches central points was 8.97 ± 0.83 mm. Patients with dolichognathic macrodontic dental arches had the distance between the arches' central point's equal to 9.11 ± 1.02 mm, while the dental arches were of medium size. In people with mesognathic macrodontic dental arches, the distance between the arches central points was 8.79 ± 0.93 mm, but the dental arches were wide. Given that, regardless of the dental arches width, in patients of Group 3 the distance between the central points of the dental and alveolar arches with protruded position of the incisors was about 9 mm.

CONCLUSION

In view of the above, the shape and size of the dental arches do not have a significant impact on the distance between the central points of the dental arch and the alveolar arch. The inter-arch distance is determined by the teeth inclination in the vestibular-lingual direction. The distance between the central points of the dental and alveolar arches with the retrusive position of the incisors was about 4 mm, with the neutral position of the incisors — 7 mm, and with the incisors protrusion — 9 mm. The results of the study can be employed in orthopedic dentistry for simulating artificial dental arches in people with complete secondary edentulism.

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SPECIFIC FEATURES OF GRINDER TEETH ROTATION AT PHYSIOLOGICAL OCCLUSION OF VARIOUS GNATHIC DENTAL ARCHES

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ABSTRACT — The article offers a view on the outcomes of analyzing the convergence angles of conditional occlusal midlines passing through the middle of the occlusal surface and intersecting with the median sagittal plane, which allows determining the specifics of the upper and lower jaws grinder teeth rotation. The subject of the study was the cast models of the jaws obtained from 64 persons with physiological occlusion, a full set of permanent teeth and various gnathic (mesognathia, brachygnathia, dolichognathia), and dental (macrodontia, normodontia, microdontia) types of dental arches. Detailed examination of the data regarding grinder teeth rotation in view of the gnathic and dental type of dental arches in people with physiological occlusion, allowed us to develop, substantiate and test an examination algorithm for patients with torsioversion to be found in almost all types of anomalies and deformations of dental arches. This algorithm allowed increasing the reliability of measurements (linear, angular) in the transversal plane; identifying the degree of complexity, and selecting the treatment tactics; describing the variability of the convergence angles for grinder teeth with different gnathic and dental types of dental arches, and evaluating the effectiveness of orthodontic treatment in patients featuring dentition abnormalities and deformations.

KEYWORDS — teeth torsioversion; physiological occlusion; tooth convergence angle; gnathic, dental type of dental arches; biometry of jaws cast models.

INTRODUCTION

Lack of teeth rotation (torsioversion, torto-occlusion) is one of "the six keys to normal occlusion" proposed by Andrews L.F. in 1967 [1]. That was mentioned by specialists who described the major types of physiological occlusal relationships [2, 3, 4].

Based on the occlusion keys, the major gnathic and dental arches types were identified where the basic parameters were offered in the sagittal and transversal directions. The obtained data allowed the authors to identify wide, narrow and medium dental arches [5, 6]. There has been a relationship detected between the main dimensions of the dental arches and the facial parameters, which is reflected in the modern face and dental arches classifications [7, 8, 9].

Numerous studies have revealed age- and race-related features of the craniofacial structure [10,11]. Gender-bound dimorphism of human dental arches in view of the head measurements has been identified [12, 13].

This, in turn, determined the indications for the choice of orthodontic and orthopedic treatment methods, taking into account individual specifics of the dental system [14, 15, 16]. The effectiveness of the proposed research methods for treating children with congenital face and jaw disorders has been demonstrated [17, 18].

Modern methods of biometric study of jaws cast models are diverse, reliable and meet the examination standards for people with different anomalies [19, 20]. The data of a biometric study focusing on jaw models served the basis for mathematical modeling and graphic construction of individual shapes of dental arches [21–30]. Methods of X-ray examination, including cone-beam computed tomography for studying dental arches, jaw bones and the craniofacial complex as a whole [31, 32, 33, 34], have been proposed. The need for studying the temporomandibular joint has been demonstrated taking into account the front teeth location and the dental arches shape [35, 36].

The variety of research methods and clinical types of maxillofacial anomalies urge the authors to developing examination algorithms that are recommendatory in nature [37–43]. Torsioanomalies have been observed in almost all types of dental arches anomalies and deformations; however, there is virtually no information available regarding the methods for determining this anomaly [44–52]. In the available literature, we found no data on teeth rotation magni-

tude at physiological occlusion, which determined the aim of our study.

Aim of study:
to identify the specifics of grinder teeth rotation at physiological occlusion of various gnathic types of dental arches.

MATERIALS AND METHODS

The study involved 64 persons with physiological occlusion and a full set of permanent teeth. The studies were carried out in an age group belonging to the first mature age (21–35 years), in view of the biomedical ethics principles with a voluntary informed consent obtained. The patients were divided into three groups depending on the dental arches shape. 20 people of Group 1 had the shape of the dental arches identified as wide, while the gnathic and dental indicators corresponded to mesognathic macrodontic, brachygnathic normodontic and brachygnathic macrodontic types of dental arches. Group 2 included 23 persons with a medium size of the dental arches, while the main types of dental arches were mesognathic normodontic, brachygnathic normodontic and dolichognathic macrodontic ones. Group 3 consisted of 21 patients with narrow dental arches, which were to be found in people with mesognathic microdontic, dolichognathic micro- and normodontic types, which is consistent with the classification of experts who pointed at the size of dental arches as an important factor for selecting the treatment method [2].

All the patients received cast models of the jaws, which were photographed for further analysis. On the photograph, in occlusion norm, reference points and lines were applied. In the transversal direction, a line was drawn connecting the points located on the distal vestibular tubercles, than the center of the line was identified, where the reference point was set. That point was connected to the interincisal (central) point of the dental arch located between the medial incisors near the crowns occlusal contour. The resulting line was marked as the median sagittal plane (line) of the dental arch in question (Fig. 1).

On each tooth's vestibular and lingual surface, the midpoints position was identified with target points put, through which the median occlusal lines were drawn until the intersection with the model median sagittal line. The convergence angles of the indicated lines were measured. The data obtained through the study were subjected to statistical processing using the methods of parametric and non-parametric analysis following the outcomes of testing the compared sets for their normal distribution. The statistical analysis was performed using IBM SPSS Statistics 23 software.

RESULTS AND DISCUSSION

The study of the dental arches allowed obtaining data on grinder teeth rotation in view of the gnathic type of dental arches in people with physiological occlusion. It showed that the lateral teeth are located in the dental arch in such a way that their chewing surface' conditional occlusal midlines intersect in the mid-sagittal plane projection thus shaping an angle turned towards the front teeth. During that, the convergence angle of the first premolars in people with wide dental arches was 120.21±1.28°, with medium dental arches it was 125.13±1.16°, and for narrow arches the similar index was 130.09±1.28°. Moving further away from the dental arch center towards the lateral teeth, the convergence angle increased, and for the second upper premolars, its size in case of wide arches was 125.13±1.22°, for the medium arches it was 130.11±1.12°, and in people with narrow dental arches the value was 135.08±1.31°. A similar pattern was observed for the first molars located sixth in the dental arch. In case of wide, medium and narrow arches, the convergence angle was 135.23±1.14°, 140.03±1.08° and 145.13±1.06°, respectively. The upper jaw second molars had the largest rotation of all the grinding teeth. At the same time, the convergence angle in people with wide dental arches was within 144.84±1.31°. With medium and narrow types of dental arches, the angle was 150.02±1.34° and 155.11±1.43°, respectively (Fig. 2).

Therefore, the upper jaw grinding teeth feature the rotation of the mesial part to the median sagittal. The rotation was the highest in people with wide arches where the convergence angle of the conditional occlusal midline of the chewing surface was the smallest. In case of narrow dental arches, the rotation was the lowest in case of an increased convergence angle.

A similar situation was observed in the lower jaw grinding teeth (Fig. 3).

The rotation of the first lower premolars in people with wide arches was higher than in people with narrow dental arches. The convergence angle was 125.16±1.19° and 134.88±1.25°, respectively ($p \leq 0.05$). In patients with medium dental arches, the convergence of conditional occlusal lines was 130.05±1.27°. In the second premolars, compared with the first ones, the rotation increased, which led to an increase in the convergence angle: 130.21±1.17° for wide dental arches; 135.26±1.24° for medium arches, and in cases with narrow dental arches it was 145.18±1.26°. For the first lower molars with wide, medium and narrow dental arches, the convergence angle was 139.83±1.21°, 144.94±1.09° and 150.04±1.14°, respectively. For the second lower molars, the convergence of teeth with

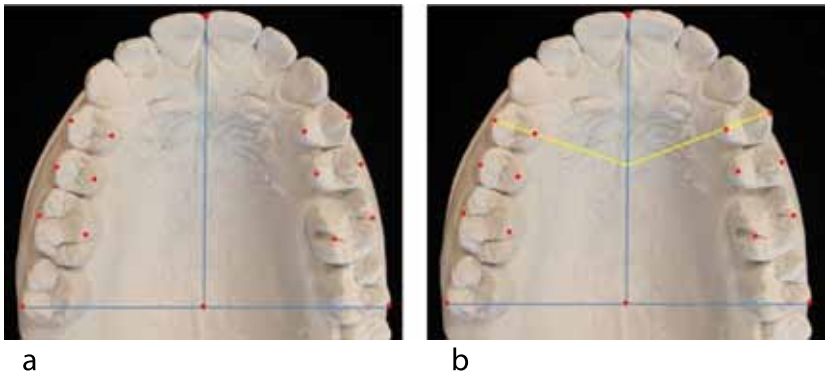

Fig. 1. Applying reference lines and points on the model image (a) to identify the convergence angle for median occlusal lines (b)

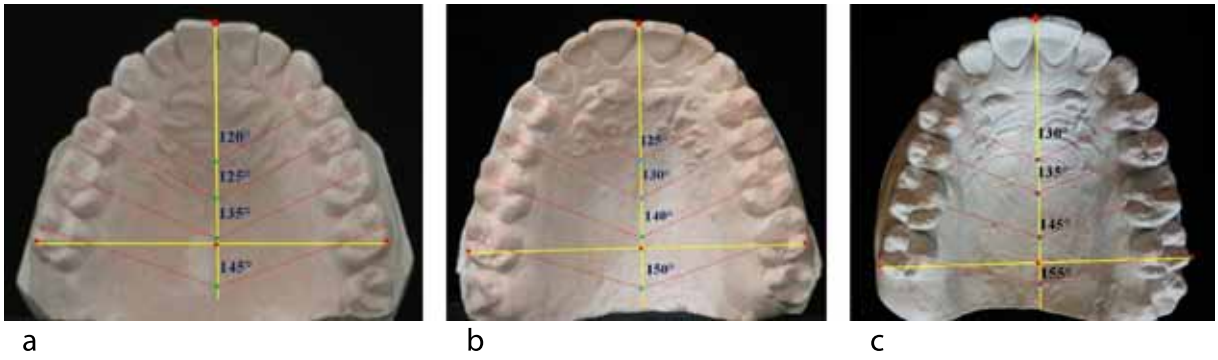

Fig. 2. Rotation specifics of upper jaw teeth in people with wide (a), medium (b) and narrow (c) dental arches

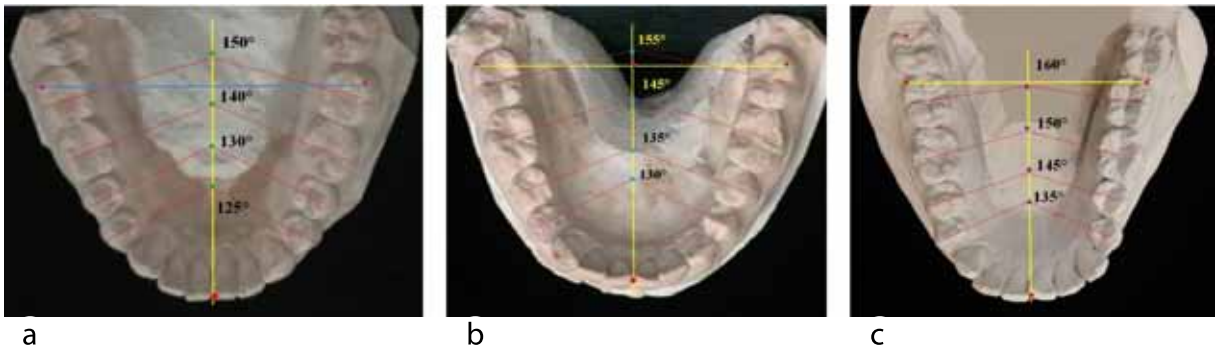

Fig. 3. Rotation specifics for lower jaw teeth in people with wide (a), median (b) and narrow (c) dental arches

wide dental arches was 150.12±1.14°. With medium and narrow types of dental arches, the angle was 154.99±1.31° and 160.16±1.02°, respectively.

Given the above, as far as the lower jaw is concerned, almost all the parameters that determine the teeth rotation, were on average 5 degrees above those relate to the antagonists.

CONCLUSIONS

1.Based on the measurement of the convergence angle for the conditional lines passing through the middle of the occlusal surface and intersecting the median sagittal plane, the new method for identifying the grinder teeth rotation is reliable, diagnostically meaningful, and can be employed as an additional criterion to describe the physiological occlusion of dental arches belonging to various gnathic types, for diagnosing lateral teeth torsioanomalies, as well as for evaluating the effectiveness of orthodontic treatment in patients with dentofacial pathology.

2.Regardless of the gnathic and dental types of arches of both jaws, almost the all indicators that

determine the teeth rotation in the lower jaw exceed similar parameters in the upper jaw, on average by 5 degrees, which can be used in clinical orthodontics to identify treatment tactics and its effectiveness in people with various gnathic and dental types of arches.

3.The introduction into clinical orthodontics the data obtained through this study regarding the convergence angles for the upper and lower jaws premolars and molars in patients with various gnathic and dental types of arches, will allow reducing the time spent at the early diagnostics stage, and improve the efficiency of monitoring the orthodontic correction outcomes.

4.People with wide dental arches with the smallest convergence angle of the grinding surface conditional occlusal median lines feature the highest level of the grinding teeth rotation in both jaws. People with narrow dental arches who had the largest convergence angle were found to have the lowest grinding teeth rotation in both jaws.

5. Improving the existing methods used for diagnosing dental anomalies resulted in the development of algorithms that can be employed to examine patients with torsioanomalies that occur in virtually all types of dental arches issues and deformations, and introducing the algorithms in question may allow not only optimizing biometric instrumental measurement techniques on cast jaw models yet also ensuring optimal functional and aesthetic results due to the predictability of orthodontic treatment.

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SPECIFIC FEATURES OF TRANSVERSAL AND VERTICAL PARAMETERS IN LOWER MOLARS CROWNS AT VARIOUS DENTAL TYPES OF ARCHES

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ABSTRACT — The article offers a view on the outcomes of morphometric studies focusing on vertical, transverse parameters of the lower large molars in patients with various dental types of arches. The subject of the study was the lower molars segments obtained from jaws cast models of 65 people with physiological occlusion, a full set of permanent teeth and various types of dental system (Group 1 — normodontia; Group 2 — macrodontia; Group 3 — microdontia). For transversal direction morphometry, the vestibular-lingual size of the tooth crown and neck were used, as well as the intertubercular distance. To measure the vertical parameters, the height of the vestibular, lingual odontomere and that of the tubercle were identified. The results of the study show that people with physiological occlusion and permanent teeth macrodontia, the vertical, transversal parameters exceed those featured by people with normodontia and microdontia types of the dental system. The obtained odontometric features can be used through diagnosing occlusal relationships and identifying various pathological, physiological abrasions of varying degrees of occlusal surface loss.

KEYWORDS — jaws cast model biometry, lower jaw molars, physiological occlusion, tooth morphological structure.

INTRODUCTION

The specific feature about the large molars group is the multitubercle shape of the occlusal surface. The complexity of the odontomers structure and their variability have been presented from the point of gender and race dimorphism, as well as in view of age-related changes in the masticatory apparatus [1–6]. Odontometry issues have key positions in clinical dentistry, morphology, odontology, and forensic medicine. At the same time, people with different types of face and dental arches reveal variability of morphometric odontological parameters [7–13]. Modern classifications of face types and dental arches in people with physiological occlusion have been presented [14–19]. Teeth measurements in orthodontic clinics are the basic positions when it comes to diagnosing pathology of occlusal relationships and selecting treatment methods [20–26].

Methods of dental arches biometric study are diverse and imply not identifying linear dimensions alone, yet also allow reproducing the shape of dental arches via mathematical simulation [27–29].

Methods for maxillofacial X-ray examination have been presented, which allow identifying the key teeth position, molars in particular, to further choose the treatment method [30–39]. It has been noted that the size of the teeth is largely determined by the head facial part parameters, in particular, the diagonal dimensions that correlate with the size of the teeth and the dental arches in both jaws [40, 41].

The morphometric features of the teeth and dental arches in people with various types maxillofacial anomalies and deformations have been shown, as well as differential diagnostics algorithms have been presented [42–46].

Odontometric studies serve the basis for the choice of methods to treat patients with occlusion issues and dental arches defects of various length and localization, including full edentulism, and offer criteria for determining the effectiveness of treatment [47–50]. However, the presented works, as a rule, have odontometry measuring the mesial-distal size of the teeth crowns. It is very rarely that studies focus on measuring the transversal and vertical size of teeth, and even if so, they are carried out on the dental-maxillary segments isolated from native preparations [51].

Physiological abrasion, which is a loss of occlusal surface within the enamel, is of adaptive nature and is a factor preventing teeth functional overload. Physiological abrasion, as a slow-progressing compensated process, improves the chewing function and establishes

conditions for the lower jaw free movement, as well as for the smooth sliding of the tooth rows through various articulation stages. Pathological abrasion, as a rapidly-progressing process of the teeth enamel and dentine abrasion, causes changes in the dental and nearby tissues, and is characterized by functional disorders of the temporomandibular joint and masticatory muscles. Reducing height of the tooth crown with pathological abrasion affects the size of the face gnathic part, which requires prosthetic measures and use of structural materials [52–55].

Identifying the boundaries between the physiological and pathological teeth abrasion is an urgent task for clinical dentistry. Given that, relevant are the issues of studying the chewing surface tubercles in case of physiological norm, which determined the aim of this study.

Aim of study:
to evaluate the height of crowns and tubercles of the lower large molars with permanent teeth physiological occlusion.

MATERIALS AND METHODS

For this study, 65 people (29 men, 36 women) aged 21–35 years old were examined with physiological occlusion and integral dentition. After receiving the prints, diagnostic models from super-gypsum class III "Elite Model" ("Zhermack", Italy) were cast. Measurements of the parameters of the teeth and dental arches were carried out on the obtained diagnostic models. A digital caliper "NORGAU ABS" was used as a toolkit (division value 0.01mm). Odontometric studies were carried out according to the method of A.A. Zubov (1968), and included the definition of mesio-distinal and vestibular-lingual size of the crown. The longitudinal length of the dentition was calculated by the Nance method, as the sum of the mesial-distal diameters of the teeth forming it. The third molars were not taken into account in the measurements, since they are as variable as possible (Fig. 1).

Measurement was carried out on the lower molars segments obtained from 65 pairs of jaws cast models. The models were divided into three groups — with normodontia, macrodontia and microdontia.

Given the data from previous studies, dental arches were referred to normodontia where the length (or the sum of the mesial-distal crown size of 14 teeth) ranged from 112 to 118 mm (24 pairs of cast models). Sizes below or above the specified range were attributed to micro- (22 pairs of cast models), or to macrodontia (19 pairs of cast models) (Fig. 2).

In view of the specific five-tubercle shape of the first lower molars, a cut of the distal segment was performed between the spots located at the apexes of the distal vestibular and distal lingual tubercles. The distal tubercle height (hypoconulide) was not taken into account within our study, since its size varied over a wide range, up to the complete reduction of the tubercle.

On the tooth segment picked out from the cast model, spots were made with the reference lines drawn. The main points for the study were the teeth necks and the tubercle apexes on the chewing surface (Fig. 3).

The major reference was the line connecting the neck of the vestibular and the lingual contours of the crown, marked as the neck line (1). From the middle of the cervical line and perpendicular to it, a conditional median tooth vertical was drawn (2). Perpendicular to the conventional median vertical, a line was drawn through the point located at the junction of the chewing surface tubercles, which was marked as the occlusion plane (3).

The conditional tubercle lines (4 and 5) pointed at the location of the tubercles relative to the conventional median vertical and occlusal planes of the tooth.

In the transversal direction, the vestibular-lingual (VL) crown and cervical sizes were measured, as well as the intertubercular distance.

To measure the vertical parameters, the above-mentioned reference lines were used. The height of the vestibular (a) and lingual (c) odontomers was measured from the cervical line to the reference points on the articular tubercles tops. The height of the tubercles (b and d) was measured to the occlusal plane level. The data obtained through the study were subjected to statistical processing using the methods of parametric and non-parametric analysis following the outcomes of testing the compared sets for their normal distribution. The statistical analysis was performed using IBM SPSS Statistics 23 software.

RESULTS AND DISCUSSION

An analysis of the height parameters of the lower chewing teeth crowns and the transversal dimensions between the vestibular and the lingual contours helped reveal the morphometric features of the molars in various types of dental systems.

Measuring the mesial segment of the first lower molar, isolated from jaws cast models obtained from Group 1 patients (normodontic dental system), revealed that the crown width in its widest part was 10.83±0.07 mm. In the cervical region, the buccal-lingual size was 10.35±0.05 mm, while between the tubercles tops it was 7.39±0.03 mm. In the distal segment, the crown and neck dimensions were 10.56±0.08 mm and 10.25±0.06 mm, whereas the intertubercular distance was 7.25±0.04 mm (Table 1).

Vertically, the height of the crown mesial part on

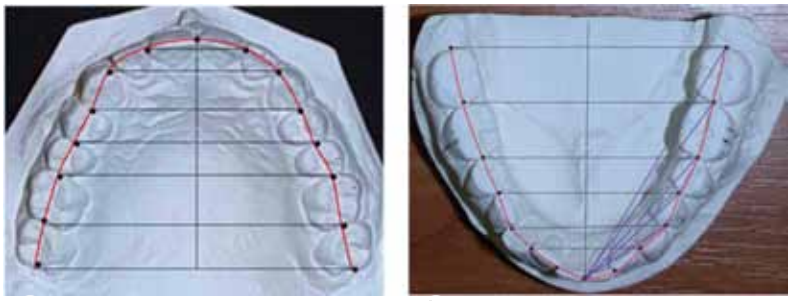

Fig. 1. *Photos of plaster models of the upper (a) and lower (b) jaws with contours for measuring the longitudinal length of the dental arch*

Fig. 2. *Photos of plaster models of the upper jaw of patients with normodontia (a), microdontia (b), macrodontia (c).*

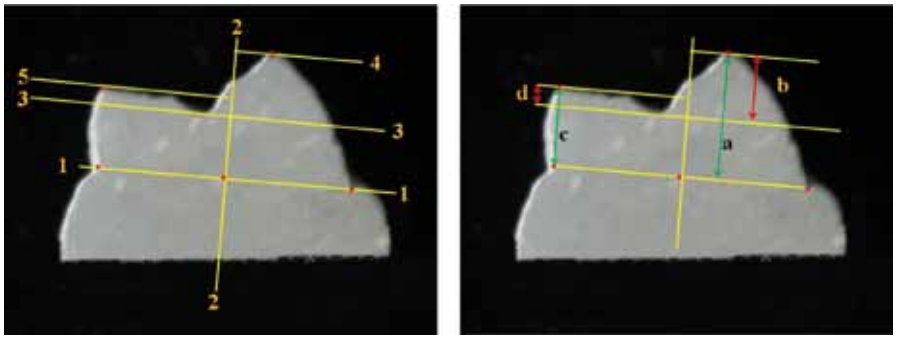

Fig. 3. *Images of the right lower molar mesial segment with marked reference lines and points for measurement (see text)*

the vestibular side was significantly above ($p \leq 0.05$) that on the lingual side of the tooth, which affected the height of the chewing surface tubercles. The height of the mesial vestibular tubercle was 2.20±0.01 mm, and that of the mesial lingual tubercle was 1.32±0.01 mm.

The second molar represented two sectors as well, the anterior (mesial) and the posterior (distal) ones. The crown width of the anterior segment was 10.32±0.06 mm, distal — 9.85±0.07 mm. Therefore, the second molar was slightly smaller in size than the first one, which confirms the ideas expressed by most respective experts.

The vertical parameters of the second lower molar in the crown anterior sector were 5.35±0.04 mm from the vestibular side and 4.23±0.02 mm from the lingual side. The height of the anterior vestibular tubercle was

Table 1. *Lower molars crowns in people with physiological occlusion and normodontia of permanent teeth, (mm), (M ± m)*

The parameters studied	The size of the segment isolated from the lower jaw:			
	at the first molar in the segment:		the second molar in the segment:	
	mesial	distal	mesial	distal
Vestibular lingual size of the tooth crown	10,83±0,07	10,56±0,08	10,32±0,06	9,85±0,07
Vestibular lingual size of the tooth neck	10,35±0,05	10,25±0,06	9,87±0,04	9,71±0,06
Inter-hill distance	7,39±0,03	7,25±0,04	6,10±0,05	5,22±0,04
Height of buccal odontomer	5,87±0,02	4,86±0,03	5,35±0,04	4,62±0,02
Height of buccal tubercle	2,20±0,01	1,73±0,02	1,25±0,02	0,97±0,01
Height of lingual odontomer	5,21±0,02	5,26±0,03	4,23±0,02	3,67±0,03
The height of the lingual tubercle	1,32±0,01	1,32±0,02	1,51±0,03	1,36±0,01

1.25±0.02 mm, and the mesial lingual tubercle height — 1.51±0.03 mm.

In Group 2, the patients had larger teeth than with normodontia, which was quite obvious. The length of the dental arch in the group as a whole was 118.54±1.39 mm, which was typical of the macrodontic dental system.

The width of the first molar crown between the vestibular and lingual contours equators was 10.90±0.05 mm. In the cervical area, the buccal-lingual size was 10.68±0.06 mm, while between the tubercles peaks it measured 8.12±0.04 mm. Therefore, the tooth sizes in the transversal direction were significantly above those in Group 1.

Table 2 offers a view on the major vertical dimensions in Group 2.

type of dental system. Besides, there was an increase observed in the height of the tubercles chewing surface as compared with Group 1.

In the second permanent lower molar, at the anterior segment, the crown height and that of the tubercle from the vestibular side was 5.33±0.06 mm and 2.29±0.02 mm, and from the lingual side, the crown height was 5.03±0.06 mm, while the tubercle height was 1.65±0.04 mm. In the distal sector, the height of the vestibular contour of the crown and of the tubercle was 5.49±0.07 mm and 2.30±0.04 mm, respectively. On the lingual side, the height of the crown and tubercle was 4.48±0.05 mm and 1.81±0.03 mm, respectively.

In Group 3, the teeth were significantly smaller than in the other groups. The length of the dental arch in the group as a whole was 98.73±1.39 mm, which

Table 2. *Lower molars crowns in people with physiological occlusion and macrodontia of permanent teeth, (mm), (M ± m)*

The parameters studied	The size of the segment isolated from the lower jaw:			
	at the first molar in the segment:		the second molar in the segment:	
	mesial	distal	mesial	distal
Vestibular lingual size of the tooth crown	10,90±0,05	10,70±0,08	10,67±0,06	10,16±0,07
Vestibular lingual size of the tooth neck	10,68±0,06	10,25±0,07	10,46±0,05	9,97±0,09
Inter-hill distance	8,12±0,04	7,75±0,03	7,06±0,06	6,58±0,08
Height of buccal odontomer	6,03±0,05	6,01±0,07	5,33±0,06	5,49±0,07
Height of buccal tubercle	2,39±0,01	2,41±0,03	2,29±0,02	2,30±0,04
Height of lingual odontomer	5,83±0,05	5,47±0,07	5,03±0,06	4,48±0,05
The height of the lingual tubercle	1,78±0,02	1,83±0,02	1,65±0,04	1,81±0,03

The mesial height of the first large molar crown from the vestibular side was 6.03±0.05 mm, from the lingual side — 5.83±0.05 mm. The obtained data on the vertical dimensions of the crown indicated that during macrodontia, the vertical parameters were slightly above that in people with the normodontic

was typical of the microdontic type of the lower dental system.

The size of the first molar crown between the equator points of opposite contours was 10.59±0.06 mm. In the cervical area, the vestibular-lingual area measured 9.45±0.04 mm, while between

the tubercles tops it was 5.28±0.02 mm. Therefore, the teeth sizes in the transversal direction were significantly smaller (p≤0.05) compared to Group 1 (Table 3).

behind the development of pathological abrasion in hard dental tissues, and the choice of optimal treatment tactics with a minimum likelihood of complications development.

Table 3. *Lower molars crowns in people with physiological occlusion and microdontia of permanent teeth, (mm), (M ± m)*

The parameters studied	The size of the segment isolated from the lower jaw:			
	at the first molar in the segment:		the second molar in the segment:	
	mesial	distal	mesial	distal
Vestibular lingual size of the tooth crown	10,59±0,06	10,37±0,08	10,09±0,05	9,64±0,09
Vestibular lingual size of the tooth neck	9,45±0,04	10,12±0,03	9,41±0,08	9,22±0,06
Inter-hill distance	5,28±0,02	6,32±0,05	5,38±0,03	4,88±0,07
Height of buccal odontomer	4,62±0,08	3,95±0,04	3,71±0,07	3,52±0,04
Height of buccal tubercle	1,81±0,02	1,56±0,06	1,05±0,04	1,34±0,06
Height of lingual odontomer	4,20±0,07	3,45±0,03	3,78±0,08	3,97±0,05
The height of the lingual tubercle	1,62±0,03	1,56±0,04	1,57±0,02	1,46±0,03

On the cast model mesial sectors of the first lower molars, the crown height from the vestibular and lingual sides were smaller than that in Group 1, and measured 4.62±0.08 mm and 4.20±0.07 mm, respectively, which had its effect on the height of the vestibular and lingual tubercles.

In the second molar, the following measurements were obtained: the height of the vestibular contour of the mesial and distal sectors was 3.71±0.07 mm and 3.52±0.04 mm, and at the lingual contour — 3.78±0.08 mm and 3.97±0.05 mm, respectively.

CONCLUSIONS

1. The results of odontometry for lower permanent molars in people with different dental arches have revealed a direct relationship between the teeth size (macrodontia, normodontia, microdontia) and their transversal (vestibular-lingual size of the crown, tooth neck and intertubercular distance), as well as the vertical (height of the vestibular, lingual odontomer and of the tubercle) parameters.
2. The obtained odontometric data can be used for diagnosing occlusive relationships and identifying various pathological, physiological abrasion types with varying degrees of occlusal surface loss.
3. The morphometric features of the teeth transverse and vertical parameters can offer meaningful data and be clinically significant in terms of diagnosing and predicting the course of the disease, as well as they are of reasonable scientific value and can be employed to clarify the mechanisms

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SPECIFIC FEATURES OF JOINT SPACE IN PATIENTS WITH PHYSIOLOGICAL OCCLUSION ON COMPUTED TOMOGRAM HEAD IMAGE

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Aim: to develop a method for constructing an articular triangle on head CT transversal sections, which would allow assessing the spatial location of the mandible articular heads.

The biomechanics of the lower jaw movement is subject to the articulation laws, which follow the temporomandibular joint morphology. The major elements of the joint bone structures include the temporal bone articular fossa and the articular head of the mandible condylar process. The lower jaw articular heads have an ellipsoid shape, elongated in the horizontal (transverse) direction. The articular heads axes are directed toward the large occipital foramen and, when crossing, shape an angle with a very variable magnitude [3, 4, 8]. Numerous studies have proven the relationship between the articular heads shape and the dental arches. Modern classifications for dental arch shapes distinguish gnathic and dental types, which differ in their morphometric parameters. It is a proven fact that pathological occlusion has an effect on the location of the temporomandibular joint elements and the tactics for complex treatment [1, 2, 5, 6, 7, 10]. The main methods for studying the temporomandibular joints include radiography, where the most objective one is the method of cone-beam computed tomography [6, 9, 11, 12]. The software of the tomographs available nowadays allows measuring angular and linear parameters of various structures comprising the craniofacial complex. Tomogram image sections construct articular heads' transversal axes and then the angle of their intersection is measured. However, we could find no data on the methods for linear measurements of inter-articular relationships, which was the purpose of this work.

MATERIALS AND METHODS

Cone-beam computed tomography (cephalostat PaX-i3D SC, VATECH Global) was employed to carry out a survey involving 28 people (both males and females) in their first period of mature age with a full set of permanent teeth and an orthognathic bite. The CT sections passing through the mandible articular heads were used to make measurements using landmarks represented as dots and lines. The articular heads had the medial and lateral poles of the ellipse marked on them, which were interconnected with a straight line thus producing the articular heads longitudinal axis. The obtained marks were further used to develop a research method.

RESULTS AND DISCUSSION

The method for constructing an articular triangle implied connecting the longitudinal axes of the articular heads thus shaping the apex of the triangle. The base of the triangle was a line connecting the articular heads' lateral poles. The proposed method allowed measuring the distance between the articular heads in millimeters as well as measuring the articular heads angle. In case of physiological occlusion, all the examined articular triangles, as a rule, were equilateral. In addition, the proposed method allowed us to measure the triangle height and calculate its area as the product of half the inter-articular distance by the articular triangle height. The proposed method of constructing an articular triangle will help understand the articulation laws and identify the dental system individual specifics. The data can be used in orthopedic dentistry when designing artificial dental arches.

CONCLUSION

Given the above, the shape of the articular triangle can be used when selecting a method for treating

occlusion anomalies in the transversal direction, for diagnosing anomalies of the articular heads location, and for evaluating their symmetrical spatial arrangement.

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