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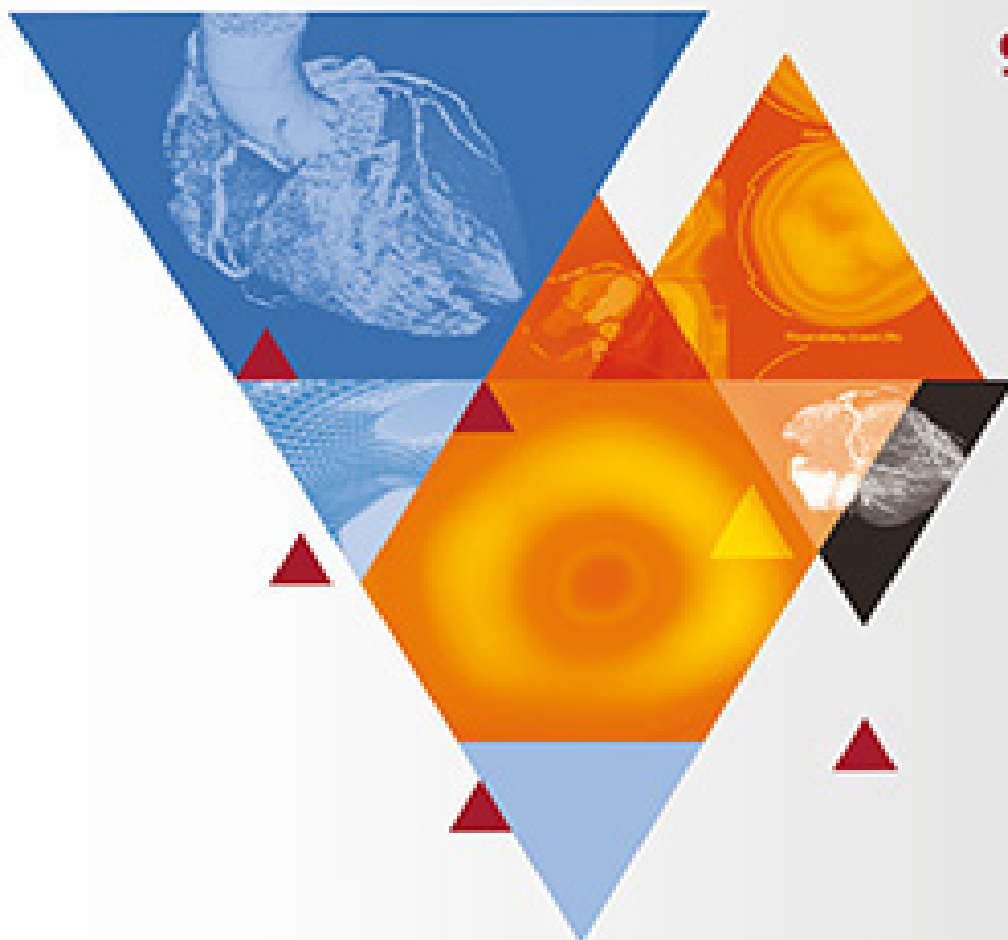
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Adaptive Changes in the Psyche of Homo sapiens during the Period of the Singularity

Alexander G. Kruglov, PhD, ScD*; Andrey A. Kruglov, PhD

*Central Research Institute of Radiation Diagnosis
Moscow, the Russian Federation*

Abstract

The perception of environmental images that are not in causal relationships and are not interconnected sequences forms what is called clip thinking (ClipT). The concomitant decrease in the analytical-synthetic psychic functions is the result of “images” from the environment (not constructs of the psyche of the subject) being crowded out by a sequence of other images, and so are not kept in the field of voluntary attention for sufficient time for fixation. As a result, along with analytical and synthetic functions, from the sequential series of operational functions of conceptual thinking (ConceptT), come the closing steps of the perception phase—the default mode network (DMN) activity. The result is changes in the reciprocal relationship between the perception and processing of incoming information. The deficiency changes in DMN cause an imbalance of structures that provide “focus and defocus,” difficulty fixing attention and filtering information, interruption of social relations, and other disruptions. ClipT forms the ability to perceive a larger (compared to ConceptT) amount of short volumes of information per unit of time, with an increase in the total number and range of heterogeneous units of incoming information, increasing the multitasking of thinking and allowing the simultaneous execution of several types of activities. The prospective dynamics in the development of ClipT forms global unified communicative codes with the potency of the functional association of subject groups that do not have stratification and quantitative restrictions. The distribution of information in these associations, which have almost identical characteristics of operational symbols (OSs), and the high speed of distribution of content on an unlimited scale of social networks, is similar to infection mechanisms during epidemics. ClipT is at the initial stages of development, adapting the psyche to accelerating changes in the environmental information parameters. We believe that ClipT and ConceptT are various interfering processes of the transforming psyche of Homo sapiens. The development of ClipT creates the conditions for the psyche to transition to a new qualitative level, with a change in structure and a transition to a new class of systems. In the conditions of increasing density, speed, randomness of the information flow and increasing degree of uncertainty, we consider the new phase transition as the attractor of the described changes in thinking (the psyche as a whole) of HS—the singularity period determining the changes (transformation of thinking is the most accessible integral research feature of significant, but less noticeable changes in the psyche as a whole) that take place with the future final state of a unified information system, including external devices and adaptive integral changes in the properties of the psyche as a whole. (**International Journal of Biomedicine. 2020;10(2):95-100.**)

Key Words: clip thinking • conceptual thinking • singularity • attractor • evolutionary development

Abbreviations

CC, creative construct; **ClipT**, clip thinking; **ConceptT**, conceptual thinking; **DMN**, the default mode network; **HS**, Homo sapiens; **OS**, operational symbol; **PT**, paralogical thinking; **TC**, thinking in complexes

Basic Part

The aim of the present work was to identify some vectors of the development of the human psyche by analyzing the interconnections of ConceptT and ClipT during the period of evolutionary phase transition.

Materials and Discussion

We consider ConceptT in the current period of time as a derivative of syllabic writing, having a standard order of signs: the alphabet (in contrast to the subject, pictographic, hieroglyphic, and other types of communication): i.e. as a

variant of a textual civilizational cultural code that brought together oral and written speech. Throughout the history of the development of HS, the development and dissemination of “textual” ConceptT in various historical eras was limited to social groups where writing was predominantly utilitarian in nature (with exceptions that are not significant in the aspect under consideration). The total spread of writing and the concomitant development of the “text” ConceptT arose only with the beginning of printing (Gutenberg, 1439). The global spread of book printing has triggered the emergence of print (“textual”) culture; the perception, processing, and dissemination of information; and the development of ConceptT as the dominant form of thinking that forms the psyche as a whole.

We consider thinking as an integral function of the psyche, a special kind of mental and practical activity of HS, including the operational activity of a cognitive and transformative nature, which has several successive stages. In its most general form, this activity is visual-effective thinking (object-manipulative) at an early age; visual-figurative thinking, in which the subject thinks only with specific images (up to ~7 years of age); figurative thinking (verbal-logical), using concepts without having had specific experience of them (>12 years of age). Operational functions of thinking are a) comparison – detecting similarities and differences between objects and phenomena; b) analysis – allocating essential features of objects; c) synthesis – combining the selected properties into a single whole; d) abstraction – allocating the main (from the point of view of the subject) signs separated from secondary signs; e) generalization – an ability to combine the main distinguished properties of an object into a single whole. “Concepts” are formed by the consistent dynamics of psychological changes (>12 years of age),^(1,2) which are the result of independent activity (operating with a word). All mental functions (associations, memory, attention, etc.) participate in this process, but do not create it. A “concept” is formed by a word acquiring a certain semantic meaning. ConceptT does not exist outside of speech-thinking, without words, in contrast to instincts and drives. The signifiatory meaning of a word, its use as a means of “concept” formation means a transition to operational activities, mediated by semantic meanings, hence to ConceptT. ConceptT forms the possibility of finding and highlighting significant features of an object, and qualifying and sorting those features into a homogeneous series of signs characterizing the object.^(1,2) The next stage is the sorting of objects, which allows one to combine in homogeneous groups not signs, but objects, forming an integral picture of the visible world and building a continuum of the environment as a whole.

The evolutionary bifurcation of hominids took place, presumably, as a result of the emergence of a new subsystem of the psyche—“creativity”—in the HS subspecies (~50,000 years ago).⁽³⁾ We understand creativity as a specific feature of the psyche of HS, the ability to produce prognostic hypotheses that cannot be derived directly from the initial conditions. As a result of the development of creativity, there appeared the ability to think symbolically, as well as to construct abstract images and adaptive forms of purposeful (non-reactive)

behavior corresponding to the conditions and degree of uncertainty. The creative construct (CC), originally “image of the goal,” was transformed into a fragment of the environment with the functions of a controlling object.⁽³⁾ The combination of images and meaningful meanings in CC created abstract thinking, the development of which led to the development of symbolic thinking. Being the final segment of the pyramid, consisting of representations, mythologies, myths, logical constructions, etc., the final product of thinking was formed—the “symbol” (Image+Sense),⁽⁴⁾ which has a finite number of signs, correlated with the amount of voluntary attention (consciousness) and perceived as an indivisible object. Each CC, arising as a neural association that realizes an actualized need,^(3,5) acquires the properties of a dominant focus with the corresponding equivalents. Dominant foci displaced from the “conscious” area constitute structured neural constellations that are in interferential relationships and are actualized by repeated invariant sensory patterns from the sensory organs or arsenal of needs (neural pathways). The totality of these functional neural constellations makes up the content of the “subconscious”, where each structural component has the potential for “readiness” to motivate the subject during actualization. Creating a system of virtual representations and reducing the degree of uncertainty, the CC sequence structures and organizes the system of subject-object relations. Thus, in the phylogenesis of HS, a precedent has been noted for integrating a new psyche subsystem, creativity, with reformatting of the psyche as a whole and the emergence of qualitatively new operational functions (creation and dissociation of a symbol with behavioral equivalents).^(3,5)

ClipT is formed by the perception of environmental images that are not in causal relationships, as well as by those that are not interrelated sequences but being in sufficient time in this environment.^(6,7) A 6-fold excess of the efficiency of presenting the sequence of the imagery (suggesting a ready-made solution) over the text (with sequential conceptual processing and the final conclusion) is established. A decrease in the level of analysis and synthesis of information obtained in the form of a finished image associated with ClipT is a consequence of the fact that the “image,” not being a construct of the psyche of the subject and not holding in the field of voluntary attention (consciousness) long enough for fixing (and subsequent processing), is crowded out by a sequence of other images, often outside of logical and sequential relationships. We believe that the sequential series of operational functions of thinking changes, which are the closing steps of the perception phase (the DMN activity) in ConceptT. Thus, the reciprocal relationships between the brain’s systems for perceiving and processing incoming information are changes, and their imbalance arises. In connection with a deficiency of changes in DMN, the balance of the joint work of structures that ensure “focus” and “defocus” is upset.⁽⁸⁾ The results are a) difficulty in fixing attention; b) disturbances of filtering (necessary/unnecessary) information; c) an easy distraction from the main current task; d) a decreased flexibility of thinking;^(9,10) e) disruption of the chain of “social ties”.^(11,12) The time lag of a sequence of ConceptT is much longer than ClipT. Possessing an advantage in the speed and volume of perceived fragmented

information, ClipT loses in the perception and processing of a long linear sequence, possibly playing the role of a “filter” in the conditions of information overload and randomness of the growing information flow.⁽¹³⁾ In other words, ClipT forms the ability to perceive a larger (compared to ConceptT) amount of short volumes of information per unit of time, with an increase in the total number and range of heterogeneous units of incoming information, increasing the multitasking of thinking and allowing the simultaneous execution of several types of activities.⁽¹⁴⁾

We note, in solidarity with the “theory of incompleteness,”⁽¹⁵⁾ that any depth of penetration into the studied object, even for the most developed ConceptT, is always relative, reaching the limits possible for a given subject, being only a “striving” in this direction, with limited achievement. In other words, the productive results of ConceptT, despite the obvious effectiveness in all areas of human activity, are superficial with respect to the structure of any object of study (limited by the current level of knowledge). We believe that a comparison of the effectiveness and productivity of ConceptT and ClipT is premature, since important factors are still not sufficiently known—the development vectors and the distant characteristics of ClipT in the initial phases of development, the proportionality limits of relationships with external devices (forming a new configuration of both personality and society), and the long-term results of the practical, operational effectiveness of ClipT in the face of accelerating changes in the information and social environments.

Many reasons are advanced for the formation of ClipT in modern man. We consider, without considering it sufficiently complete, one of the classifications:⁽¹⁶⁾ the development of modern technologies, which are accompanied by a significant increase in the information flow; 2) the need to receive a large amount of information more quickly; 3) multitasking and expanding the range of information flow; 4) the accelerating rhythm of life and increased diversity of simultaneously arising tasks; and 5) the growth of dialogicity at different levels of the social system. ClipT differs from ConceptT in that what is absent in ClipT is context—i.e. semantic completeness,⁽¹⁷⁾ the meaning of the components of the text (speech), and the semantic connections of fragments of the context; and as a result, ClipT lacks analytical and synthetic functions with the subsequent difficulty in conjugating the perceived fragments into a generalized picture. A fragmented (without internal semantic connections) mosaic of perceived images, compared with each other, according to an algorithm unknown to us, forms ideas about the environment, which may not correspond to reality, ideas about which (i.e. reality) are formed with the participation of ConceptT. We believe that the subjective interpretation of the reflection of environmental phenomena (i.e. “experience,”⁽¹⁸⁾ which is formed with the participation of ClipT) constructs ideas about the environment that are different from those created through ConceptT, on the basis of which new forms of adaptive behavior are formed that are adequate to the current ideas (different from those that have become standard). In other words, the gradients of personality development and the formation of adaptive (including social) behavior under the influence of ConceptT and ClipT, having

differences in content and speed (with a predominance of ClipT) characteristics, also have diverging development paths.

The transformation of modern society into “electronic, informational,” through electronic means of communication, forming ClipT, partially returns human thinking to the pre-text era, with the exhaustion of the role of a linear sequence of characters as a basis for cultural development.⁽¹⁹⁾ For a quantitatively growing part of the population that has undergone informational transformation, which will become the general population of the future, what is fundamentally new is the constructive formation of perception and modeling of the image of the environment, based on the perception of information segments (“slicing”), as the preferred method of perception. A preference for perception is given to multiple incoherent episodes, pieces of information, in contrast to the completed plot media models.^(20,21)

Given the growing global homogeneity (informative and figurative) and the similarity of information codes that form and format ClipT (unification of degrading informational occasions, informative and figurative media products, the unifying impact of social networks and entertainment, memes, etc.), we consider the following conclusion to be logical. Thus, in contrast to ConceptT, the development gradient and the main vector of perspective dynamics of ClipT leads to the achievement of a finite volume of unified OSs, the narrowing range of which will cover significant numbers of subjects (possibly populations), regardless of geographical, linguistic and other differences. In other words, global unified communication codes are gradually being formed, creating network associations that are not scalable and are organized by horizontal connections. Features of ClipT (reduction of analytical and synthetic functions, unification of OSs, etc.) form a functional union of a “homogeneous series” of subjective unstratified associations that contain an identical cultural communication code, where the mechanism of information dissemination (proximity of meanings and OSs with reduced filtering functions, high speed of content distribution on an unlimited scale of social networks, etc.) is close to that of infection mechanisms during epidemics.

Discrete signs of pre-book (pre-text) style in cultural communication, forming ClipT, arose long before the advent of computer games and social networks.⁽²²⁾ Functionally, this style is the perception of the connections of one’s own perceptions with equivalent connections of the objects of perception, i.e. partial identification of oneself with the object of perception. In ontogenesis, this is the phase of adolescent “thinking in complexes” (TC), where complexes are not concepts, but their functional equivalents. TC is a functional precursor of ConceptT, structurally included in the design of ClipT. In TC, each item entering a complex does not merge with another one, retaining its identity (for example, ancient languages: Chinese, Latin, etc.). In this phase, any connection can arbitrarily group actual (non-abstract, non-logical) objects. That is, in contrast to ConceptT, the basis of which is a single significant connection (including abstract, logical); in the TC, regardless of the type of complex, the basis for combining objects is actual (random) communication. TC is diffuse, and does not have certain outlines and boundaries. The form

of TC is ambivalent, representing: a) generalization, but different in structure from the concept; b) an image, «mental drawing of the concept.»⁽²⁾ The result of TC is a difference in the values that define different objects with the same word. In other words, each specific object entering the complex retains its identity, not combining with others. The result is a participation (relationship) that primitive thought establishes between different objects, considered either as partially identical, or as influencing each other, but in reality having neither spatial contact nor any other causal connection.^(2,23) At the basis of the etymological definitions of a “word.” a very significant part did not have logic or connections established in “concepts.” That part was replaced by figurative complexes with the allocation of a random attribute that determined the name of the object.

Without bringing all phases of the formation of ConceptT in ontogenesis, we note the main, final stage: the ability to synthesize abstracted signs, where the word (sign) is the basis for distinguishing the sign and the subsequent synthesis of signs into a higher operational function—a symbol (Image+Sense). ClipT, surpassing ConceptT in speed and range characteristics, while being deficient in the depth of analysis, synthesis, and filtering of values, differs in the final result—the OS. The OS of ClipT is different from the OS of ConceptT. With the identical initial information field, the semantic content of the OS changes. OSs of ConceptT and ClipT, preserving the identity of the “image,” have substantial (semantic) differences, as a result of differences in principles of constructing fragments of the information field in the OS during ClipT. An OS with ClipT has a superior (in comparison with ConceptT) range of perceived objects and a large information capacity with less analytical and synthetic generalization. Insufficient conceptual, analytical and synthetic depth at a high processing speed, suggests the fragmentation of the final informational figurative resource with arbitrary plot integration into a single whole. The intra-plot connections in ClipT create the conditions for the formation of logical circuits that combine figurative series in way according to an algorithm unknown to us. ClipT arises, in our opinion, as an adaptive version of the development of the psyche with a rapid chaotic increase in the quantitative characteristics and variability of the choice of stimuli from the information field, as well as with many alternatives and the absence of clear criteria for optimality and efficiency, increasing the degree of uncertainty of the system as a whole. We note the existence of the concept of uncertainty as a fundamental property of nature.⁽²⁴⁾ The general tendency for the development of ClipT at the present stage, according to formal features, can be defined as the archaization of thinking. Constructively, ClipT includes significant fragments of TC and PT,⁽²⁵⁾ including the possibility of identifying the subject “I” with an external object by abstraction and “transfer” (imagination, empathy) of personal properties to an external (external) object, i.e. splitting the representation of the integral personality.⁽²⁶⁾

We believe that we describe the development of a new type of thinking of HS, conventionally called ClipT, that has arisen and is being formed in the general cultural environment with the participation and on the basis of ConceptT, in

conditions of rapid growth and increasing randomness of the information flow. HS at the next stage of technological development, in the dynamics of the rapid development of the electronic digital environment, received external devices that provide inexhaustible memory resources, computational speed, and synthesis of previously incomparable objects, creating a virtual environment with a migration of a decision center, the possibility of dialogue (with an opponent whose logic, memory and speed of thinking are qualitatively and quantitatively different from the subjective decisions), and other characteristics. Interaction with an external device has created the possibility of relationships that can be partially defined as “interpersonal,” which already leads to depletion (crowding out) of social ties and a change in the whole configuration and parameters of the social spectrum of the external environment. In other words, the dynamics of the development of ClipT (with the gradual displacement of ConceptT) indicates the HS psyche is adapting to the conditions of an increasing information flow, its randomness, and a gradual change in the ratio of roles in a pair: operator-device. We intentionally do not give quantitative values for the increase in density, speed and quality of the information flow, taking them as a fact confirmed by many studies. Our goal is high-quality adaptive changes in the psyche of HS in the conditions of the information environment with a rapid change in parameters.

ClipT is at the initial stages of formation, being a measurable indicator of the adaptation of the HS psyche to accelerating changes in the basic information parameters of the environment. We believe that we do not describe the transformation of ConceptT into ClipT; these are two different, partially interfering processes of the psyche that adapts to significant and accelerating changes in the environment. Considering the psyche as an active, distributed system consisting of functional subsystems, we believe that the emergence of a new integrating subsystem, ClipT, creates (partially crowding out ConceptT) conditions for the transition to a new qualitative level of the system as a whole, with a change in the structure of functional properties and transition to a new class of systems.

The theory of evolution of the biosphere and society is formulated as a synergistic model of development,⁽²⁷⁻²⁹⁾ where resource and technological-humanitarian crises, as well as environmental degradation, can be productive (producing phase transitions). One of the decisive factors for survival during phase crises is the excessive internal diversity of the system. That is, at the onset of the evolutionary crisis, species forms that did not have evolutionary advantages before found a deterministic equivalent response, an adaptive response to the crisis, which made them a new system-forming factor;⁽²⁸⁾ one of the signs of such an adaptive reaction, we believe the formation of ClipT.

Modification of HS, through the emergence and development of new mental functions (creativity, symbolic thinking, variable adaptive behavior that initiated technological development), which were phylogenetically transformed into a significant and then leading factor in evolution.^(3,5) In our earlier data (including graphs and velocity characteristics of

evolutionary phase transitions),⁽⁵⁾ we considered the effects of the attractor on the evolutionary development of HS. We understand the attractor as the final area of the inevitable convergence of the phase trajectories of a complex system, the attraction of which draws into it the many possible states of the trajectories of the systems, determined by different initial conditions. In other words, the final state has not yet been achieved, but it is already having an effect, like the goal chosen by the system. The dynamics of the accelerating phase transitions (decreasing over time intervals) of HS evolution is defined as a sequence that has a limit (singularity of evolution).^(30,31) Since the “singularity point” is a mathematical abstraction, it is proposed to consider it not a “point,” but a “period of singularity.”⁽³²⁾

We believe that the singularity of evolution, as an attractor, affects the changes in the HS psyche, including the formation of changes in thinking—the most obvious, accessible for perception and study (in large samples), is an integrated set of signs of changes in the psyche as a whole for a historically short (observable) period of time. We consider the complex of the described changes in thinking, deviations from the standards of ConceptT, accompanied by changes in the structure, content and basic vectors of the development of thinking (conventionally called ClipT), as a developing, adaptive, systemic change in the HS psyche as a whole. These systemic changes in the psyche are in the process of adapting to the development vectors of the singularity, with subsequent transformation into a single information system, including external devices and concomitant changes in the personality’s integral properties. That is why we do not support the alarmist conclusions of some psychologists about the transformation of thinking as presumably degrading; rather, we believe that the psyche is at one of the initial stages of adaptation to the information environment that is developing at an accelerating pace.

Conclusion

Thinking, as an integral function of the psyche, is currently being transformed under conditions of a significant increase in the density, speed, and randomness of the information flow. On the basis of the dominant ConceptT, a new type of thinking is being formed, conditionally called ClipT, which adapts the psyche to informational changes in the environment. ClipT emerged as a result of the increasing speed and randomness of the information flow, which in turn led to the perception of environmental images that are not in causal relationships. The formation of a new type of thinking with higher speed characteristics and other principles of the formation of structural elements, and changes in the construction of the semantic part of the final product (“symbol”), is accompanied by changes (possibly partial loss) in the closing steps of the perception phase, the DMN activity, which leads to a contravention in the reciprocal connection of the perceiving and processing information systems of the brain. OSs of ConceptT and ClipT, while preserving the identity of the image, have substantial semantic differences, as a result of the construction (according to an algorithm unknown to us) of fragments of the OS information field in

ClipT. Gradients of the development of the psyche and forms of adaptive behavior under the influence of ConceptT and ClipT have differences in content and speed characteristics, as well as diverging development trajectories. We believe that we are not observing the transformation of ConceptT into ClipT, but two different, partially interfering processes of the HS psyche, which adapts to accelerating changes in the information environment. The integration of a new subsystem (ClipT) creates the conditions for the transition of the psyche to a new qualitative level, with a change in functional properties and a transition to a new class of systems. In the conditions of increasing density, speed, randomness of the information flow and increasing degree of uncertainty, we consider the new phase transition as the attractor of the described changes in thinking (the psyche as a whole) of HS—the singularity period determining the changes (transformation of thinking is the most accessible integral research feature of significant, but less noticeable changes in the psyche as a whole) that take place with the future final state of a unified information system, including external devices and adaptive integral changes in the properties of the psyche as a whole.

Competing Interests

The authors declare that they have no competing interests.

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*Corresponding author: Alexander G. Kruglov, PhD, ScD. Central Research Institute of Radiation Diagnosis. Moscow, the Russian Federation. E-mail: krag48@mail.ru

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Assessment of Anatomical Variation of Coronary Arteries for Sudanese Patients Using Cardiac Computed Tomography Angiography

Salem Saeed Alghamdi, PhD¹; Rowa Aljondi, PhD¹; Ikhlas Abdelaziz, PhD¹;
Mohamed Yousef, PhD²; Mustafa Z. Mahmoud, PhD^{3*}; Mugtaba Elgazali, MSc⁴;
Abdulrahman Tajaldeen, PhD⁵

¹University of Jeddah, College of Applied Medical Sciences, Department of Medical Imaging and Radiation Sciences, Jeddah, Saudi Arabia

²Radiologic Sciences Program, Batterjee Medical College, Jeddah, Saudi Arabia

³Radiology and Medical Imaging Department, College of Applied Medical Sciences, Prince Sattam bin Abdulaziz University, Al-Kharj, Saudi Arabia

⁴College of Medical Radiological Science, Sudan University of Science and Technology, Khartoum, Sudan

⁵Radiological Science Department, College of Applied Medical Science, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

Abstract

Background: The aims of this study were to assess the prevalence of anatomical variation of coronary arteries among Sudanese patients using cardiac computed tomography angiography (CTA) and to identify the pathological finding for patients undergoing cardiac CTA.

Methods and Results: This prospective cohort study was conducted with 87 Sudanese patients subjected to cardiac CTA examination, using a 64-slice CT scanner (Aquillon 64, Toshiba Medical Systems, Tochigi, Japan) with retrospective ECG gating. Data were collected from 3D reconstruction at optimal phase. The numbers of diagonal and obtuse marginal branches were counted. The presence of ramus intermedius and origination of the right coronary artery (RCA) from right coronary sinus (RCS) were also reported.

Findings showed that most patients had one (33.3%) or two (47.1%) diagonal artery branches. In addition, 3.4% of patients had no diagonal branches, 12.6% had three and 3.4% had more than three diagonal branches arising from the left anterior descending artery (LAD). About 8% of our patients had no obtuse marginal branch. The majority of patients had one, two and three branches of the obtuse marginal arising from a left circumflex artery, which amounted to 24.1%, 41.4% and 25.3%, respectively. Only one patient had more than three obtuse marginal branches. Furthermore, 94.3% of patients presented with RCA originating from RCS, and 5.7% of patients had an ectopic origin of the left coronary artery (LCA).

Conclusion: This study concluded that there is a wide variation in the number of diagonal and obtuse marginal branches arising from the left circumflex artery among Sudanese patients. Ramus intermedius was the most common anatomical variation affecting the coronary artery. Ectopic origination of the RCA is a very rare condition in Sudanese patients. (*International Journal of Biomedicine*. 2020;10(2):101-103.)

Key Words: anatomical variation • coronary arteries • cardiac computed tomography angiography

Abbreviations

CAD, coronary artery disease; CTA, computed tomography angiography; ECG, electrocardiography; LAD, left anterior descending artery; LCA, left coronary artery; RCA, right coronary artery; RCS, right coronary sinus; ROI, region of interest; 3D, three-dimensional

Introduction

The arterial supply to the heart is provided by the right and left coronary arteries, which arise from the ascending aorta immediately above the aortic valve. The coronary arteries and their major branches are distributed over the surface of the heart, lying within sub-epicardial connective tissue. The RCA arises from the anterior aortic sinus of the ascending aorta and runs forward between the pulmonary trunk and the right auricle. It descends almost vertically in the right atrioventricular groove, and at the inferior border of the heart, it continues posteriorly along the atrioventricular groove to anastomose with the LCA in the posterior interventricular groove. The branches from the RCA supply the right atrium and right ventricle, parts of the left atrium and left ventricle, and the atrioventricular septum.^(1,2) The LCA, which is usually larger than the RCA, supplies the major part of the heart, including the greater part of the left atrium, left ventricle, and ventricular septum. It arises from the left posterior aortic sinus of the ascending aorta and passes forward between the pulmonary trunk and the left auricle. It then enters the atrioventricular groove and divides into an anterior interventricular branch and a circumflex branch.^(1,3)

Cardiac CTA has emerged as a less invasive imaging modality for the diagnosis of CAD and is often used to avoid conventional coronary angiography, particularly, in low- and intermediate-risk patients.⁽⁴⁻⁶⁾ Continuous improvements in CT detector technology and in temporal and spatial resolution that have resulted in clinical examinations with cardiac CT are similar to examinations obtainable with conventional catheter coronary angiography.^(7,8)

The aims of this study were to assess the prevalence of anatomical variation of coronary arteries among Sudanese patients using cardiac computed tomography angiography (CTA) and to identify the pathological finding for patients undergoing cardiac CTA.

Materials and Methods

After we received approval from the local ethics committee, a group of 87 Sudanese patients undergoing cardiac CTA, presenting at the Radiology Department, were recruited between March 2019 and April 2020 for this prospective study. The current study adhered to the Declaration of Helsinki and Title 4, US Code of Federal Regulations, Part 46, Protection of Human Subjects.

All cardiac CTA examinations were performed using a 64-slice CT scanner (Aquillon 64, Toshiba Medical Systems, Tochigi, Japan) with retrospective ECG gating. Each patient was injected with 80-85 mL high iodine concentration contrast media with a flow rate of 5 mL/s. In addition, an injection of 20 mL saline solution was given. Scanning timing was determined by the automated bolus-tracking technique by placing the ROI over the proximal descending aorta and setting the trigger threshold to 180HU. Images were reconstructed at the optimal phase and transferred to another workstation. The 3D reconstruction was performed with high resolution and multiple views in order to count the number of diagonal and

obtuse marginal branches in a coronary artery. The presence of ramus intermedius and the origin of the RCA from RCS were reported. One experienced consultant radiologist interpreted images for diagnosis, while all images obtained from patients with surgical bypass and stents were excluded.

Statistical analysis was performed using the standard Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) version 20 for windows.

Results and Discussion

Table 1 shows the distribution of gender in our study samples. Of the 87 patients, there were 42(48%) males and 45(51%) females. The distribution of age groups in our study patients is shown in Table 2. The age group of 40-50 years (21%) had cardiac CTA performed with minimum frequency. On the other hand, the age group of more than 60 years (42.5%) had a higher percentage due to the increased risk of CAD with the increase of patient age, as reported by Huxley et al.⁽⁹⁾

Table 1.
The distribution of gender in study samples

Gender	Frequency	Percentage
Male	42	48.3%
Female	45	51.7%
Total	100	100%

Table 2.
The distribution of various age groups

Age groups	Frequency	Percentage
40-50	19	21.8%
50-59	31	35.6%
More than 60	37	42.5%
Total	100	100%

As shown in Table 3, a total of 3(3.4%) patients had no diagonal branch, 29(33.3%) patients presented with one diagonal, 41(47.1%) patients had two diagonal branches, 11(12.6%) patients had three diagonal branches, and 3(3.4%) patients had more than three diagonal branches arising from the LAD. These results are in agreement with a previous study by Abdelrahman et al.⁽¹⁰⁾

Table 4 shows that 7(8%) patients presented with no obtuse marginal branches, 21(24.1%) patients had one obtuse marginal branch, 36(41.4%) patients had two obtuse marginal branches, 22(25.3%) patients had three obtuse marginal branches, and 1(1.1%) patient had more than three obtuse marginal branches arising from the left circumflex artery. These results were not similar to a previous study conducted by Abdelrahman et al.,⁽¹⁰⁾ in which the researchers found that most of their study samples had one obtuse marginal branch. This difference was due to the large age groups distributed in their study samples.

Table 3.**Distribution of the diagonal branches**

Diagonal branches	Frequency	Percentage
Absent	3	3.4%
One	29	33.3%
Two	41	47.1%
Three	11	12.6%
More than three	3	3.4%
Total	87	100%

Table 4.**Distribution of obtuse marginal branches**

Obtuse marginal branches	Frequency	Percentage
Absent	7	8.0%
One	21	24.1%
Two	36	41.4%
Three	22	25.3%
More than three	1	1.1%
Total	87	100%

Table 5 demonstrates that about 17(19.5%) Sudanese patients in the current study had no ramus intermedius branches arising from the LCA, compared to the rest of the 70(80.5%) patients, who presented with intermedius branches arising from the LCA. Table 6 shows that 82(94.3%) patients had the RCA originating from RCS and 5(5.7%) patients had ectopic origin from the LCA. Both findings presented in Table 5 and Table 6 were in line with other studies conducted by Abdelrahman et al.⁽¹⁰⁾ and Erol et al.⁽¹¹⁾

Table 5.**The frequency (n) and percentage (%) of patients with and without ramus intermedius branches arising from the LCA**

Ramus intermedius branch	Frequency	Percentage
Patients with ramus intermedius	70	80.5%
Patients without ramus intermedius	17	19.5%
Total	87	100%

Table 6.**RCA origination in the study samples**

RCA origin	Frequency	Percentage
Normal from RCS	82	94.3%
From LCA	5	5.7%
Total	87	100%

Conclusion

The present study concluded that there is a wide variation in numbers of diagonal branches arising from the left anterior descending and on obtuse marginal branches arising from the left circumflex artery in Sudanese patients. Finally ectopic origination of the RCA is a very rare condition in Sudanese patients.

Competing Interests

The authors declare that they have no competing interests.

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***Corresponding author:** Professor Dr. Mustafa Z. Mahmoud, Radiology and Medical Imaging Department, College of Applied Medical Sciences, Prince Sattam bin Abdulaziz University, E-mail: m.alhassen@psau.edu.sa, PO Box: 422, Zip Code: 11942, Al-Kharj, Saudi Arabia.

Markers of Endothelial Damage, Inflammation, Oxidative and Cellular Stress in Patients with Coronary Artery Disease and Type 2 Diabetes

Julia A. Kotova, PhD*^{*}; Anna A. Zuikova, PhD, ScD; Aleksander N. Pashkov, PhD, ScD; Natalia V. Strahova, PhD; Olga N. Krasnorutskaya, PhD, ScD; Veronika I. Shevzova, PhD; Elena Yu. Esina, PhD, ScD

*Voronezh State Medical University named after N.N. Burdenko
Voronezh, the Russian Federation*

Abstract

Results of this study present the features of the severity of coronary atherosclerosis, evaluated by the Gensini score, depending on the presence of T2D (type 2 diabetes). The presence of T2D aggravates the course of coronary heart disease due to the more pronounced processes of inflammation, endothelial dysfunction, and oxidative and cellular stress. (**International Journal of Biomedicine. 2020;10(2):104-107.**)

Key Words: coronary heart disease • type 2 diabetes • oxidative stress • endothelial dysfunction • inflammation

Abbreviations

ADPH, aldehyde derivative of DNPH; **BMI**, body mass index; **CVDs**, cardiovascular diseases; **CHD**, coronary heart disease; **CAG**, coronary angiography; **DNPH**, 2,4-dinitrophenylhydrazine; **ED**, endothelial dysfunction; **FPG**, fasting plasma glucose; **GS**, Gensini score; **hsCRP**, high-sensitivity C-reactive protein; **HDL-C**, high-density lipoprotein cholesterol; **Hsp70**, heat shock protein 70; **KDPH**, ketone derivative of DNPH; **OS**, oxidative stress; **OMP**, oxidative modification of proteins; **SOD**, superoxide dismutase; **T2D**, type 2 diabetes; **tHcy**, total homocysteine; **WC**, waist circumference.

Introduction

Usually caused by atherosclerosis, CHD constitutes a high level of mortality among CVDs—a leading cause of morbidity and mortality globally.⁽¹⁻⁴⁾ Atherosclerotic plaque formation is a complex process that involves several mechanisms, including lipid accumulation, ED, OS, vascular proliferation, matrix degradation, chronic inflammation, and thrombosis.⁽⁵⁻⁷⁾ Oxidation of low-density lipoprotein cholesterol is one of the key factors for the development of atherosclerosis. Many factors are involved in the progression of atherosclerosis in patients with T2D; however, the most important factors are insulin resistance and hyperglycemia.⁽⁸⁻¹⁰⁾ T2D is characterized by accelerated atherosclerosis with widely distributed vascular

lesions. People with diabetes are more likely to have carotid plaque with calcification and lipid-rich necrotic cores than people without diabetes.⁽¹¹⁾ People with diabetes have a high incidence of two or more vessel diseases, compared with subjects without diabetes.⁽¹²⁾

Hyperglycemia is an important factor in cardiovascular damage, working through different mechanisms, such as activation of protein kinase C, polyol and hexosamine pathways, and production of advanced glycation end-products. All of these pathways, in association with hyperglycemia-induced mitochondrial dysfunction and endoplasmic reticulum stress, promote accumulation of reactive oxygen species and activation of OS.^(13,14) The impact of chronic hyperglycaemia might induce damage on vascular homeostasis, mainly attributable to the endothelium function. Numerous observational studies have found increased levels of the mediators of inflammation, such as C-reactive protein (CRP), interleukin-6 (IL-6), plasminogen activator inhibitor 1 (PAI-1) Many studies have demonstrated an

**Corresponding author: Julia Kotova, PhD. Voronezh State Medical University named after N.N. Burdenko. Voronezh, Russia. E-mail: kotova_u@inbox.ru*

association between insulin resistance and accelerated CVD in patients with T2D.⁽¹⁵⁻¹⁸⁾ The strong association between insulin resistance and adverse cardiovascular outcomes in non-diabetic individuals and individuals with T2DM has been summarized in several meta-analyses.⁽¹⁹⁻²¹⁾

The aim of the present study was to evaluate the changes in the markers of endothelial damage and oxidative and cellular stress in CHD patients with T2D.

Materials and Methods

We examined 336 patients (178/53% women and 158/47% men aged between 47 and 75 years, mean age of 61.8±8.1 years) with CHD verified by standardized validated criteria and clinical-functional methods. T2D was detected in 70 out of 300 CHD patients.

All patients underwent the following examinations: assessment of traditional risk factors, physical examination, clinical and biochemical laboratory methods, 12-lead ECG, echocardiography, Holter ECG monitoring, treadmill test, and coronary angiography. Blood samples were obtained in the morning after a 12h overnight fast. The levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), and FPG were determined in the blood plasma. All patients underwent OGTT.

CAG was performed using General Electric Innova 3100 (GE Healthcare, USA). The transfemoral approach (the Seldinger technique) was used.

The severity of coronary atherosclerosis was evaluated by the GS.⁽²²⁾ The severity of the disease is expressed as the sum of the scores for individual lesions and the functional importance index of the area of each lesion in the coronary tree. The sample was continuous. According to the calculated GS, patients were divided into three groups: GS0 – 162 patients with normal coronary arteries, GS1 (1-15 points) – 80 patients with hemodynamically insignificant coronary atherosclerosis, and GS2 (>15 points) – 94 patients with hemodynamically significant coronary atherosclerosis. As markers of OS, oxidized modified proteins and the SOD activity were determined. The determination of OMP in the blood serum was carried out using the methods by Dubinina et al.⁽²³⁾ The assay is based on the spectrophotometric detection of the reaction between 2,4-dinitrophenylhydrazine (DNPH) with protein carbonyl to form protein hydrazone. The optical density of 2,4-dinitrophenylhydrazones derivatives was recorded on an SF-36 spectrophotometer. The optical density of aldehyde- and ketone derivatives of a neutral character was recorded at 356 nm and 370 nm, respectively (ADPHn and KDPHn). The optical density of aldehyde- and ketone derivatives of a basic character was recorded at 430nm and 530nm, respectively (ADPHb and KDPHb).

The SOD activity was determined by the spectrophotometric method. The serum level of tHcy was determined by EIA using «Axis-Shield» test kit.

Hsp70 and their chaperone activity were considered as markers of cell stress. Extracellular Hsp70 was measured by ELISA (Elisa Kit for Hsp70, Cloud-Clone Corp.) in

blood samples. Hsp70 chaperone activity was measured by monitoring the DTT-induced aggregation of insulin using recombinant Hsp70 (HSPA1A) (Cloud-Clone corp.).⁽²⁴⁾

The level of high-sensitivity C-reactive protein (hsCRP) was determined using the High Sensitive Elisa Kit for CRP (Cloud-Clone Corp., USA).

Statistical analysis was performed using statistical software package SPSS version 20.0 (SPSS Inc, Chicago, IL). Median values are presented with interquartile (IQ) ranges (IQR; 25th to 75th percentiles). The Mann-Whitney test was used to compare median values. The frequencies of categorical variables were compared using Pearson χ^2 . The Spearman correlation coefficient (r_s) was used to assess the relationship between variables. A probability value of $P<0.05$ was considered statistically significant.

Results

All patients were further divided into two groups, depending on the presence of T2D. Group 1 included 70 CHD patients (45.7% women and 54.3% men) with T2D; Group 2 included 266 CHD patients (54.9% women and 45.1% men) without T2D. The characteristics of the compared groups are presented in Table 1. GS was significantly higher in Group 1 ($P=0.005$) (Table 1). In Group 2, GS0 was determined in 51.8% of patients, GS1 in 25.6%, and GS2 in 22.6%. In Group 1, we saw the opposite trend: GS0 was determined in 23.3% of patients, GS1 in 17.1%, and GS2 in 48.6%.

Table 1.

The characteristics of the compared groups

Variable	Group 1	Group 2	P-value
Age, yrs	60.1±6.9	58.8±5.8	0.236
BMI, kg/m ²	27.6 [25.7;31.4]	26.7 [25.7; 27.9]	0.118
WC, cm	92 [82;110]	90 [80;98]	0.071
SBP, mmHg	160 [140;180]	140 [140;160]	0.116
DBP, mmHg	90 [90;100]	90 [80;98]	0.005
GS, score	11 [0;29]	0 [0;12]	0.005

According to the parameters of the lipid spectrum, no significant differences between the two groups were found, with the exception of HDL-C, the level of which was lower in Group 1 ($P=0.032$). The glucose level in Group 1 was 1.5 times higher than in Group 2. The levels of tHcy and hsCRP were significantly higher in Group 1 than in Group 2. At the same time, the SOD activity was lower in Group I than in Group 2 ($P=0.002$). Markers of cell stress showed a tendency to increase in Group 1, compared with Group 2, indicating a more pronounced mitochondrial dysfunction and endoplasmic reticulum dysfunction in CHD patients with diabetes (Table 2). When evaluating OMP, a significant difference was established between these two groups in the blood levels of ADPHn ($P=0.009$), KDPHn ($P=0.004$), and KDPHb ($P=0.045$) (Fig.1). As is known, an increase in ADPHn indicates the activation of free radical processes, and an increase in KDPHn/b indicates a depletion of the adaptive performance.

Table 2.

Markers of endothelial damage, inflammation, oxidative and cellular stress in the compared groups

Variable	Group 1	Group 2	P-value
TC, mmol/L	5.9 [4.5;6.9]	5.5 [4.55;6.2]	0.311
LDL-C, mmol/L	2.7 [2.3;3.7]	2.6 [2.1; 3.1]	0.384
HDL-C, mmol/L	1 [0.9;1.1]	1.1 [0.9;1.1]	0.032
TG, mmol/L	1.3 [1;1.4]	1.2 [1.1;1.5]	0.757
FPG, mmol/L	7.9 [6.4;9.8]	5.4 [4.9;5.8]	5.96E-13
tHcy, μ mol/L	10.9 [9.8;12]	10 [9.5;11.34]	0.020
hsCRP, mg/L	0.15 [0.01;0.76]	0.02 [0.005;0.21]	0.022
SOD, %	35.21 [32.1;37.6]	38.3 [35.21;39.26]	0.002
Hsp70, ng/ml	1.43 [1.02;1.9]	2.12 [1.52;2.88]	0.0002
Hsp70 chaperone activity, %	65.9 [52.7;68.5]	71.2 [61.5; 76.1]	0.001

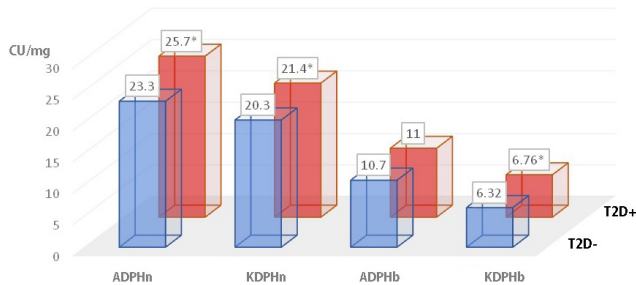


Fig.1. Parameters of OMP in the compared groups.

The lower chaperone activity was found in Group 1. As is known, chaperones play a huge protective role during cellular stress and pathologic conditions. Important redox modifications of chaperone proteins have been described in OS conditions. The activation of Hsp70 may play a role in protecting the cells against OS and inflammatory damage.⁽²⁵⁾ HSP70 is susceptible to S-glutathionylation under oxidative stress conditions. It has been suggested that S-glutathionylation of HSP70 may potentiate its chaperone activity.⁽²⁶⁾ In the setting of inducibly elevated Hsp70, cardiomyocyte protection was identified.⁽²⁷⁾ Additionally, other studies have found a lower incidence of post-operative atrial fibrillation in patients with high levels of HSP70 (also known as HSP70-1a, HSP70-1b), in contrast to those with low HSP70 (or a HSP70 polymorphism with decreased function) who have an increased risk of post-operative atrial fibrillation.^(28,29) Hsp70 participates in cardioprotection induced by exercise preconditioning, early and late protection, where Hsp70 repairs unfolded proteins or may stabilize the function of the endoplasmic reticulum.⁽³⁰⁾

Correlation analysis revealed the relationships between the presence of T2D and GS ($r_s=0.217$, $P=0.005$), the blood levels of ADPHn ($r_s=0.201$, $P=0.009$), KDPHn ($r_s=0.221$, $P=0.004$), KDPHb ($r_s=0.155$, $P=0.044$), SOD activity ($r_s=-0.237$, $P=0.002$), Hsp70 ($r_s=-0.284$, $P=0.000$), and chaperone activity ($r_s=-0.249$, $P=0.0001$).

Thus, our study showed that the course of coronary atherosclerosis in CHD patients with T2D is more severe than in patients without T2D. The presence of T2D aggravates the course of CHD due to the more pronounced processes of inflammation, endothelial dysfunction, and oxidative and cellular stress.

Competing Interests

The authors declare that they have no competing interests.

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Some Questions of the Mixed Anxiety-Depressive Disorders in Patients with Cerebellar Stroke in the Early Period of Convalescence

V. A. Kutashov, PhD, ScD; O. V. Ulyanova, PhD; I. S. Protasov, PhD*;
A. P. Skorokhodov, PhD, ScD; O. V. Zolotaryov, PhD; L. S. Nemykh, PhD;
E. S. Ananyeva, PhD; A. A. Dudina, PhD; M. V. Uvarova

*Voronezh State Medical University named after N.N. Burdenko
Voronezh, the Russian Federation*

Abstract

Background: The objective of this study was to investigate the medico-social and mixed anxiety–depressive disorders (MADD) in patients with cerebellar stroke (CS) in the early recovery period.

Methods and Results: The study included 140 patients (87/62% men and 53/38% women) with average age of 57.2 ± 2.2 years. Patients were examined in the early recovery period (6 months from the onset of the disease). The level of anxiety and depression was assessed using the Hamilton Rating Scale for Depression. In the early recovery period from CS, the different types of MADD were diagnosed. A high level of depression was found in 3.57% of patients, the average level - in 27.86%, mild depression - in 33.57% of patients, and the absence of depressive symptoms in 35% of patients. A high level of anxiety in 17.50% of CS patients, a moderate level of anxiety in 47.86%, a low anxiety level in 45.0%, and no anxiety in 2(1.43%) patients.

Conclusion: Different levels of MADD in CS patients in the early period of convalescence need individual specific treatment. (*International Journal of Biomedicine. 2020;10(2):108-111.*)

Key Words: acute cerebrovascular accident • cerebellar stroke • mixed anxiety–depressive disorders

Abbreviations

BP, blood pressure **CS**, cerebellar stroke; **HRDC**, the Hamilton Rating Scale for Depression; **HT**, hypertension; **MADD**, mixed anxiety–depressive disorders.

Introduction

More than 450,000 cases of stroke are registered in Russia annually. The incidence of acute cerebrovascular accident in Russia is 2.5–3.52 cases per 1000 population per year, and mortality in the acute period of stroke reaches 35%, increasing by 12%–15% by the end of the first year; within 5 years 44% of stroke patients die. The highest mortality rate is recorded for acute strokes in the carotid artery supply (60% during the first year).⁽¹⁻⁴⁾

Cerebellar stroke (CS) is a relatively rare cerebrovascular pathology. It accounts for 1.5% to 2.3% of all acute cerebrovascular accidents. The signs and symptoms of different CSs are quite familiar to neurologists. Rehabilitation prognosis is also better than in patients who have strokes in the carotid supply or in the brain stem.⁽¹⁻¹⁰⁾ However, there is insufficient data about the recovery of patients with CS, taking into account the peculiarities of medical and social characteristics and the presence of affective and anxiety disorders. CS is a sudden and serious illness, which can cause psychological trauma and is accompanied by a neurotic, hypochondriac and depressive reaction. In this context, great importance is given to the study of personal characteristics of patients who have undergone CS, in order to determine

*Corresponding author: Igor S. Protasov, PhD. Voronezh State Medical University named after N.N. Burdenko. Voronezh, Russia. E-mail: protasovis@mail.ru

personality traits that inhibit the formation of a positive attitude to treatment.^(1,3,4,5,7,9,10)

According to several studies, among patients with CS, 25% have serious limitations that affect their everyday life. First of all, they need rehabilitation. A major factor affecting the result of the treatment and rehabilitation is the formation of complex psychopathological conditions.⁽⁷⁻¹⁰⁾ Often, psychiatric pathology advocates for a significant factor in the restoration of destroyed social functions—household adaptation. Due to the development of stroke, the most common psychiatric disorder is mood changes, the prevalence of which varies from 30%-60%. Recently, MADD as a form of affective disorders is attracting more and more attention, not only from psychiatrists, but also from neurologists since the combination of MADD and somatic pathology is included among the factors that adversely affect the treatment process and rehabilitation.^(3,5,7,9)

The objective of this study was to investigate the medico-social and mixed anxiety–depressive disorders in patients with cerebellar stroke in the early recovery period.

Materials and Methods

The study was conducted in accordance with ethical principles of the WMA Declaration of Helsinki (1964, ed. 2013) and approved by the Ethics Committee of Voronezh State Medical University. Written informed consent was obtained from all participants.

Our study included patients with CS verified by modern diagnostic methods and undergoing treatment from 2013 to 2019 in the Voronezh Regional Clinical Hospital #1, and City Emergency Medical Hospitals #1 and #10. The study was prospective in character; it included patients with a favorable course of the disease. The study included 140 patients (87/62% men and 53/38% women) with average age of 57.2 ± 2.2 years. Patients were examined in the early recovery period (6 months from the onset of the disease). In the earliest period of convalescence, all CS patients were assessed for a stroke scale, and given a neurological examination and psychometric testing. The level of anxiety and depression was assessed using HRDS.

The causes of CS were often several factors: HT, atrial fibrillation, atherosclerosis of cerebral arteries, degenerative-dystrophic changes in the cervical spine, and compression of the vertebral arteries.

Statistical analysis was performed using the *Statistica* 6.1 software package (Stat-Soft Inc., USA).

Results

Duration of illness when the study started was on average 39.0 ± 8.0 days. Nine (6.4%) patients had relatives who have undergone a cerebrovascular accident. We analyzed the features of patients after CS: 50% of all patients live with spouse and children, 27.2% with relatives, and 22.8% live alone. Only 20% of patients were not satisfied with their housing conditions, such as the small size of their housing, lack of water supply, crowded housing, and poor heating system.

Most of the patients suffered from HT. However, only 25.5% of patients were monitored daily for BP; 17.8% were monitored several times in a week, and 17.8% - several times in a month. Moreover, 16.4% of patients were forced to measure BP in medical institutions, since they did not have a personal BP monitor; 26.4% of patients did not measure BP at all, and 16.4% measured BP 1 time per year. An analysis of indicators characterizing compliance to HT therapy showed that 59 (58.4%) patients out of 101 who were prescribed antihypertensive therapy, for various reasons, would skip prescribed medicine. It was found that 65.7% of patients have never obtained special training for patients with HT and other vascular problems; at the same time 47.8% of people admitted that they lacked information about their previous disease conditions. Despite ongoing measures to provide information for the population to prevent the development of stroke, some patients did not receive the necessary information or did not receive it in full format.

Before CS development, 92.9% of patients had complaints, yet 26.4% of them did not see a doctor for help, and did not control the BP level, even with the following symptoms: high BP, headache, chest pain, dizziness, tinnitus, hearing loss, and slight numbness or weakness in the limbs. Thus, it can be concluded that the therapeutic compliance prior to the development of CS was very low: patients took drugs irregularly and did not follow any diagnostic or preventive regimen. Moreover, most patients had one or more complaints preceding the development of CS.

In the early recovery period from CS, the following types of MADD were diagnosed: 20% of patients with an ergopathic and 15.7% of patients with a hypochondriac type of relationship to the disease. Less often were neurasthenic (8.5%) and sensitive (10%) types. A small percentage of patients, 7.1%, were identified with harmonious, anosognostic, and anxious types of attitudes towards the illnesses. We further identified 5.7% of the egocentric type, 6.4% each of the paranoid and depressive types, and 2.8% of the melancholic and apathetic types of relationship to the disease.

Based on the analysis, we can conclude that the observed patients with CS were predisposed to such personality traits as neurotic response, low self-esteem, anhedonia, and depression, correlated with personal characteristics, stress response to ordinary life situations that proceeds according to a passive-defensive type. Identified: anxiety, abstinence, and lack of confidence. As a result, difficulties arise in forming social contacts, as well as a decrease in the degree of spontaneous aggression, which most often indicates difficulties in expressing negative emotions. Low grades on the scale of “sociability” and “extraversion-introversion” indicate that this group of patients had low social activity. Based on the received results it can be concluded that the observed CS patients revealed a low level of quality of life, as well as a non-adaptive type of relation to the disease, in view of the presence of personality traits predisposing them to neurosis and stress response.

The most common complaints in the psycho-emotional sphere that were presented by CS patients in the early recovery period were decreased mood, tearfulness, and increased

fatigue. Often, patients complained of sudden mood swings, excitement, anxiety at the slightest occasion and without reason, difficulty or impossibility of performing daily activities, decreased concentration, obsessive self-derogatory thoughts, disturbed sleep, anxiety about the future, thoughts of suicide, and thoughts of impending death. All patients experienced manifestations of general physical and mental weakness (asthenia). These complaints prevailed in the clinical picture. Patients complained of fatigue that did not go away after rest, for both long and short periods of time, general weakness, lack of desire to deal with any everyday issues, and incredibly rapid exhaustion. Most patients refused to perform any kind of hard and complex work. Only 17(12.4%) patients decided to continue work but transferred to another position, in connection with expressed asthenic phenomena to a great extent. Patients who potentially preserved working capacity really wanted to work. They wanted to be helpful by applying extraordinary efforts to do any kind of work. A number of patients felt guilty for forced idleness and considered themselves an excessive burden on the family, which undoubtedly worsened their psycho-emotional status. Very often, patients complained of a reduced need for communication, a weak desire to maintain mutual relations with friends, difficulties in communicating with loved ones and colleagues. Many patients wanted to stay alone longer, explaining that they lacked the strength to see anyone. Many patients had emotional lability in the form of "inability to control emotions."

When conducting a special study, we encountered resistance to our efforts. Patients reluctantly agreed to psychological testing. Many patients tried to embellish their subjective state. The majority spoke negatively of psychiatry and psychological help.

Patients maximally fixed their attention on the smallest manifestations of CS. Many of the patients significantly exaggerated the significance and severity of neurological and other somatic disorders. A number of patients insisted to doctors that they "did not correctly establish the diagnosis." Many spoke out that the disease is very severe, and the treatment "cannot change anything, the outcome is clear." In the hospital, patients believed that the doctors did not pay enough attention to their "incurable disease," and that doctors prescribe drugs that are too "simple, cheap" or the completely wrong treatment. Many patients and their relatives in the early recovery phase of CS turned to alternative medicine. Patients expressed that they do not rely on the effectiveness of academic treatment.

Based on the results obtained, the presence of MADD in patients with CS was revealed in the early days of the recovery period. According to the results obtained during the initial psycho-diagnosis, according to the HRSD, a high level of depression was found in 3.57% of patients, the average level - in 27.86%, mild depression - in 33.57% of patients, and the absence of depressive symptoms in 35% of patients.

Thus, it can be noted that with minimal and moderate depression, patients experience a dreary mood, a feeling appears that their life is at an impasse, and they experience bad feelings in the soul. They see the bad slightly more than usual in their past, thus hope for the best decreases, there appears

the fear that there are no reasons for joy, they do not enjoy life as before, and they feel that life is meaningless. However, with mild to moderate depression, these conditions pass more quickly; patients can independently cope with them, find positive moments in life, and return to the normal course of their lives. The situation is different for people with severe depression. Such patients note that almost all the time they have bad feelings in the soul, they worry about their future, and they lose hope for the best. They constantly feel like their life is meaningless, noting that this condition never goes away. They see in themselves and others only the bad; they are constantly irritable, have no strength to do anything, no appetite, and a constant desire to cry. A depressed mood is regular for them, and in the morning, it can be worse than in the evening. It is very difficult to cope with a depressed status; it can last a long time and can develop into a normal state of constant depression.

So, the study showed that deep depressive states were not inherent in a significant number of studied patients.

A study of anxiety levels showed a high level of anxiety in 17.50% of CS patients, a moderate level of anxiety in 47.86%, a low anxiety level in 45.0%, and no anxiety in 2(1.43%) patients.

As we can see, patients who have undergone CS are characterized by a moderate level of anxiety. This level of anxiety indicates that different life situations tend to be perceived adequately, with the subject objectively assessing the current situation. Such subjects are confident enough in most life situations; they feel calm, collected. They are not inclined to avoid critical situations and difficulties. However, they may have periods when they greatly experience their frustrations and cannot forget about them for a long time. In such moments the subjects lack self-confidence and the level of personal anxiety may rise. In general, these subjects can be described as calm, balanced, not prone to excessive irritability and anxiety.

For subjects with a high level of anxiety, it is characteristic to have excessive anxiety in a wide range of life situations. They are afraid of all sorts of trifles and minor troubles. In situations that are not even significant, they do not feel confident and are not able to maintain balance. The occurrence of anxiety is possible even in minor situations; they rarely feel calm, collected and confident. They tend to avoid critical situations and difficulties, believing that they do not have enough strength to deal with them. Generally such patients can be described as disturbed, irritable, rarely calm and balanced, and prone to excessive anxiety. Thus, the obtained indicators characterize them as prone to exhibit excessive anxiety, worried and distracted by trifles and minor troubles. They rarely feel calm, collected and confident, and often try to avoid critical situations and difficulties, believing that they do not have enough strength to deal with them.

Discussion

Our study found that a high level of depression was inherent in only a small number of subjects (3.57%). Mild or moderate depression was more characteristic of the patients

(33.57% and 27.86%, respectively). With mild to moderate depression, these conditions pass quickly; subjects can independently cope with them, find positive moments in life, and return to the normal course of their lives.

Patients with severe depression note that they have a bad feeling in the soul almost all the time, they worry about their future, and do not have hope for the best things. They constantly feel the meaninglessness of their life and see only the bad things for themselves and others. They are constantly irritable and have no strength to do anything, no appetite, and a constant desire to cry.

Most patients (47.86%) with CS have a moderate level of anxiety. Such subjects are quite confident in themselves; in most of life situations they feel calm, collected. But they can have periods when they greatly experience frustrations and cannot forget about those feelings for a long time. In such moments, these patients lack self-confidence and the level of personal anxiety may rise.

A high level of anxiety occurred in 17.50% of patients with CS; the results characterize them as prone to be overly anxious, worried and distracted by trifles and minor troubles. They rarely feel calm, collected and confident, and are often inclined to avoid critical situations and difficulties, believing that they do not have enough strength to deal with them. Thus, the results obtained indicate that patients with CS, at the stage of recovery, have a high level of anxiety and depressive manifestations. That finding determines the need for early detection and prescription of specific therapy for anxious-affective pathology in CS. Different levels of MADD in CS patients in the early period of convalescence need individual specific treatment.

Competing Interests

The authors declare that they have no competing interests.

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Application of Exogenous Luteinizing Hormone in IVF Patients of Late Reproductive Age

N. V. Protopopova^{1,2,3}; E. B. Druzhinina^{1,2,3}; N. A. Boldonova³; A. V. Labygina²;
Y. V. Mylnikova³; N. L. Sakhyanova³; L. I. Maschakevich³; K. V. Krylova³; N. A. Kurashova^{2*}

¹Russian Medical Academy of Continuous Professional Education

²Scientific Centre for Family Health and Human Reproduction Problems

³Irkutsk Regional Clinical Hospital, Regional Perinatal Center
Irkutsk, the Russian Federation

Abstract

Background: A decreased concentration of luteinizing hormone (LH) in women of late reproductive age contributes to disorder in adequate steroidogenesis and impairs oocyte/embryo quality, which results in fewer embryos suitable for cryopreservation, a lower implantation rate, and increased rate of early miscarriage. The purpose of our study was a retrospective analysis of the effectiveness of applying exogenous LH (urinary and recombinant) in ART programs in women of late reproductive age with previous IVF failures.

Methods and Results: We studied how adding exogenous LH (urinary and recombinant) from Days 2-3 and 6-7 of menstrual cycle in ovulatory stimulation affects the outcomes of *in vitro* fertilization cycles. The study included 324 women (mean age of 35.7±3.8 years) receiving treatment for tubal-peritoneal infertility as part of IVF programs. All patients were divided into 4 groups depending on the stimulation day (Day 2-3 or Day 6-7) when exogenous LH-based inductor (human menopausal gonadotropin or recombinant LH) was added. In patients with a risk of “poor response,” supplementing rLH at the initial stages of folliculogenesis (Day 2-3 of menstrual cycle) promotes optimization of oogenesis processes, obtaining a high percentage of good quality embryos (78%) and increasing pregnancy rate (30%). The good quality frozen embryos obtained in stimulation with recombinant LH and human menopausal gonadotropin added, had the same good quality and provided the PR comparable to transfer of fresh embryos.

Conclusion: It is possible that LH injection restores the microenvironment of developing follicles in women of late reproductive age, which is reasonable for the patients studied. (**International Journal of Biomedicine. 2020;10(2):112-115.**)

Key Words: *in vitro* fertilization • exogenous luteinizing hormone • pregnancy • embryo cryopreservation

Abbreviations

AMH, anti-Müllerian hormone; ART, assisted reproductive technology; CG, chorionic gonadotropin; FSN, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; hMG, human menopausal gonadotropin; hCG, human CG; IVF, *in vitro* fertilization; LH, luteinizing hormone; MC, menstrual cycle; OHSS, ovarian hyperstimulation syndrome; PR, pregnancy rate; rFSH, recombinant FSH; rLH, recombinant LH.

Introduction

Preservation and restoration of the population's reproductive health are problems far from a permanent solution, yet are the most important medical challenge of national standing. The rate of infertile couples in Russia ranges from 8% to 20%, which exceeds a 15% critical level as determined by WHO.⁽¹⁻³⁾

The most important step in infertility treatment is to elaborate the ART methods and apply them to clinical practice. According to Russian Association of Human Reproduction, in 2017 the pregnancy rate in Russia was 33.9% per cycle, and 38.4% per embryo transfer in programs of *in vitro* fertilization (IVF). After age 35, the response of ovaries to ovulation stimulation is impaired, quality of the retrieved oocytes and their fertilization pattern is affected up to complete absence,

and the nature and rate of embryo cleavage are transformed—all of which finally leads to worse IVF results.^(4,5)

Ovarian stimulation is the basis of assisted reproduction programs, which is thought to be optimal if one succeeds in retrieving a minimum of 7 preovulatory follicles. Maturation of 5-6 or less than 5 follicles is regarded as a suboptimal or “poor” response; it is found in nearly a third of the women of late reproductive age (over 35 years).⁽⁶⁾ Inadequate ovarian response leads to decreased IVF effectiveness.

In normal MC, in follicular phase first half, the follicle development implies FSH-dependent growth; in the second half, LH-dependent growth begins when a dominant follicle is selected.⁽¹⁾ Regimens with GnRH agonists and antagonists successfully solve the problem of LH early peak; use of rFSH devoid of LH activity leads to a significant decrease in the concentration of endogenous LH. On the other hand, in patients of late reproductive age we noted a decreased number of functional LH-receptors, which may lead to lower ovarian response.^(4,7)

Depletion of the LH concentration contributes to disorder in adequate steroidogenesis and impairs the oocyte/embryo quality; this results in a lower implantation rate, fewer embryos suitable for cryopreservation, and increased rate of early miscarriage.⁽⁸⁾ For the above reasons, the absolute or functional insufficiency of LH to improve the effectiveness of IVF calls for supplemental administration of exogenous LH preparations in stimulation cycles, such as highly purified HMG and rLH as well.⁽⁹⁻¹¹⁾

The purpose of our study was a retrospective analysis of the effectiveness of applying exogenous LH in ART programs in women of late reproductive age with previous IVF failures.

Materials and Methods

We examined 324 women receiving treatment for tubal -peritoneal infertility as part of IVF programs in the ART Department of Irkutsk Regional Clinical Hospital. All women signed an informed consent for use of their personal data. All patients were comparable with respect to their medical history and clinical data, and their laboratory and ultrasound parameters. Their average age was 35.7±3.8 years; they had reduced ovarian reserve (number of antral follicles 6.3±3.3) and previous IVF failures, which was the predisposing cause of poor ovarian response during stimulation. The exclusion criteria were as follows: severe somatic pathology, concentration of functional sperm below 30%.

We divided all patients into 4 groups depending on the stimulation day (Day 2-3 or Day 6-7) when exogenous LH-based inductor (hMG or rLH) was added. Gonadotropin doses and duration of stimulation were determined individually, taking into account the past medical history, age and dynamic ultrasound data. We stopped the rLH injections 2 days prior to the hCG trigger.⁽¹²⁾ We assessed the embryo stage parameters, pregnancy rate, “poor response,” and OHSS.

For transvaginal ovarian puncture, we used a standard technique 36 hours after injection of a CG ovulatory dose with COOK double-lumen needles. Follicles were flushed with Flushing Medium buffer solution (“ORIGIO”) in an

amount not exceeding the amount of the retrieved follicular fluid. For cultivation of gametes and embryos, and for freezing and thawing of embryos, we used the ORIGIO medium line (MediCult Media), four-well trays “Nunc,” and the CO²-incubator ThermoForma, according to the manufacturer’s recommendations. The applied freezing method was vitrification. We selected embryos for cryopreservation on Day 2-5 of the cultivation based on “good quality” standard criteria; the thawed embryos were transferred 2 hours after thawing. The embryos were transferred using a standard technique.

The studied patients were distributed as follows:

Group 1A included 123 patients: hMG was added on Day 2-3 of rFSH stimulation

Group 1B included 68 patients: hMG was added on Day 6-7 of rFSH stimulation

Group 2A included 70 patients: rLH was added on Day 2-3 of rFSH stimulation

Group 2B included 63 patients: rLH was added on Day 6-7 of rFSH stimulation

All the studied patients were comparable with respect to their medical history and clinical data, and their laboratory and ultrasound parameters (Table 1).

Table 1.

Description of initial parameters in patients with various stimulation protocols

Variable	Group 1A (n=123)	Group 1B (n=68)	Group 2A (n=70)	Group 2B (n=63)	P-value
Age, years	36.1±3.9	35.5±3.6	35.5±5.5	35.6±3.7	≥0.05
MC duration, days	28.9±6.1	28.4±2.1	28.6±4.3	28.4±4.5	≥0.05
Duration of infertility, years	7.6±4.2	8.1±4.2	9.3±5.3	9.2±5.2	≥0.05
Primary infertility	37/30.1%	21/30.9%	25/35.7%	19/30.2%	≥0.05
Secondary infertility	86/69.9%	47/69.1%	45/64.3%	44/69.8%	≥0.05
Basal LH, mIU/ml	5.0±3.3	5.2±2.8	5.1±3.0	5.3±3.2	≥0.05
Basal FSH, mIU/ml	8.2±3.8	7.8±2.8	8.4±4.1	7.9±4.2	≥0.05
Basal AMH, ng/ml	1.9±1.7	2.0±1.9	1.7±1.6	1.9±1.6	≥0.05
Number of antral follicles in both ovaries	6.0±2.9	6.0±3.2	5.8±3.1	6.1±3.1	≥0.05

The statistical analysis was performed using the statistical software STATISTICA 6.1 (StatSoft Inc., USA). The mean (M) and standard deviation (SD) were calculated. For data with normal distribution, inter-group comparisons were performed using Student's t-test. Mann-Whitney U test was used to compare means of variables not normally distributed. A probability value of $P < 0.05$ was considered statistically significant

Results and Discussion

The day of LH injection was chosen based on recommendations from a great deal of published data. It is known that LH begins to impose the stimulating effect on the growing follicles once they reach a minimum of 10 mm in diameter, i.e. by the 6th day of stimulation.

The analysis of the embryo stage in 4 LH-stimulation protocols shows that supplementing with hMG or rLH during the ovarian stimulation process at different phases of follicular maturation has various effects on folliculogenesis and oogenesis (Table 2).

Table 2.

Description of embryo stage basic parameters

Variable	Group 1A (n=123)	Group 1B (n=68)	Group 2A (n=70)	Group 2B (n=63)	P-value
Number of punctured follicles	5.0±2.9	4.8±2.9	4.5±3.1	5.0±3.4	$P \geq 0.05$
Number of oocytes retrieved	3.6±2.9	3.6±2.9	2.9±2.7	4.0±3.1	$P_{2A-2B} = 0.009$
Number of mature oocytes	3.0±2.6	3.0±2.4	2.3±2.1	3.1±2.6	$P \geq 0.05$
Number of immature oocytes	0.5±0.7	0.6±0.8	0.4±0.8	0.8±1.1	$P_{1A-2B} = 0.049$ $P_{2A-2B} = 0.011$ $P_{1B-2A} = 0.018$
Number of degenerative oocytes	0.2±0.4	0.1±0.3	0.1±0.3	0.1±0.3	$P \geq 0.05$
Number of unfertilized oocytes	0.8±0.9	0.8±1.0	0.5±0.8	1.0±1.4	$P_{1A-2A} = 0.048$ $P_{2A-2B} = 0.016$ $P_{1B-2A} = 0.049$
Number of embryos obtained	2.7±2.4	2.8±2.3	2.3±2.1	2.9±2.3	$P \geq 0.05$
Number of good quality embryos	1.8±1.3	2.0±2.5	1.8±1.1	2.0±1.8	$P \geq 0.05$
Proportion of good quality embryos	65.9%	70.5%	78.0%	67.1%	$P_{2A-2B} = 0.034$ $P_{1A-2A} = 0.009$
Embryo cryopreservation	16.3%	19.1%	14.3%	15.9%	$P \geq 0.05$
Number of frozen embryos	0.4±1.1	0.4±0.9	0.3±0.9	0.6±1.7	$P \geq 0.05$

In all groups, there were no statistical differences in the average number of punctured follicles, retrieved mature and degenerative oocytes, and the number of embryos obtained, including those of good quality. However, the smallest number of oocytes (2.9±2.7), including immature oocytes (0.4±0.8), was retrieved in Group 2A. This fact evidences that in rFSH stimulation with rLH added from Day 2–3, the growth of preovulatory follicles was more uniform than in other groups, where with a greater number of the oocytes retrieved, most of them were immature. Group 2A gave the lowest number of unfertilized oocytes (0.5±0.8), a fact that can be attributed to this group having the smallest number of immature oocytes, and to better quality of oocytes retrieved

as well. The proportion of good quality embryos in Group 2A was the largest among the 4 groups (78%, 65.9; 70.5 and 67.1%, respectively). Good quality extra embryos were frozen in 16.3% of cases in Group 1A, 19.1% – in Group 1B, 14.3% – in Group 2A, and 15.9% – in Group 2B ($P > 0.05$). The average number of frozen embryos in all groups was not statistically different.

In recent years there has been a trend toward selective transfer into the uterine cavity of one good quality embryo that has reached the blastocyst stage, in order to reduce the risk of multiple pregnancy and to improve pregnancy rates. However, this can hardly be related to women with reduced parameters of ovarian reserve who have the risk of “poor” response, when it is safer to transfer 3-day embryos, as their further development is not guaranteed. In our study, the number of embryos that reached the blastocyst stage in patients of the 4 groups was 4%, 3.7%, 3.8% and 4.7%, respectively ($P > 0.05$).

In Group 2A, there were the highest pregnancy rates per stimulated cycle and per embryo transfer, and the rate of term births (30%, 36.8%, and 25.7%, respectively), which counts in favor of the best quality of oocytes and embryos obtained, and the endometrium readiness for implantation in the protocol with rLH added on Day 2-3 of MC (Figure 1).

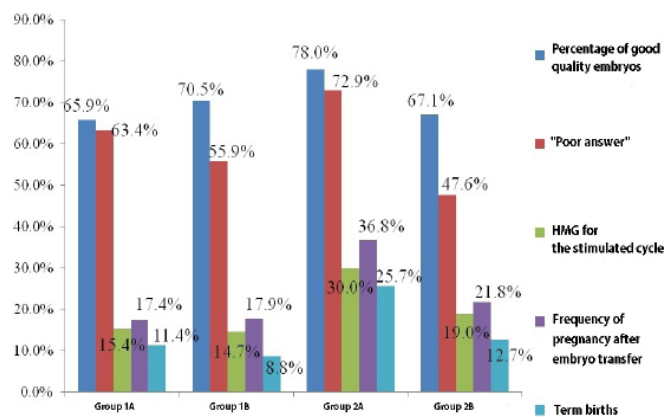


Figure 1.

In Group 2A, such an IVF effectiveness parameter as “poor” response was noted at the highest level (72.9%), which was further accompanied by retrieving the lowest number of oocytes in this group, but the greatest proportion of good quality embryos (78%). In other words, the 2A protocol did not increase the number of follicles punctured, but improved the quality of oocytes and embryos retrieved.

OHSS was registered at statistically the same low level in all groups (3.3%, 5.9%, 5.7%, and 3.2%). The OHSS low rate is conditioned by the initial low ovarian reserve in the patients studied.

Our study shows that supplementation of rLH at the initial stages of folliculogenesis is more physiological, since in the natural menstrual cycle, follicles grow in the presence of both endogenous FSH and LH from the early stages. On the other hand, the increased level with age of FSH, not LH, is noted in the early follicular phase. It is possible that LH injection restores the microenvironment of developing follicles

in women of late reproductive age, which is reasonable for the patients studied.

At the next stage, we evaluated the effectiveness of transferring frozen embryos retrieved in 2 protocols (Table 3).

Table 3.

Effectiveness of transfer of frozen embryos retrieved in various stimulation protocols

	rFSH+hMG protocol (n=98)	rFSH+rLH protocol (n=46)	P-value
Pregnancy rate, case (%)	22 (22.4%)	13 (28.3%)	>0.05

The effectiveness of frozen embryo transfer does not depend on the stimulation protocol by which the frozen embryo was obtained, (i.e. whether the stimulation involved supplementation of recombinant or urine exogenous LH). In addition, PR was not statistically different in frozen and fresh embryo transfer (Figure 1).

As stated previously, in patients with the expected low ovarian response in a stimulated cycle it is safer to transfer 3-day embryos as their further development is not guaranteed. Therefore, for this cohort of patients in a stimulated cycle, we have to choose the most effective protocol that facilitates obtaining the best quality embryos (in our case, rFSH+rLH from Day 2-3). Obtaining good quality extra embryos suitable for freezing is a good indicator of stimulation in these patients. In a non-stimulated cycle, only good quality frozen embryos are transferred, those that have passed another stage of selection—vitrification. Thus, the good quality frozen embryos obtained in stimulation with rLH and hMG added, have the same good quality and provide the PR comparable to transfer of fresh embryos.

In conclusion: In patients with a risk of “poor response,” supplementing rLH at the initial stages of folliculogenesis (Day 2-3 of MC) promotes optimization of oogenesis processes, obtaining a high percentage of good quality embryos (78%) and increasing PR (30%). Good frozen embryos obtained in stimulation with rLH and hMG added are of the same good quality, and pregnancy rates are not statistically different in transfer of frozen and fresh embryos.

Competing Interests

The authors declare that they have no competing interests.

***Corresponding author:** Nadezhda A. Kurashova, PhD, ScD. Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk, the Russian Federation. E-mail: nakurashova@yandex.ru

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The Role of Immunological Mechanisms in Physiological Pregnancy and the Consequences of Disturbing Them during *in vitro* Fertilization

Eduard A. Kashuba, PhD, ScD¹; Yuliya S. Chekhova^{1*};

Svetlana V. Solovyova, PhD, ScD¹; Tatiana G. Drozdova, PhD¹; Daria D. Bekina^{2,1}

¹Tyumen State Medical University, Tyumen, Russia

²Tyumen Region Clinical Hospital #1, Tyumen, Russia

Abstract

We observed the immunologic status of clinically healthy women and women with tuboperitoneal infertility during the planning and first trimester of pregnancy. The markers of unsuccessful *in vitro* fertilization (IVF) attempts were also identified. No pathological anomalies were noted in the clinically healthy women before and after conception. The changes in the immune system of infertile women who underwent IVF were different from the women who had physiologic pregnancy. Identification of these changes could help to guide preventive measures while preparing for IVF and during the IVF procedure. (**International Journal of Biomedicine. 2020;10(2):116-119.**)

Key Words: pregnancy • infertility • *in vitro* fertilization • immunologic status

Introduction

Studying the immunological basis of the mother-fetus interaction is one of the priorities of modern medicine. Complex immune interactions that occur during pregnancy follow the principle of direct and reverse connection.⁽¹⁻³⁾ The outcome (termination or preservation of pregnancy) depends on maintaining a balance of the complex immune interactions.⁽⁴⁾ This problem of maintaining regulatory balance is more relevant in patients having pregnancy via *in vitro* fertilization (IVF). At the present stage of studying IVF outcomes, increasing the effectiveness of the IVF procedure is directly related to studies in the field of immunological regulation of the reproductive function of women.⁽⁵⁾

Immunocompetent cells (macrophages, lymphocytes) and cytokines produced by these cells (interleukines, growth factors and chemokines) play a role in folliculogenesis, ovulation, and yellow body formation and function, as well as in cyclic morphofunctional endometrial changes, fertilization and egg implantation.^(6,7) Therefore, assessment of cell-mediated and humoral immunity, as well as cytokine balance, is crucial for detection of markers that may indicate an unsuccessful outcome of IVF.

Materials and Methods

Our study was open-label, prospective and comparative. Group 1 (main group) included 70 healthy women (mean age of 25.69±0.14 years) prior to conception and in the first trimester of pregnancy. Group 2 (IVF+) included 25 women (mean age of 30.28±0.50 years) with tuboperitoneal infertility before IVF and in the first trimester of pregnancy. Group 3 (IVF-) included 45 women (mean age of 32.29±0.32 years) with tuboperitoneal infertility and an unsuccessful IVF attempt. The study was approved by the Tyumen State Medical University Ethics Committee (protocol N 68 dated 08.04.2016). Written informed consent was obtained from all participants.

The blood tests were taken twice: during the planning of pregnancy and 8 weeks after conception/IVF procedure. Blood samples were collected in accordance with the existing requirements in the morning on an empty stomach from the cubital vein in commercial heparin tubes with heparin (25 IU/ml).

The blood tests included:

- Total blood count in the blood smears, stained by the Romanovsky-Giemsa method

- Immunophenotyping of peripheral blood lymphocytes using an expanded panel of monoclonal antibodies to differentiation antigens (CD3+, CD4+, CD8+, CD16+, CD20+, CD23+, and CD38+). Lymphocytes were isolated by centrifugation on a ficoll-verographin gradient. Lymphocyte

*Corresponding author: Yuliya S. Chekhova, Tyumen State Medical University, Tyumen, Russia. E-mail: doktor-ch@bk.ru

subpopulations were determined on a FACScan (Beckton-Dickinson, USA) flow cytometer equipped with an argon laser

- Determination of the concentration of cytokines (IL-1, IL-4, IL-6, IL-10, IFN γ , and TNF α) in cell culture supernatants by flow immunofluorometry using a double-beam laser automated analyzer (Bio-Plex $\text{\textcircled{R}}$ Suspension Array System, Bio-Rad, USA, Bio-Rad, USA)

- Determination of the concentration of serum immunoglobulins (IgG, IgM, IgA) by the method of radial immunodiffusion (Mancini method)

Statistical analysis was performed using the Statistica 10.0 software package (Stat-Soft Inc., USA). The normality of distribution of continuous variables was tested by the Kolmogorov-Smirnov test with the Lilliefors correction and Shapiro-Wilk test. Results are presented as median (interquartile range [IQR]) (Me[25-75]). Differences of continuous variables were tested by the Mann-Whitney U-test. The Wilcoxon criterion was used to compare the differences between the paired samples. A probability value of $P < 0.05$ was considered statistically significant.

Results

Complex evaluation of cell-mediated immunity during the planning of pregnancy showed a statistically significant rise in the count of leukocytes with a tendency to lymphopenia in Group 3, compared to Group 1 (Table 1). Analysis of the lymphocyte subpopulations in Group 2 before the IVF procedure revealed activation of the T-system immunity due to an increase in the level of CD3+T cells and CD4+T cells.

Group 3 had a statistically significant drop in these cells. The level of effector CD8+cells was also significantly reduced in women with an unsuccessful IVF attempt. The highest level of CD16+natural killer (NK) cells was found in Group 2, and a statistically significant decrease was observed in Group 3. The level of B cells before pregnancy and the IVF program was also different in all groups. A statistically significant activation of B cells due to an increase of CD20+B cells and CD22+ B cells was found in Group 3 (Table 1).

In the first trimester of pregnancy (8 weeks after conception), in Group 1, we found a statistically significant decrease in the levels of CD3+T cells and CD4+T cells, and an increase in the level of CD20+cells and CD22+cells. Similar changes were found in Group 2, but the decrease in the level of CD3+T cells and CD4+T cells was more pronounced than in Group 1. A statistically significant increase in the level of CD16+NK cells and CD20+B cells was also found in Group 2. In Group 3, 8 weeks after an unsuccessful IVF procedure, we found a statistically significant increase in the level of CD3+T cells and a decrease in the levels of CD8+T cells and CD22+B cells (Table 1).

Cytokines are actively involved in the processes occurring in the fetoplacental complex, namely in regulating the normal development of the fetus and implementing the mechanisms of the complicated course of pregnancy.⁽⁸⁾ Blood cytokine levels are presented in Table 2. At the stage of pregnancy planning, a statistically significant increase in the level of IL-1 α was observed in Groups 2 and 3, as well as TNF α with the highest values in Group 3. The IFN γ level showed significant variability. Before conception, the IFN γ level was within normal values in Group 1 and significantly elevated in Group 2. In Group 3, the IFN γ

Table 1.

The lymphocyte subpopulations in study groups

Indicators	Group 1 (n=70)			Group 2 (n=25)			Group 3 (n=45)		
	before	<i>P</i>	after	before	<i>P</i>	after	before	<i>P</i>	after
Leukocytes, $\times 10^9/L$	6.0 ^{###} (5.6;6.2)	0.650	6.0 ^{###} (5.8;6.5)	6.1 ^{^^} (5.9;6.5)	0.002	7.0 ^{^^} (6.7;7.5)	7.2 ^{###^^} (7.0;7.4)	0.004	7.9 ^{###^^} (7.8;7.9)
Lymphocytes,%	24.0 ^{***###} (23.0;25.0)	0.004	22.0 ^{***###} (22.0;23.0)	25.0 ^{***^^} (24.0;26.0)	0.000	22.0 ^{***^^} (21.0;23.0)	21.0 ^{###^^} (20.0;22.0)	0.000	28.0 ^{###^^} (27.0;28.0)
CD3+	64.2 ^{***###} (62.2;65.4)	0.000	57.5 ^{***###} (56.5;57.6)	65.7 ^{***^^} (65.2;66.5)	0.000	56.7 ^{***^^} (55.9;57.6)	58.2 ^{###^^} (57.7;59.1)	0.030	60.2 ^{###^^} (58.8;61.1)
CD4+	34.3 ^{***###} (34.2;35.4)	0.003	29.9 ^{***###} (28.2;31.2)	35.8 ^{***^^} (33.8;36.9)	0.000	27.0 ^{***^^} (26.1;30.3)	27.9 ^{###^^} (25.9;29.0)	0.356	29.0 ^{###^^} (27.9;29.8)
CD8+	21.7 ^{##} (20.9;22.5)	0.000	27.3 ^{##} (26.8;27.8)	22.3 ^{^^} (21.0;22.7)	0.061	22.4 ^{^^} (22.2;23.3)	20.7 ^{###^^} (18.7;21.9)	0.005	18.8 ^{###^^} (17.9;19.0)
CD4+/CD8+	1.6 ^{##} (1.5;1.6)	0.000	1.1 ^{##} (1.09;1.2)	1.6 [^] (1.5;1.7)	0.001	1.2 [^] (1.3-1.4)	1.3 ^{###^} (1.3;1.5)	0.062	1.5 ^{###^} (1.2;1.3)
CD16+	15.7 ^{***###} (15.0;16.4)	0.031	16.5 ^{***###} (16.1;17.0)	17.0 ^{***^^} (16.3;17.5)	0.001	19.4 ^{###^^} (18.9;19.8)	13.9 ^{###^^} (13.6;14.0)	0.060	12.1 ^{###^^} (11.7;12.5)
CD20+	16.0 ^{###} (15.3;16.5)	0.000	22.0 [#] (20.5;23.2)	15.5 ^{***^^} (15.1;15.9)	0.000	21.0 (19.2;21.3)	21.7 ^{###^^} (20.9;22.0)	0.255	20.9 [#] (20.1;21.2)
CD22+	7.5 ^{###} (7.4;7.7)	0.042	8.7 ^{***###} (7.6;8.9)	8.8 ^{***^^} (8.2;9.3)	0.350	9.0 ^{***^^} (8.1;9.2)	9.8 ^{###^^} (9.0;10.0)	0.001	5.9 ^{###^^} (5.8;5.9)

Footnotes:

* - statistically significant differences between Groups 1 and 2: * - $P < 0.05$; ** - $P \leq 0.01$; *** - $P \leq 0.000$

- statistically significant differences between Groups 1 and 3: # - $P < 0.05$; ### - $P \leq 0.01$; ### - $P \leq 0.000$

^ - statistically significant differences between Groups 2 and 3: ^ - $P < 0.05$; ^^ - $P \leq 0.01$; ^^ - $P \leq 0.000$

level was significantly decreased against the background of high values of IL-4, IL-6 and IL-10. Groups 2 and 3 showed an increase in the IL-6 level during the preconception period, compared to Group 1 (Table 2).

In the first trimester of pregnancy, in Group 1 we found an increase in the level of IL-4 and IL-6, and a decrease in the IFN γ level. In Group 2, we observed a significant increase in the IL-1 α level against the background of decreased levels of IFN γ and TNF α . At the same time, the level of IL-4 significantly increased, and the level of IL-6 did not change, but significantly exceeded the indicator of Group I. In Group 3, 8 weeks after an unsuccessful IVF procedure, we found a decrease in the IL-1 α level, but it was significantly higher than in Group 1. Against this background, the levels of IFN γ and TNF α increased, and IL-4 and IL-10 decreased, while IL-6 did not change in dynamics.

The concentration of serum immunoglobulins is presented in Table 3. At the stage of pregnancy planning, the IgA level was decreased in Group 2 and increased in Group 3, compared

to Group 1. The IgM level in Groups 2 and 3 was significantly higher than in Group 1, with the highest values being determined in Group 3. Group 3 had also the highest IgG level.

In the first trimester of pregnancy, no significant changes in the immunoglobulin levels were found in Group 1. In Group 2, we observed a statistically significant decrease in the level of IgM and IgG, while the IgA level did not change. In Group 3, a decrease in all studied immunoglobulins was detected 8 weeks after an unsuccessful IVF procedure, at that the IgM level was significantly higher than in Group 2.

Discussion

Analysis of cell-mediated immunity showed a significant difference between the 3 groups of women at the preconception stage. Group 1 had no significant immunologic abnormalities during the study. In women of Group 3, T-cell immunity depression against the background of a tendency to neutrophilic

Table 2
Blood cytokine levels in study groups

Indicators (pkg/ml)	Group 1 (n=70)			Group 2 (n=25)			Group 3 (n=45)		
	before	P	after	before	P	after	before	P	after
IL-1 α	14.0 ^{****#} (13.9;14.3)	0.120	12.1 ^{****#} (12.0;12.2)	22.1 ^{****^} (22.0;22.2)	0.005	26.0 ^{****^} (25.9;26.2)	99.5 ^{###^^} (99.3;99.6)	0.008	96.0 ^{###^^} (95.9;96.2)
IFN γ	152.0 ^{****#} (149.0;156.0)	0.000	122.0 ^{****#} (119.0;129.0)	270.0 ^{****^} (261.0;272.0)	0.007	257.0 ^{****^} (255.0;260.0)	96.0 ^{###^^} (94.0;105.0)	0.000	165.0 ^{###^^} (163.0;166.0)
TNF α	18.1 ^{****#} (18.0;18.2)	0.000	26.1 ^{###} (26.0;26.25)	38.12 ^{****^} (36.1;42.4)	0.000	26.1 ^{^^} (26.0; 26.25)	92.0 ^{###^^} (87.0;103.0)	0.046	98.4 ^{###^^} (98.2;98.5)
IL-4	43.11 [#] (43.10;43.12)	0.005	45.1 [#] (45.07;45.14)	42.11 [^] (42.0;42.12)	0.005	46.11 ^{^^} (45.1;47.12)	59.25 ^{#^^} (56.2;60.50)	0.001	36.11 ^{#^^} (36.09;36.12)
IL-6	35.2 ^{****#} (35.1;35.4)	0.010	39.5 ^{****#} (39.0;39.8)	55.2 ^{****^} (55.0;55.4)	0.250	56.0 ^{****^} (55.7;56.3)	110.3 ^{###^^} (110.0-110.5)	0.331	109.1 ^{###^^} (103.0;113.2)
IL-10	8.8 [#] (7.5;9.1)	0.310	9.0 [#] (8.2;9.3)	9.3 ^{^^} (9.0;9.6)	0.305	9.8 [^] (8.9;10.0)	16.5 ^{#^^} (15.9;17.7)	0.001	12.6 ^{#^^} (10.4;13.5)

Footnotes:

* - statistically significant differences between Groups 1 and 2: * - $P < 0.05$; ** - $P \leq 0.01$; *** - $P \leq 0.000$

- statistically significant differences between Groups 1 and 3: # - $P < 0.05$; ## - $P \leq 0.01$; ### - $P \leq 0.000$

^ - statistically significant differences between Groups 2 and 3: ^ - $P < 0.05$; ^^ - $P \leq 0.01$; ^^ - $P \leq 0.000$

Table 3.
The concentration of serum immunoglobulins in study groups

Indicators (g/L)	Group 1 (n=70)			Group 2 (n=25)			Group 3 (n=45)		
	before	P	after	before	P	after	before	P	after
Ig A, g/L	1.55 ^{****#} (1.54;1.55)	0.786	1.55 ^{****#} (1.49;1.57)	1.11 ^{****^} (1.10;1.12)	0.444	1.2 ^{****^} (1.15;1.25)	1.89 ^{###^^} (1.87;1.91)	0.001	1.0 ^{###^^} (0.98;1.05)
Ig M, g/L	1.19 ^{****#} (1.17;1.20)	0.395	1.19 ^{****#} (1.17;1.19)	1.27 ^{****^} (1.25;1.28)	0.001	0.97 ^{****^} (0.96;0.97)	17.29 ^{###^^} (17.28;17.31)	0.000	1.64 ^{###^^} (1.64;1.65)
Ig G, g/L	15.95 ^{###} (14.98;15.98)	0.151	16.97 ^{****#} (16.95;16.98)	16.0 ^{^^} (16.56;16.06)	0.000	11.05 ^{****^} (11.05;11.07)	42.2 ^{###^^} (42.00;42.30)	0.000	9.28 ^{###^^} (9.27;9.30)

Footnotes:

* - statistically significant differences between Groups 1 and 2: * - $P < 0.05$; ** - $P \leq 0.01$; *** - $P \leq 0.000$

- statistically significant differences between Groups 1 and 3: # - $P < 0.05$; ## - $P \leq 0.01$; ### - $P \leq 0.000$

^ - statistically significant differences between Groups 2 and 3: ^ - $P < 0.05$; ^^ - $P \leq 0.01$; ^^ - $P \leq 0.000$

leukocytosis could indicate an ineffective suppression of latent chronic inflammation.⁽⁹⁾ On the contrary, an increase in the level of cells with cytotoxic and killer activity in Group 2 patients indicated a tension in the immune response that impeded the maintenance of active inflammation.^(10,11) In the first trimester of pregnancy, women of Group 1 exhibited a characteristic immunological rearrangement aimed at preventing fetal rejection. In Group 2, the changes had a similar orientation; however, a more pronounced inhibition of T-cell immunity was observed, which could be a consequence of the induction of superovulation. In Group 3, 8 weeks after an unsuccessful IVF procedure, on the contrary, we observed activation in T-cell immunity along with inhibition of B-cell immunity.

The study of cytokine status at the stage of pregravid preparation revealed an increase in the concentration of pro-inflammatory cytokines (IL-1 α , TNF α , and IFN γ) in patients of Group 2. In Group 3, along with high values of IL-1 α and TNF α , the level of IFN γ was significantly reduced. This cytokine imbalance in women of Group 3 was probably due to a statistically significant increase in the level of IL-4, IL-6, and IL-10. Secreted cytokines cause the loss of ability of Th1 cells and cytotoxic T cell to proliferate and to produce IFN γ . The balance between Th1 cells and Th2 cells and the cytokines produced by them is shifted towards Th2, which leads to the implementation of humoral reactions.

In the first trimester of pregnancy, in Group 1 the cytokine balance shifted towards immunosuppressive Th-2 cytokines, which are able to inhibit the activity of cellular immunity and stimulate the production of progesterone and chorionic gonadotropin. The increase in IL-1 α level in patients of Group 2 can probably be associated with its effect on the initial stages of embryogenesis, by enhancing the adhesive properties of trophoblast. Moreover, an increase in the production of IL-4, which ensures the dominance of humoral immunity over cellular, explains the decrease in the IFN- γ level in this group of women. In addition, no increase in the level of anti-inflammatory IL-6, IL-10 may be due to the influence of superovulation, which can level the activity of humoral immunity.^(12,13) In women of Group 3 with an ineffective IVF program, after stimulation of superovulation, the TNF α level remained high, along with high IFN- γ level and decreased IL-4 level. High values of IgM and IgG during preparation for pregnancy in patients with an IVF program, with a significant decrease after the procedure, can predict an unfavorable outcome of IVF.^(14,15)

Thus, in healthy women, immunological changes occurring after conception are aimed at ensuring the physiological course of pregnancy. The revealed shifts in the functioning of the immune system in women with infertility, depending on the outcome of IVF, are multidirectional and may serve as a prognosis factor.

Competing Interests

The authors declare that they have no competing interests.

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Pathomorphological Markers of Overcoming Radioresistance in the Treatment of Cervical Cancer

Pavel Yu. Andreev¹; Daniil Yu. Bugrimov, PhD^{1*}; Andrey A. Filin, PhD¹;
Olga V. Kashaeva, PhD²; Natalya V. Korotkikh¹; Andrey A. Klimovich¹

¹Voronezh State Medical University named after N. N. Burdenko, Voronezh, Russia

²Moscow State University of Medicine and Dentistry named after AI Evdokimov, Moscow, Russia

Abstract

The aim of this study was to study pathomorphological markers of decreasing radioresistance of cervical tumor tissue during radiation therapy and the administration of sodium deoxyribonucleate.

Methods and Results: The object of the study was patients with diagnosed cervical cancer (T1/FIGOIB, T1a1-2/FIGOIA1-2, and T1b1-2/FIGOIB1-2; NX0 and M0). All patients in the study were divided into 2 groups. The main group (MG) included 40 patients receiving combination of standard chemoradiotherapy with sodium deoxyribonucleate (5ml intramuscularly for 20 days). The comparison group (CG) included 23 patients receiving only standard chemoradiotherapy. Histological and immunohistochemical analysis was performed.

Conclusion: Against the background of complex therapy, the TLR9 expression indices in the patients of the MG were 24%-36% higher than the indices in the patients of the CG. The inclusion of sodium deoxyribonucleate—a TLR9 agonist—in the chemoradiotherapy regimen for cervical cancer has great potential for stimulating the TLR9 expression in immunocompetent cells of the tumor microenvironment. (**International Journal of Biomedicine. 2020;10(2):120-123.**)

Key Words: radioresistance • TLR9 expression • cervical cancer • pathomorphological markers

Introduction

The possibility of increasing the radiosensitivity of tumors is of high practical importance in oncology.⁽¹⁻³⁾ In radiation therapy, of particular relevance is the search for chemical compounds that not only increase the sensitivity of tumor tissue to the effects of radiation therapy, but also act indirectly, by activating their own immunocompetent cells, including in the area of the tumor microenvironment.^(4,5)

One of the ways to implement the above strategies is to analyze the expression of a group of cell receptors of the innate immune response, known as the family of PRRs (pathogen recognition receptors), capable of recognizing and binding to antigens of pathogen-associated molecular patterns.^(2,5) The main PPR family is the Toll-like receptors (TLRs), which recognize molecular patterns associated with pathogens, including bacteria, viruses, fungi and protozoa.⁽⁶⁻⁸⁾

TLRs are largely classified into 2 subfamilies based on their localization: cell surface TLRs (TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10) and intracellular TLRs localized in the endosome (TLR3, TLR7, TLR8, TLR9, TLR11, TLR12, and TLR13).^(9,10) TLR9 is activated intracellularly by bacterial DNA and synthetic oligodeoxynucleotides containing unmethylated CpG dinucleotides (CpG motifs).^(6,11)

As a rule, TLR activation leads to the production of cytokines and antimicrobial factors through common intracellular signaling pathways. The TLR family harbors extracellular leucine-rich repeat domains and a cytoplasmic domain that is homologous to that of the interleukin (IL)-1 receptor (IL-1R) family. After stimulation, TLR recruits IL-1R-associated kinase via adaptor myeloid differentiation factor 88 (MyD88) and induces activation of NF-kappaB and mitogen-activated protein kinases.^(3,4,6,12) The TLR9-MyD88 signaling pathway plays a critical role in promoting adaptive immune responses and that modulation of this pathway may have enormous therapeutic potential in enhancing vaccine potency, controlling autoimmunity, as well as improving the outcome of viral vector-mediated gene therapy.⁽¹³⁾

*Corresponding author: Daniil Yu. Bugrimov, PhD. Voronezh State Medical University named after N.N. Burdenko. Voronezh, Russia. E-mail: daniikt@mail.ru

The search for and study of TLR9 agonists is of great scientific and practical importance. According to the literature, sodium deoxyribonucleate can be classified as an agonist to TLR9.^(14,15) Thus, the mechanisms of the immunotropic effect of sodium deoxyribonucleate, which contains unmethylated CpG motifs, suggest a high tropism for TLR9 with subsequent activation of innate immunity mechanisms. The rationale for investigating TLR9 agonists as antitumor agents is based on the hypothesis that the innate immune response may have direct antitumor effects. A study of the expression of TLR9 against the background of the use of sodium deoxyribonucleate may be useful to clarify the mechanism of the sodium deoxyribonucleate action at the level of the tumor microenvironment.

The aim of this study was to study pathomorphological markers of decreasing radioresistance of cervical tumor tissue during radiation therapy and the administration of sodium deoxyribonucleate.

Materials and Methods

The object of the study was patients with diagnosed cervical cancer (T1/FIGOIB, T1a1-2/FIGOIA1-2, and T1b1-2/FIGOIB1-2; NX0 and M0). All patients in the study were divided into 2 groups. The main group (MG) included 40 patients receiving combination of standard chemoradiotherapy with sodium deoxyribonucleate. The comparison group (CG) included 23 patients receiving only standard chemoradiotherapy. The standard treatment protocol included radiation therapy (RT) in combination with weekly platinum chemotherapy (CT). RT: median doses to point A and B were 85–90 Gy and 55–60 Gy, respectively. Cisplatin 40 mg/m² with gemcitabine 125 mg/m² was prescribed weekly against the background of the remote component of RT, then after RT, 2 courses of adjuvant CT (cisplatin 50 mg/m² on Day 1 and gemcitabine 1000 mg/m² on Days 1 and 8 of a 21-day cycle with an interval of 3 weeks) were performed.

During the study period, all patients were given a threefold morphological study of biopsy material: biopsy #1 (biopsy before treatment), biopsy #2 (14 days after treatment) and biopsy #3 (28 days after treatment). A 1.5% solution of sodium deoxyribonucleate (5 ml daily) was administered intramuscularly for 20 days. For routine staining of histological preparations, Mayer hematoxylin and a 1% alcohol solution were used. Immunohistochemical analysis of TLR9 expression was performed using monoclonal antibodies (Anti-TLR9 antibody [26C593.2] ab134368 (100 µg) (manufacturer: CarlZeiss Microscopy, Germany). Tissue samples were processed using the AGT11-FMP-4 system and BenchMark XT immunostainer.

Unmasking was carried out on the Dako PT Link using EnVision™ FLEX Target Retrieval Solution, High pH (50x Tris/EDTA buffer, pH 9) (Dako Omnis); heating time of 20 minutes (t=95°C). We used the Histofine imaging system (the polymer incubation time of 20 minutes, t=22-24°C), Dako chromogen (incubation time of 10 minutes with 22-24°C, without amplification). Sections were prepared using a Slide 4004M sled microtome. Morphometry of the preparations was carried out with an Olympus microscope BX2WI; for photo documentation, we used a hardware-software complex for

biological research with a documenting system based on the upright microscope Axio Imager-2 (Carl Zeiss, Germany).

Quantification of the expression level of TLR9-positive cells in the cervical biomaterial was performed by determining the representation of TLR9-positive immunocompetent cells in the field of view using an x40 lens. During a planimetric analysis, the entire volume of biopsy material was examined; the number of fields of view could be more than 40. The results were expressed quantitatively in the average amount of expression of TLR9-positive cells in the field of view of each patient. Statistical analysis was performed using the Statistica 10.0 software package (Stat-Soft Inc., USA).

Results and Discussion

Comparison of biopsy material in patients of the CG and MG groups before treatment showed that the tumor was represented by squamous cell carcinoma (a well-differentiated tumor in 65% and 58% of cases, respectively; moderately differentiated in 35% and 42% of cases, respectively). Tumor cells were represented by layers of atypical squamous epithelium with the phenomena of invasive and infiltrative growth and mitoses, including pathological ones. The study of biopsy material during therapy showed grade IV therapeutic pathomorphosis in all cases in the MG and 84% of cases in the CG. Tumor regression was combined with pronounced fibrotic changes: 24% in the CG and 46% in the MG. In the MG, in all patients who underwent biopsy #3, there was an almost total regression (97%) with a pronounced picture of fibrosis. It is worth noting that dystrophic groups of tumor cells in the CG were rarely encountered during the third biopsy. Thus, in both groups, against the background of the therapy, a significant regression in the volume of the tumor lesion and an increase in the amount of connective tissue (fibrosis) were revealed. Slight differences were observed in the severity of changes on biopsies #1 and #2: in patients of the MG, tumor regression was observed 16% more often than in patients of the CG.

Before treatment, cells with different degrees of immunopositivity for TLR9 were detected in patients of CG and MG in the biopsy material (Photos 1A, C). The relative content of cells with high TLR9 expression was small (Photo 1A) and tissue distribution (Photo 1B) was uneven. Radiation therapy led to increasing the TLR9 expression in tumor stromal cell cytoplasm (Table 1) in both study groups. At the same time, in patients of the MG by the time of biopsy #3, a higher level of TLR9 expression in the biopsy material was noted (Table 1). In the MG after treatment, a visual increase in cells with high immunopositivity for TLR9 was noted, not only peritumorally (Photos 2C,D) but also intratumorally (Photos 2A,B).

Table 1.

Comparative indicators of TLR9 expression in patients of compared groups (CU)

Observation period	CG	P-value	MG
Biopsy #1	2.95±0.15	<0.05	3.15±0.05
Biopsy #2	4.15±0.15	<0.05	4.65±0.15
Biopsy #3	3.95±0.05	<0.01	5.15±0.05

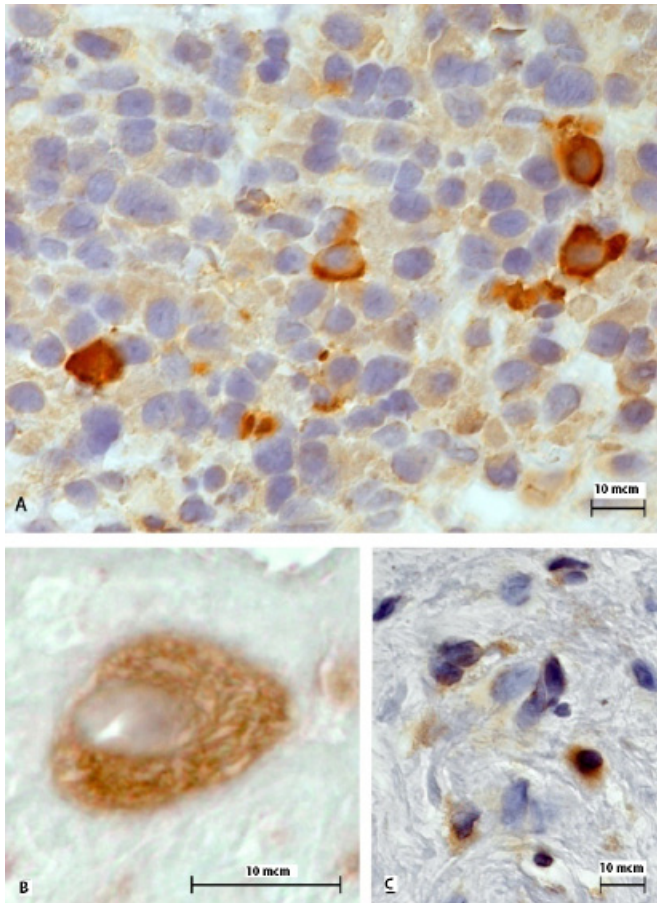


Photo 1. Expression of TLR9 by tumor microenvironment cells in the cervical biomaterial before treatment: A - tumor microenvironment cells with a high TLR9 content; B - cytological picture of tumor stromal cells, which are immunopositive for TLR9 cells; B - different intensities of TLR9 expression in tumor stromal cells.

Thus, against the background of complex therapy, the TLR9 expression indices in the patients of the MG were 24%-36% higher than the indices in the patients of the CG. The inclusion of sodium deoxyribonucleate—a TLR9 agonist—in the chemoradiotherapy regimen for cervical cancer has great potential for stimulating the TLR9 expression in immunocompetent cells of the tumor microenvironment.

Competing Interests

The authors declare that they have no competing interests.

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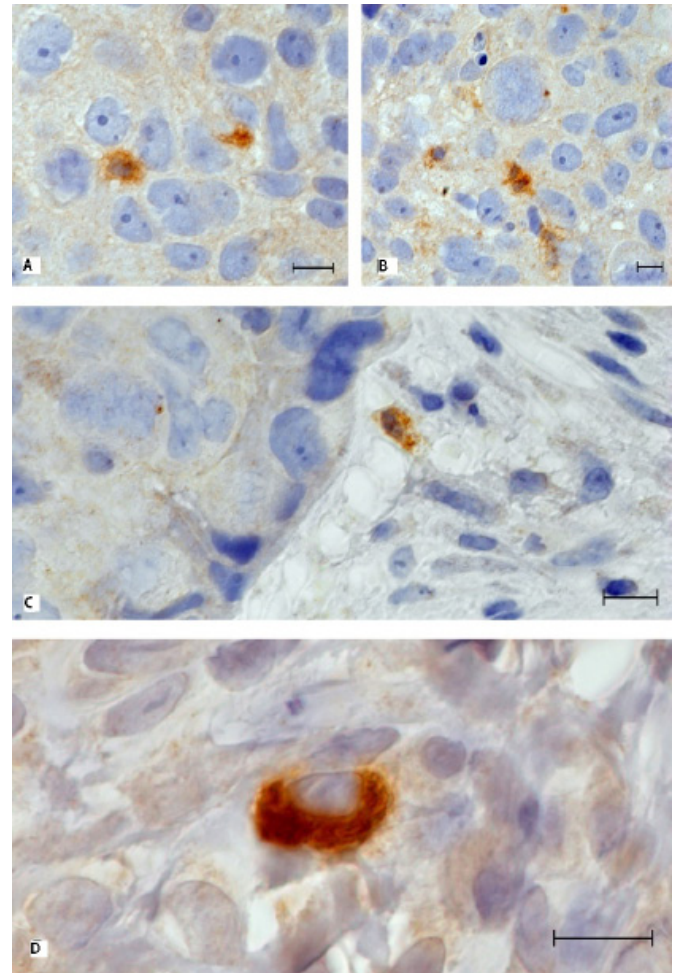


Photo 2. Expression of TLR9 by tumor microenvironment cells in the MG: A, B — localization of TLR9 positive cells in close proximity to tumor cells; C – a localization of TLR9 positive cells in the peritumoral region; D – a cell with a high degree of immunopositivity for TLR9 in the tumor stroma.

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The Phantom for Studying Foreign Bodies' Echo-Signs

Ekaterina G. Privalova, PhD¹; Yana A. Shumina¹;
Aleksandr Yu. Vasilyev, PhD, ScD^{1,2*}; Igor N. Bondarenko, PhD¹

¹Central Research Institute of Radiation Diagnostics, Moscow, Russia

²Moscow State University of Medicine and Dentistry named after A. I. Evdokimov, Moscow, Russia

Abstract

Background: The frequency of foreign bodies (FBs) is about 4% among all diseases in the maxillofacial region and more than a third of them are missed during initial examinations. Several authors have found that multi-position ultrasound examination of the soft tissues of the maxillofacial region has a high diagnostic significance. The aim of our study was to create a useful simulation model of soft tissues from durable material to study the echo-signs of FBs and to confirm ultrasonography as a method of choice for suspicion of the presence of FBs in the soft tissues of the maxillofacial region.

Methods and Results: We created a utility model. Two parts of a phantom were made of silicone-containing material with organic and non-organic FBs located in two parallel rows at a depth of 5 mm and 20 mm. The echo-signs of objects of organic and non-organic origin were determined. Ultrasonography allowed visualization of all FBs located at a depth of 5 mm. None of the organic FBs placed into the phantom were visualized by computed tomography. Compared with computed tomography, ultrasonography was more informative and preferable in the diagnosis of FBs of soft tissues.

Conclusion: The created model is a highly sensitive and accurate tool for detecting even the radiolucent FBs of different origin. (*International Journal of Biomedicine*. 2020;10(2):124-128.)

Key Words: ultrasonography • soft tissues • model • foreign bodies

Abbreviations

3DR, 3D reconstruction; **CT**, computed tomography; **MRI**, magnetic resonance imaging; **MPR**, multiplanar reconstruction; **MIP**, maximum intensity projection; **US**, ultrasonography

Introduction

Foreign bodies (FBs) in the soft tissues of the maxillofacial region are a common pathology with which patients turn to dentists, surgeons and radiologists. The frequency of FBs is about 4% among all diseases in the maxillofacial region.⁽¹⁾ About 30% of all FBs remain undiagnosed during the initial examinations.⁽²⁾ They are detected accidentally by ultrasonography (US), or purposefully after trauma or after the onset of symptoms. In some cases, patients are referred to ultrasound for follow-up after computed tomography (CT)

or magnetic resonance imaging (MRI) because of its high resolution and sensitivity of 95% for detection of FBs.⁽³⁾

FBs could be divided into two groups: of organic and non-organic origin. It is highly important to determine the nature of FBs because their nature affects the strategy of patient management and surgical treatment planning.

There are only a few scientific works on this theme, and there are still no precise echo-signs of FBs of the maxillofacial area, despite the obvious urgency, a high risk of complications and significant interest in patients with the presence of FBs in the soft tissues.

The aim of our study was to create a useful simulation model of soft tissues from durable material to study the echo-signs of FBs and to confirm US as a method of choice for suspicion of the presence of FBs in the soft tissues of the maxillofacial region.

*Corresponding author: Prof. Alexander Yu. Vasilyev, MD, PhD, ScD. Department of Radiology, Moscow State University of Medicine and Dentistry named after A. I. Evdokimov, Moscow, Russia. E-mail: auv62@mail.ru

Materials and Methods

Phantom features

We created a utility model. Two parts of a phantom were made of silicone-containing material with organic and non-organic FBs located in two parallel rows at a depth of 5 mm and 20 mm. The phantom for assessing the FB echo-signs of organic and non-organic origin consists of two bodies and 20 small parts. The bodies of the phantom are made of silicone-containing material and have the form of a cuboid measuring 210x50x100 mm. Placed inside, FBs are arranged in pairs in two parallel rows at a depth of 5 mm and 20 mm. The material of which the phantom consists has acoustic properties similar to the soft tissues of a person with a hypersthenic type of build (~1450m/s). The material is durable, as it does not contain gelatin, glycerine or other materials usually used in the creation of ultrasound phantoms.

The first phantom (with non-organic FBs) includes the following parts (Fig.1):

1. Glass splinters (n=2)
2. Fragments of plastic (n=2)
3. Drain tubes (n = 2)
4. Metal screws (n=2)
4. Dental filling material (n=2)



Fig. 1. The phantom with non-organic FBs.

The second phantom (with organic FBs) includes the following parts (Fig.2):

1. Husks of a sunflower seed (n=2)
2. Rose thorns (n=2)
3. Blades of grass (n = 2)
4. Pieces of a wooden toothpick (n=2)
5. Helminths (n=2)

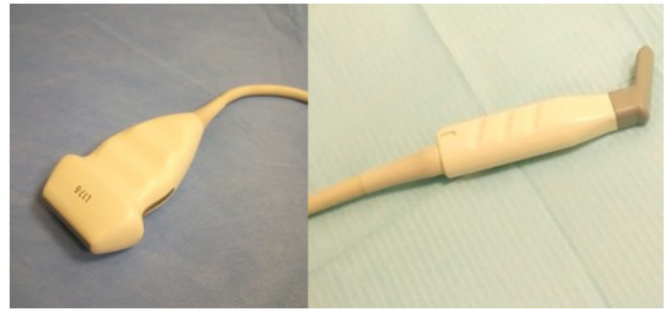


Fig. 2. The phantom with organic FBs.

The variety of objects allows establishing the echo-signs of each material, both organic and non-organic, including their artifacts.

Methods

Both phantoms underwent US and CT. US was performed on an IU-22 device (Philips, the Netherlands) using a pencil transducer, with a frequency of 7-15 MHz and a linear transducer with a frequency of 5-17 MHz in B-mode (Fig.3).



(a) (b)
Fig. 3. Linear (a) and pencil (b) transducers.

A special gel pillow was used for ultrasound examinations to improve the visualization (Fig.4), then a CT was performed on both phantoms by a 64-slice tomograph with the evaluation of the obtained images in the axial plane, using MPR, MIP, and 3DR (Fig.5).

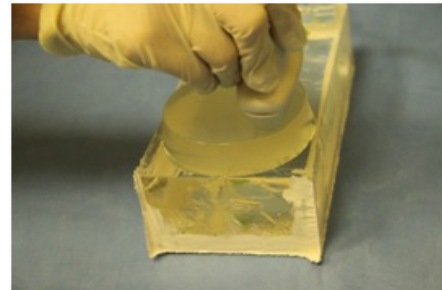


Fig.4. The gel pillow.

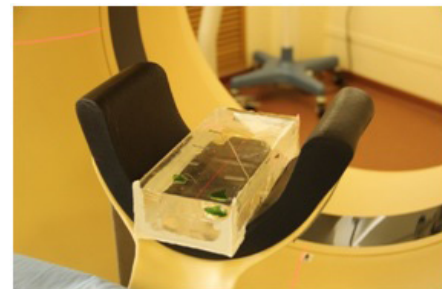


Fig. 5. CT of the model.

Results and Discussion

Ultrasonography

As a result of the research, the following ultrasound picture of the phantom was visualized: three layers separated by two layers. The upper layer (gel pillow) had increased echogenicity, a quite inhomogeneous echostructure because of tiny inclusions. The first interlayer, between the gel pillow and the top layer of the model, was visualized like a hyperechoic linear structure with a slight reverberation artifact. The second

layer (the upper layer of the model, located at a depth of 0–20 mm) was visualized as an anechoic, moderately homogeneous space with single inclusions (artifacts). The second interlayer (between two layers of the model) was visualized as a hyperechoic linear structure. The third layer (the bottom layer of the model, located at a depth of 20–50 mm) was visualized as an anechoic layer (Fig.6).

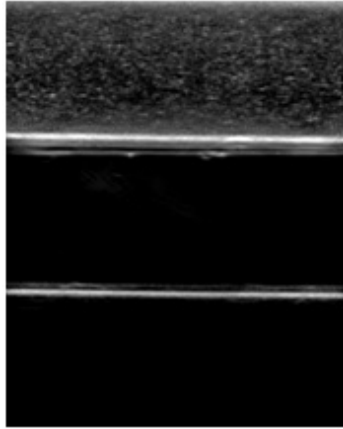


Fig. 6. US picture of the model (part without FBs)

1 – gel pillow, 2 – the first interlayer, 3 – the upper layer of the model, 4 – the second interlayer, 5 – the bottom layer of the model.

All FBs located at a depth of 5 mm were clearly visualized. But due to technical aspects (increased material density and physical properties of the interlayer), objects located at a depth of 20 mm were not convincingly visualized. Non-organic FBs were glass splinters (n=2), fragments of plastic (n=2), drain tubes (n=2), metal screws (n=2), and dental filling material (n=2).

The glass splinter was visualized as an isoechoic structure with hyperechoic, even sometimes fuzzy contours, a reverberation artifact and acoustic amplification (Fig.7). The fragment of plastic was visualized as a linear anechoic structure with clear and even hyperechoic contours (Fig.8). The drainage tube was visualized as a curly linear anechoic structure with clear and quite uneven hyperechoic contours (Fig.9). The metal dental screw was visualized as a structure with alternating bands of high and low echogenicity, with clear uneven contours and artifacts such as posterior shadow and reverberation (Fig.10). The dental filling material was visualized as a hyperechoic structure with unclear and quite uneven contours and a reverberation artifact (Fig.11).

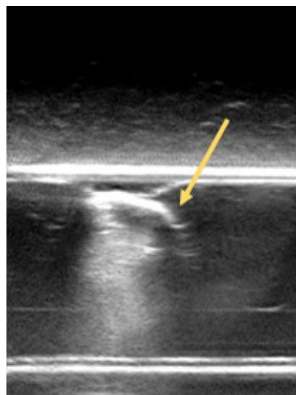


Fig. 7. US of the model. B-mode. The glass splinter.

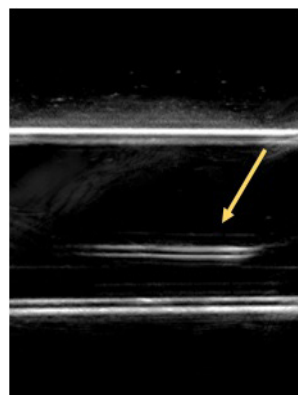


Fig. 8. US of the model. B-mode. The fragment of plastic.

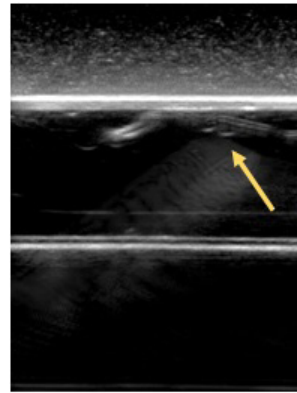


Fig. 9. US of the model. B-mode. The drainage tube.

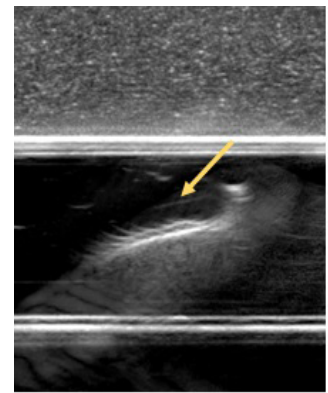


Fig. 10. US of the model. B-mode. The dental screw.

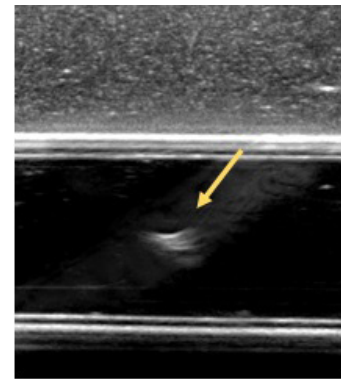


Fig. 11. US of the model. B-mode. The dental filling material.

Organic FB were husks of a sunflower seed (n=2), rose thorns (n=2), blades of grass (n=2), pieces of a wooden toothpick (n=2), and helminths (n=2).

The husk of a sunflower seed was visualized as a linear hyperechoic structure with even and sufficiently clear contours. It was also possible to trace the curved shape of the husk (Fig.12). The rose thorn was visualized as a structure that looked like an elongated triangle, with increased echogenicity and clear, even contours and homogeneous echostructure (Fig.13). The blade of grass was visualized as a curly structure with increased echogenicity with even and somewhat unclear contours (Fig.14). The piece of a wooden toothpick was visualized as a linear hyperechoic structure with clear and even contours (Fig.15).

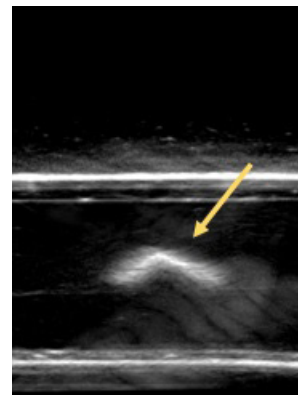


Fig. 12. US of the model. B-mode. The husk of a sunflower seed.

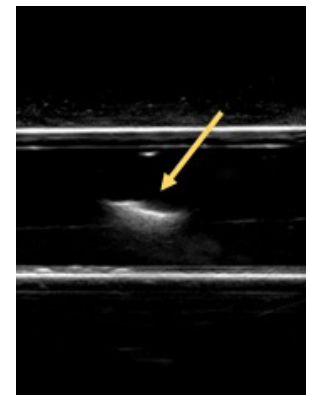


Fig. 13. US of the model. B-mode. The rose thorn.

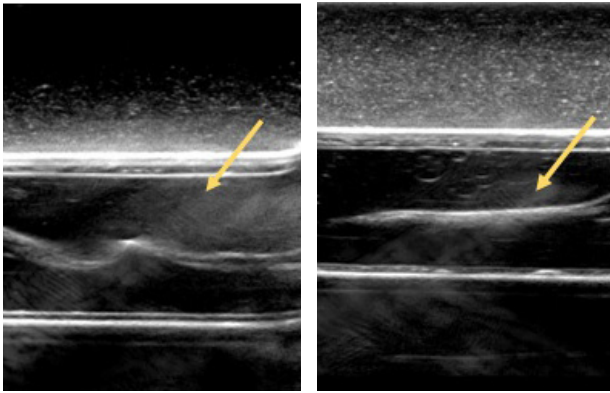


Fig.14. US of the model. B-mode. The blade of grass. **Fig.15.** US of the model. B-mode. The piece of a wooden toothpick.

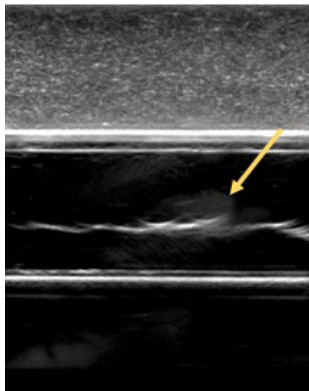


Fig. 16. US of the model. B-mode. The helminth.

The helminth (dead) was visualized as a curved structure with increased echogenicity contours and hypoechoic content. When the helminth was strongly decomposed, it was visualized as an anechoic tubular cavity with contours of somewhat increased echogenicity and with heterogeneous hyperechoic content along the lower edge – it is detritus resulting from the decomposition of the helminth (Fig.16). If the helminth were alive, its movement and peristalsis would be determined in Doppler modes.

US allowed visualization of all FBs located at a depth of 5 mm. The visualization of structures located at a depth of 20 mm was difficult due to the technical aspects (increased material density and physical properties of the interlayer). In addition, US allowed estimation of the echo-signs of all FBs, with the exact characteristics of the echo-signs, including artifacts.

Computed tomography

None of the organic FBs placed into the phantom were visualized by CT (Fig.17).



Fig.17. CT of the model with organic FBs.

However, glass splinters, metallic dental screws and a filling material were visualized by the CT of the phantom with non-organic FBs (Fig.18-20).

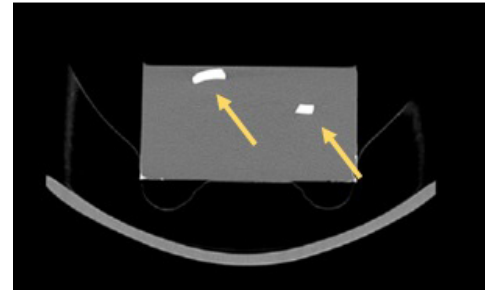


Fig. 18. CT of the model. Glass splinters.

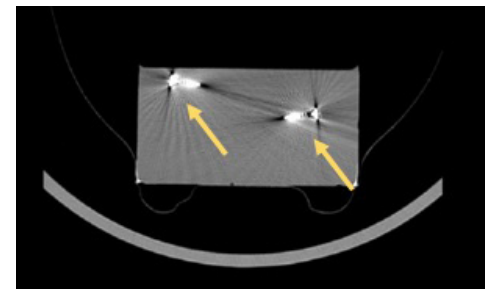


Fig. 19. CT of the model. Metallic dental screws.

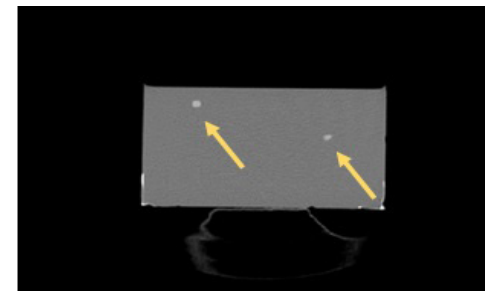


Fig. 20. CT of the model. The dental filling material.

A 3DR was presented for clarity (Fig.21). There were also zones with air in both models, which got there during manufacturing (Fig.22). High density materials, such as metal dental screws, filling material and glass splinters, were clearly detected. Drain tubes and fragments of plastic were not visualized because they are radiolucent.



Fig. 21. 3DR. Glass splinters, dental screws and filling material were visualized.

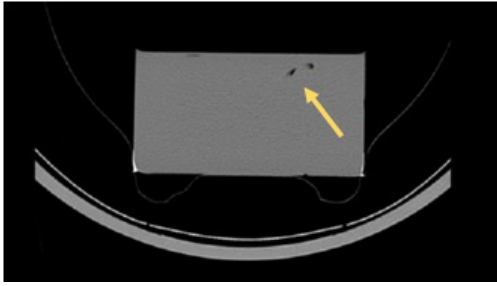


Fig. 22. CT of the model. Air.

In conclusion, as a result of the experiment, a utility model was created for the study of ultrasonic signs of FBs of various origin, which can be a useful tool for this type of study. The echo-signs of objects of organic and non-organic origin were determined. Compared with CT, US was more informative and preferable in the diagnosis of FBs of soft tissues. Thus, the created model is a highly sensitive and accurate tool for detecting even the radiolucent FBs of different origin.

Competing Interests

The authors declare that they have no competing interests.

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Combined Use of Biologically Active Hemostatic and Granulated Sorbent in Endoscopic Cytoprotective Hemostasis in Patients with Bleeding Gastroduodenal Ulcers

Evgeniy F. Cherednikov, PhD, ScD¹; Sergey V. Barannikov, PhD¹; Alexandr I. Zhdanov, PhD, ScD¹; Ivan P. Moshurov, PhD, ScD¹; Galina V. Polubkova, PhD¹; Yuri V. Maleev, PhD, ScD²; Evgeniy S. Ovsyannikov, PhD^{1*}; Darya S. Myachina, PGS¹

¹Voronezh State Medical University named after N. N. Burdenko

²Voronezh Basic Medical College

Voronezh, the Russian Federation

Abstract

Background: The treatment of ulcerative gastroduodenal bleeding remains one of the most acute problems in emergency surgery. The aim of our research was to improve the results of treatment of patients with bleeding gastroduodenal ulcers through the use of endoscopic pneumatic applications of platelet-rich auto-plasma and the cytoprotective sorbent Aseptisorb-A.

Methods and Results: The study included 112 patients with bleeding gastroduodenal ulcers. All patients were divided, by random sampling, into two equivalent groups: the main group (n=57) and the comparison group (n=55). In the treatment of patients in the main group, an individual approach was applied that used Aseptisorb-A and biologically active hemostatic agent platelet-rich auto-plasma in the complex endoscopic treatment of gastroduodenal ulcers complicated by bleeding. In the comparison group, traditional methods of endoscopic hemostasis were used without platelet-rich auto-plasma and granular sorbents. After that, complex treatment of patients in the main group and comparison group did not differ. The relapse rate and operational activity was 3.5% in the main group and 10.9% in the comparison group ($P=0.04$); mortality rate was 1.75% in the main group and 5.45% in the comparison group; the duration of inpatient treatment in the main group and the comparison group was noted within 6.0(5.0;7.0) and 9.0(8.0;10.0) days, respectively ($P<0.01$).

Conclusion: The developed method for treatment of ulcerative gastroduodenal bleeding with the combined use the cytoprotective sorbent Aseptisorb-A and a biologically active hemostatic agent platelet-rich auto-plasma, for the reliability of endoscopic hemostasis, reduces the number of emergency operations by 2.6 times, mainly due to a decrease in the number of rebleedings and, as a consequence, leads to a decrease in postoperative mortality by 3.1 times. (**International Journal of Biomedicine. 2020;10(2):129-132.**)

Key Words: bleeding gastroduodenal ulcers • endoscopic hemostasis • cytoprotective sorbents • hemostatic agents • platelet-rich auto-plasma

Introduction

The treatment of ulcerative gastroduodenal bleeding remains one of the most acute problems in emergency surgery. The number of patients with gastroduodenal bleeding does not decrease, and even tends to increase.⁽¹⁻⁴⁾ The leading treatment of patients with gastroduodenal bleeding is therapeutic

endoscopy. However, despite the variety of methods for endoscopic hemostasis, the number of relapses of bleeding, even when using modern combined methods of endoscopic hemostasis ranges from 10% to 46%. Mortality in patients with ulcerative gastroduodenal bleeding reaches 5%-15%, and with the recurrence of bleeding, it increases to 30%-40%. These circumstances show an urgent need to develop new, highly effective methods for gastrointestinal bleeding treatment.⁽⁵⁻⁹⁾

Many authors note that it is important not only to stop gastroduodenal bleeding, but also to create conditions for the rapid healing of a peptic ulcer, which is a possible source

*Corresponding author: Evgeniy S. Ovsyannikov, PhD, Department of faculty therapy, Voronezh State Medical University named after N.N. Burdenko, Voronezh, Russia. E-mail: ovses@yandex.ru

of complications.⁽¹⁰⁻¹²⁾ To accomplish this important task, biologically active drainage sorbents that have pronounced hydrophilicity and adhesiveness, endowed with cytoprotective, hemostatic, antibacterial and other properties, have been successfully used for more than 27 years. However, a number of authors note that these sorbents do not have such a pronounced hemostatic activity and their use as monotherapy in patients with bleeding gastroduodenal ulcers may be accompanied by a relapse of hemorrhage.⁽¹³⁻¹⁵⁾

The use of biologically active cytoprotective sorbents in combination with hemostatic drugs seems to be promising. One of the highly effective hemostatic agents is platelet-rich plasma. As a hemostatic and reparative drug, it has been successfully used in various fields of clinical medicine: for example, cardiovascular surgery, thoracic surgery, traumatology, and purulent surgery. However, platelet-rich plasma has not been previously used for endoscopic treatment of gastrointestinal bleeding of ulcerative etiology.

The aim of our research was to improve the results of treatment of patients with bleeding gastroduodenal ulcers through the use of endoscopic pneumatic applications of platelet-rich auto-plasma and the cytoprotective sorbent Aseptisorb-A.

Materials and Methods

A clinical study was conducted in the Voronezh City Specialized Center for the treatment of patients with gastrointestinal bleeding. The study included 112 patients with bleeding gastroduodenal ulcers.

Among all patients with gastroduodenal bleeding, there were 79(71%) men and 33(29%) women. The average age of patients was 57.5 (44.5;67.5) years. Upon admission, all patients underwent emergency esophagogastroduodenoscopy (EFGDS). Depending on the source of bleeding, patients were divided as follows: symptomatic gastroduodenal ulcers -78(69.6%); peptic ulcer and duodenal ulcer - 34(30.4%). According to the endoscopic classification,⁽¹⁶⁾ patients were divided as follows: continued bleeding (Forrest Ia-Ib) - 20(17.9%); threat of rebleeding (Forrest IIa-IIb) - 64(57.1%); signs of completed bleeding (Forrest IIc) - 28(25.0%). The severity of blood loss was evaluated according to the classification of A.I. Gorbashko (1982): mild severity was observed in 41(36.6%) patients, moderate in 61 (54.5%) and severe in 10(8.9%).

All patients were divided, by random sampling, into two equivalent groups: the main group (n=57) and the comparison group (n=55). Patients were comparable in etiology of ulcerative bleeding, age, gender, size of bleeding defect, degree of severity of bleeding, nature of bleeding according to endoscopic classification, and duration of observations.

In the treatment of patients in the main group, an individual approach was applied that used Aseptisorb-A and biologically active hemostatic agent platelet-rich auto-plasma in the complex endoscopic treatment of gastroduodenal ulcers complicated by bleeding (Patent RF № 2632771). In particular, in patients with ongoing hemorrhage (Forrest Ia-Ib), active bleeding was stopped first by injection with aminocaproic acid, then with vasoconstrictor drugs, followed by argon-

plasma coagulation; and then Aseptisorb-A was pneumatically insufflated onto the defect area followed by application of platelet-rich auto-plasma. In patients with the threat of rebleeding (Forrest IIa-IIb), the argon-plasma coagulation of the thrombosed vessel (Forrest IIa) was first performed, and in Forrest IIb, the clot was first removed from the ulcer defect by washing it, then the argon-plasma coagulation of the bleeding source was also performed. After that, with the help of an insufflator, Aseptisorb-A powder was applied to the area of these ulcerative defects, followed by the application of the patient's platelet-rich auto-plasma. With the Forrest IIc type of bleeding, with the aim of preventing the recurrence of hemorrhage, Aseptisorb-A and platelet-rich auto-plasma were applied to the defect according to the developed technique.

In the comparison group, traditional methods of endoscopic hemostasis (injection method, argon plasma coagulation, etc.) were used without platelet-rich auto-plasma and granular sorbents. After that, complex treatment of patients in the main group and comparison group did not differ.

The main criteria in assessing the results of treatment were both clinical and endoscopic indicators: the timing of the final hemostasis, the frequency of rebleeding, dynamic monitoring of the size of ulcerative defects, the quality of healing of ulcers, the presence of emergency operations, and mortality rates.

Statistical analysis was performed using Microsoft Excel software package. For descriptive analysis, results are presented as mean±standard deviation (SD), median (Me), interquartile range (IQR), minimum and maximum values. Wilcoxon rank sum test was used to test for difference in medians. Group comparisons with respect to categorical variables were performed using Fisher's exact test. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

When evaluating the results of treatment of patients in the main group with ongoing bleeding (Forrest Ia-Ib), it was noted that primary endoscopic hemostasis was achieved in all 10 patients. Clinical observations showed that in patients who received endoscopic treatment according to the developed technique, after pneumo-insufflation on a bleeding ulcer defect of Aseptisorb-A, followed by the application of platelet-rich auto-plasma, the granular sorbent swelled, turning into a soft elastic hydrogel that was tightly fixed in the area of the bleeding source due to its properties, protecting it from the effects of aggressive factors of the gastric and duodenal contents. With repeated esophagogastroduodenoscopy, it was found that the drug hydrogel was retained on the ulcer defect for up to 4 days. In this case, rebleeding and emergency operations in this group of patients was not detected.

Endoscopic primary hemostasis in the comparison group was also achieved in all 10 patients; however, in 1 patient, a ulcer rebleeding clinic appeared on Day 3 of treatment. This patient was urgently operated on "at the height of bleeding." The postoperative period was complicated by pneumonia and on Day 6 after the operation, the patient died.

Regarding patients with unstably stopped bleeding (Forrest IIa-IIb), 2 of 34 patients (5.9%) in the main group experienced a relapse of bleeding. These patients were operated on, on an emergency basis. The cause of rebleeding was penetrating ulcers of the duodenal bulb and stomach. One patient was discharged home in satisfactory condition on Day 12 of treatment, and another patient died in the postoperative period against the background of severe concomitant pathology.

Among 30 patients of the comparison group with the Forrest IIa-IIb bleeding, recurrence of ulcerative bleeding was observed in 4(13.3%) cases. Due to the failure of repeated endoscopic hemostasis, three patients were operated on "at the height of bleeding," and two of them died in the presence of severe concomitant pathology and increasing multiple organ failure in the postoperative period.

In 13 patients of the main group with Forrest IIc, there was no recurrence of hemorrhage, and there were no operations or fatal outcomes. In the comparison group, among 15 patients with bleeding, 1 patient on Day 2 of inpatient treatment revealed a relapse of bleeding, which was stopped endoscopically. However, due to the high risk of rebleeding, the patient was operated on urgently and was discharged after surgery for outpatient treatment.

In patients of the two compared groups, studies were conducted on the dynamics of indicators of a general blood test (hemoglobin, red blood cells, white blood cells) at the stages of the study. The level of hemoglobin upon admission in patients in the main group and comparison group was comparable: 109.0(95.0;120.0)g/L and 110.0(100.0;122.0) g/L, respectively ($P>0,05$). In patients of both groups, the hemoglobin and erythrocyte counts decreased for 2-3 days, which is associated with developing hemodilution; however, in the comparison group, a decrease in the level of hemoglobin occurred more significantly [94(82.0;105.0)g/L] than in the main group [100.0(90.0;106.0)g/L], which is explained by a large number of rebleedings. Starting from Days 4-5, there was an increase in the hemoglobin level up to 109.0(98.0;116.0)g/L in the main group and 95.0(85.0;108.0)g/L in the comparison group. When patients were discharged from the hospital, the hemoglobin level was 117.0(104.0;125.0)g/L in the main group and 98.0(86.0;112.0)g/L in the comparison group ($P<0.001$). A similar dynamics was observed when studying the levels of red blood cells and hematocrit in the compared groups.

In the final assessment of the results of the clinical study, it was found that the final endoscopic hemostasis was achieved in 55(96.5%) patients of the main group and in 49(89.1%) patients of the comparison group ($P>0.05$). The relapse rate and operational activity was 3.5% in the main group and 10.9% in the comparison group; operational activity was 3.5% in the main group and 9.1% in the comparison group. Moreover, mortality rate was 1.75% in the main group and 5.45% in the comparison group; the duration of inpatient treatment in the main group and the comparison group was noted within 6.0(5.0;7.0) and 9.0(8.0;10.0) days, respectively ($P<0.01$).

In conclusion, the developed method for treatment of ulcerative gastroduodenal bleeding with the combined use

the cytoprotective sorbent Aseptisorb-A and a biologically active hemostatic agent platelet-rich auto-plasma, for the reliability of endoscopic hemostasis, reduces the number of emergency operations by 2.6 times, mainly due to a decrease in the number of rebleedings and, as a consequence, leads to a decrease in postoperative mortality by 3.1 times.

Competing Interests

The authors declare that they have no competing interests.

Sources of Funding

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Changes in the Parameters of Systemic Hemodynamics and Perfusion during Laparoscopic Cholecystectomy in Patients with Acute Cholecystitis and Possible Correction Methods

Alexander N. Golomidov, MD; Serhiy H. Hryvenko, MD, PhD, ScD*;
Evelina R. Kondratiuk, MD, PhD; Yuriy V. Artemov, MD, PhD;
Alexander V. Kosenko, MD, PhD; Idris V. Dzhemilov

*Medical Academy named after S.I. Georgievsky of Vernadsky CFU
Simferopol, Crimea*

Abstract

This article presents a clinical and laboratory assessment of the effectiveness of the author's method for pharmacological correction of hypoxic changes at acute pneumoperitoneum in patients with acute calculous cholecystitis (ACC) during laparoscopic cholecystectomy (LCE) (Patent of Ukraine No. 119602). The application of the developed technique allows leveling of ischemic and reperfusion changes that occur during LCE in ACC patients by protectively affecting blood lactate level, statistically significantly reducing it after desufflation. The proposed method reduces the number of cardiovascular complications provoked by ischemic disorders on the background of pneumoperitoneum by 3.79 times. (**International Journal of Biomedicine. 2020;10(2):133-137.**)

Key Words: acute calculous cholecystitis • laparoscopic cholecystectomy • systemic hemodynamics • perfusion • hypoxia

Abbreviations

ACC, acute calculous cholecystitis; AR, anesthetic risk; BLL, blood lactate level; CVC, cardiovascular complication; LCE, laparoscopic cholecystectomy; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDH, lactate dehydrogenase; MBP, mean blood pressure; PP, pneumoperitoneum

Introduction

The most significant, revolutionary surgical event of recent decades was the rapid development and introduction into wide clinical practice of endoscopic surgical methods that radically changed the face of modern surgery. Of all sections of endoscopic surgery, laparoscopy is the most developed. Huge opportunities have opened up for the use of endo-surgical technologies, not only in planned, but also in emergency abdominal surgery.⁽¹⁻⁵⁾

In the structure of urgent surgical diseases of the abdominal cavity organs, acute calculous cholecystitis (ACC) is one of the most common nosologies. The incidence of ACC in the Russian Federation is second only to acute appendicitis and amounts to 1-1.5 cases per 1,000 adults.⁽⁶⁾ A modern method for ACC treatment is laparoscopic cholecystectomy (LCE).⁽⁷⁻¹¹⁾ One of the characteristic features of laparoscopic operations is the need to create pneumoperitoneum (PP), which provides optimal visualization of anatomical structures. Most often, carbon dioxide is used as the insufflated gas. A combination of prolonged abdominal pressure and effects of the intra-abdominal presence of carbon dioxide has a complex effect on the patient's body. Pathophysiological shifts arising during the pneumoperitoneum creation, primarily from the cardiovascular and respiratory systems, increase the risk of the operation.⁽¹²⁾ In

*Corresponding author: Serhiy H. Hryvenko, MD, PhD, ScD.
Medical Academy named after S.I. Georgievsky of Vernadsky CFU,
Simferopol, Crimea. E-mail: hryva@mail.ru

this regard, it is relevant to search for ways to reduce the negative impact of pneumoperitoneum on bodily functions when using laparoscopic surgery.⁽¹³⁾ Since the leading pathogenetic link in the disorders accompanying intense pneumoperitoneum is hypoxia, its pharmacological correction is of particular interest.

The aim of this study was to increase the efficiency of surgical treatment of patients with ACC during LEC on the basis of the development and implementation in clinical practice of a new pathogenetically substantiated method of pharmacological correction of hypoxic changes in cases of intense pneumoperitoneum.

Materials and Methods

The study was based on the analysis of clinical and laboratory monitoring of the results of surgical treatment of 102 patients with a verified diagnosis of ACC who underwent LCE in Surgical Department No.1 of Simferopol Clinical Emergency Hospital No. 6 in the period from 2017 to 2019.

The study was carried out in compliance with Ethical Principles for Medical Research Involving Human Subjects, Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the 59th WMA General Assembly, Seoul, Republic of Korea, October 2008. Written informed consent was obtained from all patients before inclusion in the study.

The criteria for inclusion of patients in the study were these: over 18 years of age and informed consent of the patient. Exclusion criteria: body mass index over 38 kg/m², decompensated organ failure, and conversion to laparotomy due to complications or technical difficulties that developed or were found during the laparoscopic procedure.

All patients included in the study were admitted in order of urgency. In accordance with the design of the work, on the basis of simple randomization, depending on the method of preoperative preparation, intraoperative support and management of the postoperative period, two groups of patients were formed and structured. The two groups were similar with regard to age, gender, and nosological characteristics. In the comparison group (CC, n=58), patients underwent traditional preoperative preparation, intraoperative support and postoperative management. In the main group (MG, n=44), in addition to the stages of preoperative preparation, intraoperative support and postoperative management, the proposed author's method for the prevention and treatment of hypoxic changes, a succinate-containing drug, meglumine sodium succinate (Fig.1) (brand name "Reamberin"), was used.⁽¹⁴⁾

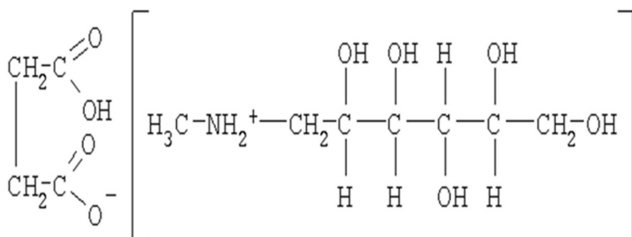


Fig.1. The chemical formula of meglumine sodium succinate

Meglumine sodium succinate in the form of a solution for infusion is included in the list of vital and most important drugs for medical use in the Russian Federation.

The average age of the patients included in the study was 56.55±1.24 years. The largest group comprised elderly patients, who made up more than half of all those studied—51.96%. The average age of the patients in MG and CG was 58.39±1.98 years and 55.16±1.78 years, respectively. Women were prevalent in the gender structure of patients in the study groups (34/77.3% in MG and 45/77.6% in CG).

In both groups, patients with destructive forms of ACC predominated (41/88.6% in MG and 42/72.4% in CG). Patients with acute phlegmonous cholecystitis predominated in the structure of destructive forms of ACC in both groups (35/81.8% in MG and 38/65.5% in CG). Accordingly, patients with a catarrhal form of ACC in MG amounted to 5/11.4% and 16/27.69% in CG.

The existing, concomitant somatic pathology increases the risks when creating PP, operational and anesthetic risk, and creates the prerequisites for postoperative complications and prolongation of the patient's hospital stay. Concomitant chronic diseases in patients of both groups were in the stage of compensation and did not constitute contraindications for surgery. None of the identified concomitant diseases at the time of treatment and at the treatment stages had a pronounced manifestation and did not significantly affect the results of the presented study. Among the concomitant diseases, cardiovascular pathology was the leading one, which is explained by the prevalence of elderly patients in the age structure of both groups. Among all cardiovascular diseases, arterial hypertension prevailed. The number of patients with this concomitant pathology in MG and CG was 16/36.4% and 23/39.7%, respectively. Patients in both groups were compensated for hemodynamic parameters before surgery.

The average Charlson comorbidity index in MG was 3.00±0.26 (Me=3.00), and 2.59±0.21 (Me=3.00) in CG. Before surgery, AR was assessed in patients of both clinical groups, according to the American Society of Anesthesiologists scale. The most numerous patients in both clinical groups were patients with AR II (29/65.9% in MG and 39/67.2% in CG). AR I was found in 11/18.97% of patients in CG and in 5/11.4% of patients in MG; AR III in 7/12.1% and 10/22.7%, respectively.

Patients in both groups underwent a standard 4-port LCE using a clipper under endotracheal anesthesia and mechanical ventilation. The working space was created by carboxyperitoneum at a pressure of 10-12 mmHg. The average duration of surgery was 84.20±5.22 minutes (Me=80.00 minutes) in MG and 76.88±5.16 minutes (Me=65.00 minutes) in CG.

In all patients, using the MINDRAY IPM-10 monitor (China), the most informative hemodynamic parameters were assessed before surgery, 30 minutes after the start of surgery, and after desufflation. The state of blood circulation was also assessed by MBP defined by the formula: MBP=DBP+(SBP-DBP)/2. MBP corresponds to constant pressure in the aorta, which provides a sufficient hemodynamic effect for adequate organ perfusion. It is believed that MBP >60mmHg is

sufficient for the functioning of organs. Normally, the MBP indicators range from 70 mmHg to 110 mmHg.

SpO₂, acid-base equilibrium (pH) and lactate levels in venous blood we estimated before surgery, 30 minutes after the start of LCE and after desufflation using a GEM Premier 3500 automatic analyzer of blood gases, electrolytes and metabolites (Instrumentation Laboratory, USA). The content of LDH in the venous blood was determined at similar time periods using the UV kinetic test in units per liter (Norm 0.0–247.0 U/L).

The statistical analysis was performed using the statistical software Microsoft Excel 2010. Student's unpaired and paired t-tests were used to compare average values for data with normal distribution. A probability value of P<0.05 was considered statistically significant.

Results

The parameters of systemic hemodynamics at the stages of surgical treatment in CG are presented in Table 1. Thirty minutes after the creation of pneumoperitoneum with a pressure of 10-12 mmHg, a significant decrease in all indicators was noted. Thus, HR, SBP, DBP and MBP decreased by 2.72%, 16.11%, 12.53%, and 14.74%, respectively. After desufflation, the inertial decrease in the levels of all the studied parameters continued. Thus, at this stage, compared with the preoperative period, the decrease in HR, SBP, DBP and MBP was 5.1%, 16.2%, 13.4%, and 15.1%, respectively.

Table 1.

Parameters of systemic hemodynamics at the stages of surgical treatment in the comparison group

Stages	HR (bpm)	SBP (mmHg)	DBP (mmHg)	MBP (mmHg)
Before surgery	75.38±1.53	142.47±2.26	89.09±1.40	115.78±1.74
30 minutes after PP creation	73.33±1.30	119.52±1.94	77.93±1.49	98.72±1.62
After desufflation	71.51±1.24	119.36±1.55	77.19±1.33	98.28±1.35
<i>P</i>	<0.05	<0.05	<0.05	<0.05

The parameters of systemic hemodynamics at the stages of surgical treatment in MG are presented in Table 2. Thirty minutes after the creation of pneumoperitoneum with a pressure of 10-12 mmHg, a significant decrease in all indicators was noted. Thus, HR, SBP, DBP and MBP decreased by 6.05%, 22.39%, 18.38%, and 20.18%, respectively. After desufflation, the inertial decrease in the level of HR continued (by 6.75% compared with the preoperative period). In contrast to CG, the remaining indicators of systemic hemodynamics showed steady growth, although they did not reach the initial

preoperative values by this time period. Thus, at this stage, compared with the preoperative period, the decrease in SBP, DBP and MBP was 17.8%, 16.8%, and 17.4%, respectively.

Table 2.

Parameters of systemic hemodynamics at the stages of surgical treatment in the main group

Stages	HR (bpm)	SBP (mmHg)	DBP (mmHg)	MBP (mmHg)
Before surgery	79.41±1.70	146.61±2.45	93.66±1.51	120.14±1.84
30 minutes after PP creation	74.61±1.37	115.34±2.08	76.45±1.97	95.90±1.93
After desufflation	74.05±1.21	120.52±1.91	77.93±1.05	99.23±1.34
<i>P</i>	<0.05	<0.05	<0.05	<0.05

Analysis of SpO₂ in patients of CG showed a moderate decrease from 97.88±0.76% to 96.25±1.87% when creating intense pneumoperitoneum (Table 3). After carbon dioxide desufflation, the SpO₂ rate was restored and almost corresponded to the average level before the operation, which testified to the temporary negative effect of pneumoperitoneum. The average blood pH during carbon dioxide insufflation was normal and amounted to 7.391±0.002; during the operation, it slightly decreased to 7.386±0.012, which is explained by the development of moderate respiratory acidosis due to an increase in intra-abdominal pressure (Table 3). The shift of acid-base balance towards acidosis correlated with hemodynamic changes. After elimination of pneumoperitoneum, the average blood pH was 7.395±0.002, which indicated stabilization of the acid-base balance due to adequate ventilation.

Table 3.

Parameters of SpO₂, blood pH and biomarkers of tissue hypoxia at the stages of surgical treatment in the comparison group

Stages	SpO ₂ , %	pH	BLL, mmol/L	LDH, U/L
Before surgery	97.88±0.76	7.391±0.002	1.27±0.03	179.55±3.55
30 minutes after PP creation	96.25±1.87	7.386±0.012	1.77±0.06	174.55±3.75
After desufflation	98.87±0.23	7.395±0.002	1.96±0.04	161.25±3.83
<i>P</i>	>0.05	>0.05	<0.05	<0.05

Thus, in the patients of CG, hemodynamic and perfusion changes were noted, which were also reflected in

the shifts of the acid-base state in response to stress due to the operation and the creation of pneumoperitoneum. At the same time, when analyzing blood biomarkers of tissue hypoxia (Table 3), significant changes were found when creating pneumoperitoneum. Despite the fact that blood lactate level in all measured time intervals corresponded to the physiological norm, a significant increase was noted after performing laparoscopic cholecystectomy, relative to the initial data (1.96 ± 0.04 mmol/L versus 1.27 ± 0.03 mmol/L). Fluctuations in lactate dehydrogenase levels at the same time had the opposite character. There was a moderate decrease in the lactate dehydrogenase level after performing laparoscopic cholecystectomy, relative to the initial data (161.25 ± 3.83 U/L versus 179.55 ± 3.55 U/L).

Analysis of SpO₂ in patients of MG (Table 4) did not reveal statistically significant changes during gas insufflation. Thirty minutes after the start of surgery, SpO₂ slightly decreased from $97.63 \pm 0.58\%$ to $96.14 \pm 1.21\%$. After gas desufflation, the SpO₂ level recovered and remained within the physiological norm. The average blood pH during carbon dioxide insufflation was normal and amounted to 7.39 ± 0.002 before the operation; during the operation it remained practically unchanged (7.4 ± 0.001); therefore, pronounced respiratory acidosis with intense pneumoperitoneum was not observed. After desufflation, the average blood pH remained at the same level (7.4 ± 0.001), which indicated the complete stabilization of the acid-base balance due to the maintenance of adequate ventilation.

Table 4.

Parameters of SpO₂, blood pH and biomarkers of tissue hypoxia at the stages of surgical treatment in the main group

Stages	SpO ₂ , %	pH	BLL, mmol/L	LDH, U/L
Before surgery	97.63 ± 0.58	7.39 ± 0.002	1.19 ± 0.04	167.41 ± 5.87
30 minutes after PP creation	96.14 ± 1.21	7.4 ± 0.001	1.39 ± 0.06	161.73 ± 5.40
After desufflation	98.21 ± 0.91	7.4 ± 0.001	1.59 ± 0.05	153.34 ± 5.42
<i>P</i>	>0.05	>0.05	<0.05	<0.05

When analyzing the levels of blood biomarkers of tissue hypoxia (Table 4) in patients of MG, moderate changes were established when creating intense pneumoperitoneum. blood lactate level in all measured time intervals corresponded to the physiological norm, while a moderate increase was observed after performing laparoscopic cholecystectomy, relative to the initial ones (1.59 ± 0.05 mmol/L versus 1.19 ± 0.04 mmol/L). Fluctuations in lactate dehydrogenase levels at the same time had the opposite character. There was a moderate decrease in the lactate dehydrogenase level after performing laparoscopic cholecystectomy, relative to the initial data (153.45 ± 5.42 U/L versus 167.41 ± 5.87 U/L).

Discussion

To identify the advantages and possible disadvantages of the proposed methods of pharmacological correction of hypoxic changes during intense pneumoperitoneum, perioperative results were compared in patients of the main group and comparison groups. The analysis of cardiovascular complications associated with pneumoperitoneum during laparoscopic cholecystectomy in patients of both groups revealed significant differences. In the comparison group, cardiovascular complications were observed in 15(25.86%) patients. Heart rhythm abnormalities were diagnosed in 2 (3.45%) cases, asystole in 1(1.72%) patient, a drop in MBP by more than 20% in the intraoperative period of the initial level in 2(3.45%) patients, and tachycardia more 120 bpm in 3(5.17%) cases. In one patient, an episode of atrial fibrillation in the early postoperative period was recorded with successful medical cardioversion. One patient during ECG monitoring within 48 hours after surgery showed signs of subendocardial ischemia; one patient showed signs of subepicardial ischemia of the anterior wall of the left ventricle; and 2(3.45%) patients showed signs of transmural ischemia of the posterior wall of the left ventricle. Two patients with ischemic heart disease had an attack of angina pectoris with ST segment depression. In all cases, effective relief of ischemia was achieved by the use of nitrates.

In the main group, cardiovascular complication were observed in 3(6.82%) patients. So, in one(1.72%) patient during ECG monitoring, signs of transmural ischemia of the posterior wall of the left ventricle were recorded, which were stopped by administering nitrates and cardiometabolic drugs. Two(3.45%) patients in the early postoperative period developed a hypertensive crisis against the background of hypertension. The use of oral antihypertensive drugs provided a decrease in blood pressure to target values.

Such significant differences in the number of cardiovascular complications in patients of the studied groups are associated with adequate pharmacological correction of hypoperfusion, and tissue hypoxia resulting from it, against the background of intense pneumoperitoneum during laparoscopic cholecystectomy. The fact that reperfusion pneumoperitoneums occur in the early postoperative period (1-2 days of the postoperative period) indicates that this period creates the conditions and prerequisites for their occurrence. This is confirmed by data on monitoring the level of lactate.

The technology of intense pneumoperitoneum in cases of laparoscopic cholecystectomy in patients with acute calculous cholecystitis has a significant stressful and traumatic effect on the body due to variable changes in hemodynamic parameters (heart rate, systolic blood pressure, diastolic blood pressure, mean blood pressure), blood pH and SpO₂. These changes lead to ischemic and reperfusion changes, which create conditions and prerequisites for the development of ischemic cardiovascular complications in the early postoperative period. The proposed pathogenetically substantiated method of drug correction of hypoxic changes during intense pneumoperitoneum makes it possible to level out ischemic and reperfusion changes that occur during

laparoscopic cholecystectomy in patients with acute calculous cholecystitis, having a protective effect on blood lactate level in patients of the main group, statistically significantly ($P < 0.05$) reducing its level after desufflation. The use of the proposed method for the medical correction of hypoxic changes in cases of intense pneumoperitoneum significantly ($P < 0.05$) reduces the number of cardiovascular complications provoked by ischemic disorders on the background of pneumoperitoneum by 3.79 times.

Competing Interests

The authors declare that they have no competing interests.

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Hemostatic Agents in Combination with Diovine for Local Treatment of Simulated Bleeding Gastric Ulcers

Evgeniy F. Cherednikov, PhD, ScD¹; Alexandr A. Glukhov, PhD, ScD¹;
Mikhail N. Romantsov, PhD¹; Yuri V. Maleev, PhD, ScD²; Sergey V. Barannikov, PhD¹;
Irina A. Shkurina, PGS¹; Anastasiya T. Vysotskaya, PhD¹; Evgeniy S. Ovsyannikov, PhD^{1*}

¹Voronezh State Medical University named after N. N. Burdenko

²Voronezh Basic Medical College
Voronezh, the Russian Federation

Abstract

Background: Acute gastroduodenal bleeding is one of the urgent and complex problems of emergency surgery. The objective of our study was to investigate the performance of powder-like hemostatic agents in combination with granular Diovine for bleeding management in experimental gastric ulcers.

Methods and Results: Experimental studies *in vivo* were conducted on 15 mongrel dogs, using the model of gastric ulcer by E. F. Cherednikov. During esophagogastrosopy, all animals were exposed to 2 bleeding gastric ulcers (experimental and control). Experiments were performed with 30 bleeding gastric ulcers. In experimental ulcers, endoscopic hemostasis was carried out by sufflations on a bleeding defect of powder-like Gelplastan and NovoSeven (lyophilized powder) in combination with Diovine. In control ulcers, endoscopic treatment was not performed. The experimental studies showed that in experimental ulcers, bleeding stopped at 3.9(3.5;4.9) sec ($P<0.001$); their hemostasis was stable without recurrent bleeding. In control ulcers, bleeding stopped at 27.5(24.0;31.0) sec ($P<0.001$) and 5 episodes of recurrent bleeding were noted. The drug composition (Gelplastan, NovoSeven, and Diovine) has a hemostatic effect and accelerates healing processes of experimental bleeding ulcers. A complete healing of experimental gastric ulcers occurred in 8.0(8.0;9.0) days with a moderate scar that does not deform the stomach wall. Complete healing of control gastric ulcers occurred in 15.5(14.0;16.0) ($P<0.001$) days of observations.

Conclusion: The combined use of Gelplastan, NovoSeven, and Diovine for the local treatment of experimental bleeding gastric ulcers in dogs leads not only to an effective termination of hemorrhage, but also promotes reparative regeneration, synchronization of interaction of cell structures and acceleration of the healing time of simulated ulcers. (**International Journal of Biomedicine. 2020;10(2):138-141.**)

Key Words: experimental gastric ulcer • gastroduodenal bleeding • endoscopic hemostasis • healing processes

Introduction

Treatment of acute gastroduodenal bleeding (GIB) remains challenging in emergency surgery. A common cause of GIB is acute and chronic ulcers of the stomach and duodenum.⁽¹⁻⁵⁾ Bleeding recurrence is the main cause of unsatisfactory results for bleeding management in the upper gastrointestinal tract.⁽⁶⁻⁸⁾ Endoscopic bleeding management occupies the main place in the treatment of this category of patients.^(9,10)

The outcome of the disease is mainly determined by stable and terminative hemorrhage arrest and by the time of ulcer healing. The search for new medicines and novel drug compositions, and

the development of effective methods of endoscopic hemostasis, are urgent tasks for GIB management.⁽¹¹⁻¹³⁾

The objective of our study was to investigate the performance of powder-like hemostatic agents in combination with granular Diovine for bleeding management in experimental gastric ulcers.

Materials and Methods

The experiment investigated the powder-like hemostatic agents Gelplastan and NovoSeven, which have not been previously used to stop gastrointestinal bleeding, and granular

biologically active sorbent Diovine with cytoprotective, hemostatic and antibacterial properties.

Studies were performed on 15 mongrel male and female dogs weighing 9-14 kg. In vivo experiments were carried out in accordance with the legislation of the Russian Federation, in strict compliance with the European Convention for the protection of animals used for experimental and other purposes (Strasbourg, France, 1986), the provisions of Directive 210/63/EU of the European Parliament and the Council of the European Union of 22 September 2010 on the protection of animals used for scientific purposes (Article 27).

During the stomach endoscopy or esophagogastro-duodenoscopy under intravenous anesthesia (Zoletil 100 solution, 7.5 mg/kg), all dogs were given an injection of a 96% ethyl alcohol solution with a 3.0 ml-needle into the submucosal layer of the antral part of the stomach along a small curvature; then they were injected with a solution of Vincristine (0.01 mg/kg).^(14,15) On Day 4, all dogs demonstrated a typical round ulcer of the stomach reaching the muscle layer of fibrin coating on the bottom with an area of inflammation at the periphery. Moderate bleeding was induced by injuring the edges and bottom of the ulcer with biopsy forceps.

Each dog was subject to 2 bleeding gastric ulcers (control and experimental) at a distance of 4-5 cm from each other. Thus, all experimental studies were conducted on 30 simulated gastric ulcers.

In the experimental ulcers, endoscopic hemostasis was achieved by applying powder-like Gelplastan and NovoSeven (lyophilized powder) in equal amounts of 0.1 g in combination with 0.3 g of Diovine; the next therapeutic endoscopy was performed in 3-4 days.

Control gastric ulcers did not receive treatment; the time of spontaneous bleeding arrest was measured with a stopwatch timer. The outcome measurement involved the following indicators: time of hemorrhage arrest, signs of the hemorrhage recurrence, time of recovery of experimental ulcers, and stomach tissue reaction to applied drugs.

Biopsies of the gastric mucosa were used as a material for morphological study. Histological sections were stained with H&E/Van Gieson's method, reticular fiber was stained by nitric acid silver, and neutral glycol-proteins were identified in PAS reaction. Histological examination of the sections was performed by light microscopy at magnifications of $\times 100$ and $\times 400$; the cellular component of the connective tissue stroma of the mucous membrane was assessed at $\times 900$ magnification.

Statistical analysis was performed using Microsoft Excel software package. For descriptive analysis, results are presented as mean \pm standard deviation (SD), median (Me), interquartile range (IQR), minimum and maximum values. Wilcoxon rank sum test was used to test for difference in medians. Group comparisons with respect to categorical variables were performed using Fisher's exact test. A probability value of $P < 0.05$ was considered statistically significant.

Results and Discussion

Experimental studies of the combined use of hemostatic agents (Gelpplastan and NovoSeven) and granular sorbent

(Diovine) demonstrated that applying them to the experimental bleeding surface in the area of the ulcer caused the development of a gel-like matrix of blood-stained hydrogel, which protected the hemostatic agents and ulcers from gastric acid irritation. This matrix remained on the surface of the experimental ulcers up to 4 days due to its adhesive properties. The average time of hemorrhage arrest was 3.91 ± 1.28 sec in the experimental group and 27.45 ± 4.5 sec in the control group ($P = 0.02$). The experimental group demonstrated stable and terminative hemostasis. There was no recurrent bleeding or other complications in the experimental ulcers associated with hemostatics in combination with Diovine. In the control ulcers, there were 5 episodes of recurrent bleeding. Hematin points were detected in the bottom of the simulated ulcers in 3 cases, and there was a dark clot in the bottom of the ulcers in 2 cases. These episodes of recurrent bleeding in the control ulcers stopped on their own and did not require any endoscopic interventions.

Dynamic gastroscopy and morphological examinations demonstrated that a combined treatment resulted in a faster reparative processes than in the control bleeding ulcers. The average time for inflammation to subside under hemostatic and sorbent treatment was 3.0(2.0;4.0) days in the experimental group and 7.0(6.0;8.0) days in the control group ($P < 0.001$). The average time of purification of the ulcer bottom was 3.0(2.0;4.0) days in the experimental group and 5.0(4.0;5.0) days in the control group ($P < 0.001$). It should be noted that the fibrinous-necrotic layer from the bottom of the ulcer was partially removed when ulcerative bleeding was induced during traumatization of the bottom and edges of the ulcer with biopsy forceps. In this regard, the time of ulcer purification is more likely to be associated with Diovine's capacity to prevent inflammation and tissue necrosis than with the removal of fibrinous-necrotic masses.

The anti-inflammatory properties of the granular sorbent in the simulated gastric ulcers can be explained by several points:

First, the layer of the swollen sorbent protects the entire ulcer surface with a hydrogel coating that prevents the peptic action of gastric juice.

Second, the sorption abilities of Diovine cause a decrease in edema in the ulcer area and a decrease in the concentration of bacteria and toxins in the ulcer crater.

Third, the hydrogel coating on the surface of the ulcer after pneumoinsufflation improves blood circulation. It is non-hermetic and does not prevent gas exchange and metabolic processes.

Microscopic examination of gastrobiopsates on Day 3 after combination therapy (Gelpplastan, NovoSeven, and Diovine) showed edema, and some biopsies showed necrosis or focal mixed-cell infiltration in surface layers of the experimental ulcers. At the same time, in most fields of vision the over-epithelium was preserved as high cubic epithelium; the glands are long and straight, with an epithelial lining. On Day 5 of the therapy, the integumentary epithelium was preserved, lymphocytes and monocytes prevailed in the stromal component of the mucous layer, and fibroblasts appeared in the experimental ulcers.

In the control ulcers, on Day 5 the mucous layer was edematous, the integumentary epithelium was partially desquamated, histiocytes and neutrophils prevailed in the stromal component of the mucous layer, and by Day 15, the number of fibroblasts had increased.

On Day 8 of therapy for the experimental ulcers, the cover-pit epithelium was represented by a high cubic epithelium. The submucosal layer revealed edema, focal fibrosis and scattered mixed-cell infiltration.

On Day 15, the control ulcers showed edematous mucosa edema, mixed-cell infiltration, and fibrin fragments. The intervertebral stroma was also edematous with foci of fibrinoid necrosis. The mucous layer was moderately edematous; intervertebral stroma also revealed foci of edema and fibrosis.

A complete healing of experimental gastric ulcers occurred in 8.0(8.0;9.0) days with a moderate scar that does not deform the stomach wall. Complete healing of control gastric ulcers occurred in 15.5(14.0;16.0) ($P<0.001$) days of observations. At the same time, all control ulcers healed with the development of a severe scar deforming the stomach wall. The efficiency Gelplastan and NovoSeven in combination with Diovine for hemorrhage is presented in the Table 1.

Table 1.

The performance of Gelplastan and NovoSeven in combination with Diovine for simulated ulcer hemorrhage therapy

Variable	Experimental ulcers	Control ulcers	P-value
Hemostasis performance indicators			
Time of hemorrhage arrest, sec	3.9 (3.5;4.9)	27.5 (24.0;31.0)	<0.001
Number of recurrences	0	5	0.04
Final hemostasis	15	10	0.04
Comparative dynamics of reparative processes			
Subsiding of inflammatory phenomena, days	3.0 (2.0;4.0)	7.0 (6.0;8.0)	<0.001
Purification of the ulcer bottom, days	3.0 (2.0;4.0)	5.0 (4.0;5.0)	<0.001
Granulation development, days	2.0 (2.0;3.0)	6.0 (5.0;6.0)	<0.001
Epithelization onset, days	3.0 (3.0;4.0)	8.0 (7.0;9.0)	<0.001
Healing of experimental ulcers, days	8.0 (8.0;9.0)	15.5 (14.0;16.0)	<0.001

Thus, morphological data indicate that combination therapy (Gelplastan, NovoSeven and Diovine) for experimental gastric ulcers contributed to synchronizing the interaction of cell structures and accelerated change of phases of the ulcerative process. Thus, the rapid subsiding of inflammatory phenomena in the experimental ulcers was demonstrated by the low quantity of neutrophils in the cell population. The development of glands that were enlarged and filled with leukocytes on Day 3 of the experiment can be regarded as a positive reaction of the mucous membrane to the

hemostatic agents with a granular sorbent. At the same time, it should be noted that during therapy for the experimental ulcers, neither destruction processes nor progression of inflammation in the subsequent periods of observations were revealed. The development of fibroblasts in the granulation tissue of experimental ulcers after Day 5 of treatment can be confirming evidence of the early beginning of the healing process, finalized with a moderate scar. At the same time, in control ulcers, inflammation began to subside only on Day 10, and an increase in the number of fibroblasts was observed on Day 15 of observation and finalized with a severe connective tissue scar.

Summarizing the results of the experimental study, we have concluded that Gelplastan and NovoSeven in combination with Diovine are effective drugs with a good hemostatic effect, providing healing of experimental ulcers. There was neither damage to the stomach tissue nor development of foreign body cells during insufflation of these drugs. Morphological signs of allergy were not detected in any case. Gelplastan and NovoSeven in combination with Diovine were spontaneously rejected during healing of ulcers and were not perceived by the gastric mucosa as a foreign body.

Thus, the powder-like hemostatic agents Gelplastan and NovoSeven in combination with Diovine for the therapy of experimental gastric ulcers contributes to stable hemostasis, reduces the time of hemorrhage, and also eliminates the possibility of recurrent bleeding. The combined use of Gelplastan, NovoSeven, and Diovine for the local treatment of experimental bleeding gastric ulcers in dogs leads not only to an effective termination of hemorrhage, but also promotes reparative regeneration, synchronization of interaction of cell structures and acceleration of the healing time of simulated ulcers.

Competing Interests

The authors declare that they have no competing interests.

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*Corresponding author: Evgeniy S. Ovsyannikov, PhD. Department of faculty therapy, Voronezh State Medical University named after N.N. Burdenko, Voronezh, Russia. E-mail: ovses@yandex.ru

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Indicators of Lipid Peroxidation Reactions and State of Structural Tissues of the Dentition System in Wistar Rats under Various Stress Regimes

Marina A. Darenskaya, PhD, ScD*; Larisa R. Kolesnikova, PhD; Lyubov V. Rychkova, PhD, ScD; Lyudmila A. Grebenkina, PhD, ScD; Natalya V. Semenova, PhD, ScD; Sergey I. Kolesnikov, Academician of the RAS; Lyubov I. Kolesnikova, Academician of the RAS

*Scientific Centre for Family Health and Human Reproduction Problems
Irkutsk, the Russian Federation*

Abstract

The aim of this research was to investigate the content of lipid peroxidation (LPO) products and components of antioxidant defense (AOD), as well as to evaluate morphometric parameters of the dentition system tissues in Wistar rats when modeling acute and chronic modes of immobilization stress.

Methods and Results: The work was performed on young (2.5-3 months), sexually mature male rats of the Wistar line, weighing 200-220g. The design of the study consisted in evaluating biochemical and morphometric parameters at the stage before immobilization—intact animals (10 rats)—as well as in modeling various stress modes: acute stress (3 hours from the moment of 3-hour single immobilization) and chronic stress (1-hour immobilization with an interval of 72 hours between separate stress episodes on Days 1, 5, 9 and 13). Both modes included 20 rats. The intensity of lipid peroxidation-antioxidant defense processes was assessed using spectrophotometric and fluorometric methods. The microscopic and morphometric studies were performed in the light-optical microscope AxioScope A1 and included a detailed description of all changes in the tooth and periodontal tissues. We found that acute stress is accompanied by increased LPO reactions at the stage of primary and secondary products with simultaneous activation of antioxidant factors. In chronic stress exposure, there is an accumulation of thiobarbituric acid reactants, with a decrease in the level of non-enzymatic components— α -tocopherol, retinol, and oxidized glutathione. The changes in the state of the dentition system in Wistar rats was accompanied by an increase in the area of the microcirculatory bed of the periodontium and pulp and a natural reduction in the connective tissue area, with an increase in the thickness of vascular endothelium and an increased number of cellular elements that control metabolic processes, especially pronounced in acute stress. The change in the mode of stress exposure to chronic in animals of this line was characterized by generally similar changes with reduced reaction intensity.

Conclusion: The processes of LPO-AOD and the indicators of the dentition system in Wistar rats undergo changes depending on the mode of stress exposure. (**International Journal of Biomedicine. 2020;10(2):142-147.**)

Key Words: Wistar rats • lipid peroxidation • periodontium • pulp • acute stress • chronic stress

Abbreviations

AS, acute stress; AOD, antioxidant defense; CD, conjugated dienes; ChS, chronic stress; CT, connective tissue; DB, double bonds; GSH, reduced glutathione; GSSG, oxidized glutathione; KD-CT, ketodienes and conjugated trienes; LPO, lipid peroxidation; SOD, superoxide dismutase; TAA, total antioxidant activity; TBARs, thiobarbituric acid reactants.

Introduction

It has been established that the impact of stress factors is one of the most important causes that provoke the development of various diseases.^(1,2) Diseases of the dentition system tissues

are the most common pathologies in the population, and stress plays a significant role in their pathogenesis.⁽³⁾ The views in the existing literature on the primary effect of stress on the body (the so-called AS) suggest that a typical stress reaction ensures maintenance of homeostatic reactions of the body in

the conditions completely new for it.⁽⁴⁾ This reaction is based on a set of stress-implementing and stress-limiting systems. The latter include an AOD system that limits the action of stressors at the cellular level.⁽⁵⁻⁷⁾ The primary mediators of the stress response are LPO processes, which initiate the damaging effect of various factors, including at the level of the central links of neuroendocrine regulation.⁽⁸⁾

Among the cognition methods that are of significant scientific and practical interest, an important place is occupied by pathological processes modeling, which allows theoretically generalizing an array of data that are not explained by existing concepts of biology and medicine.⁽⁹⁻¹¹⁾ It has been established that when using immobilization as a model of stress exposure, there are changes characteristic of psychoemotional stress, invariably accompanied by activation of the sympathoadrenal system, as well as numerous transformations in the blood system.⁽¹²⁾ The simplicity of the method, the standardness of changes, and the absence of additional damaging factors make immobilization the most appropriate model for stress exposure.⁽¹³⁾ It has been found that the systemic response of the tissues of the dentition system in rats is generally similar to that of humans, which makes these animals a promising object for studying the pathogenesis of various odontogenic disorders. It is also currently known that immobilization stress has numerous damaging effects on periodontal tissue, consisting in development of degenerative changes in the CT of the gums, reducing its osteotaryngeal base, epithelium peeling, osteoporosis development, etc.⁽¹⁴⁾

In this regard, it is extremely interesting to study systemic non-specific changes (LPO system), as well as the state of the morphological picture of the dentition system tissues, and their interactions in animals when modeling various modes of immobilization stress.

The aim of this research was to investigate the content of LPO products and components of AOD, as well as to evaluate morphometric parameters of the dentition system tissues in Wistar rats when modeling acute and chronic modes of immobilization stress.

Materials and Methods

The work was performed on young (2.5-3 months), sexually mature male rats of the Wistar line, weighing 200-220g. The animals were bred at the SPF-vivarium Center for collective use of the Federal research center "Institute of Cytology and Genetics," Siberian branch of RAS (Novosibirsk). The design of the study consisted in evaluating biochemical and morphometric parameters at the stage before immobilization—intact animals (10 rats)— as well as in modeling various stress modes: AS (3 hours from the moment of 3-hour single immobilization) and ChS (1-hour immobilization with an interval of 72 hours between separate stress episodes on Days 1, 5, 9 and 13).⁽¹⁵⁾ Both modes included 20 rats. Three-hour single immobilization of the animal on its back was performed by rigid fixation of the limbs.^(15,16)

The intensity of LPO processes was evaluated based on the content of substrates with unsaturated DB: CD and KD-CT.⁽¹⁷⁾ The content of TBARS was determined fluorimetrically.⁽¹⁸⁾ The

state of the AOD system⁽¹⁹⁾ was determined by TAA of the blood, according to the content of TAA components: α -tocopherol and retinol,⁽²⁰⁾ the SOD activity,⁽²¹⁾ and the content of GSH and GSSG.⁽²²⁾ Measurements were performed on the Shimadzu RF-1501 spectrophotometer (Japan) and the Shimadzu RF-1650 spectrophotometer (Japan).

To study the state of the dentition system after euthenizing the animals, pieces of the lower and upper jaw with incisors and molars were taken and fixed in a neutral 10% solution of formalin. Tissue fragments were decalcified in a 5% solution of nitric acid for 3 to 5 days. The material was processed in a Sacura, Japan, vacuum processing machine. Filling in the modular paraffin was done by the Tissue-Tek® TEC™ 5 filling system (Sacura, Japan). Cutting standard serial sections with a thickness of 5 microns was performed on semi-automatic rotary microtomes CM-502 (Microm, Germany) using disposable knives of the Sacura company. The sections were manually stained on slides and covered with cover glass; they were stained with hematoxylin and eosin, and picro-fuchsin using the van Gieson method to identify CT fibers. The microscopic and morphometric studies were performed in the light-optical microscope AxioScope A1 (Carl Zeiss, Germany) at $\times 40$, $\times 100$, $\times 400$, and $\times 600$ magnification and included a detailed description of all changes in the tooth and periodontal tissues. Changes in the pulp and periodontal vessels (vascular lumen state, blood filling, endothelial condition) were described in detail, and changes in the pulp and periodontal tissues, as well as the state of odontoblasts, were assessed.

The dentition system was assessed using a number of quantitative criteria of the periodontium and pulp—the area (in %) of periodontal and pulp vessels, the area (in %) of periodontal and pulp CT, the thickness of the periodontal and pulp vascular endothelium (μm^2), and the total number of fibroblasts and odontoblasts in the field of view.

Work on the animals was done in compliance with the principles of the Helsinki Declaration on the humane treatment of animals, stated in normative documents of the European community(86/609/EU), "Rules of works using experimental animals" (order of MH of the USSR № 775 dated 12.08.1977), Manual on Experimental (Preclinical) Study of New Pharmacological Substances,⁽²³⁾ and "Good laboratory practice" (MHRF Order No. 708H dated 23.08.2010).

Statistical analysis was performed using the Statistica 6.1 software package (Stat-Soft Inc., USA). The normality of distribution of continuous variables was tested by the Kolmogorov-Smirnov test with the Lilliefors correction and Shapiro-Wilk test. For descriptive analysis, results are presented as mean±standard deviation (SD), median (Me), interquartile range (IQR; 25th to 75th percentiles). Differences of continuous variables departing from the normal distribution, even after transformation, were tested by the Mann-Whitney U-test. A probability value of $P \leq 0.05$ was considered statistically significant.

Results and Discussion

When modeling the AS mode in Wistar rats, unidirectional changes were observed at all stages of formation

of LPO products in the form of an increase in the values of CD ($P=0.0429$), KD-CT ($P<0.0001$) and TBARs ($P=0.0012$), relative to the intact animals (Table 1). Changes in the AOD system under the same mode were related to a decrease in the level of TAA ($P=0.0208$), an increase in the SOD activity ($P<0.0001$), and the retinol content ($P<0.0001$) (Table 1). The glutathione metabolism indicators in the AS mode also significantly changed—in the form of an increase in GSH values ($P=0.0031$).

Table 1.

The level of LPO products and components of the AOD system in Wistar rats under various stress modes (Me, IQR [P_{25} ; P_{75}])

Parameters	Intact animals (1)	AS mode (2)	ChS mode (3)	Statistical significance of $P\leq 0.05$
DB, standard units	1.81 1.54–1.87	1.66 1.45–1.80	1.82 1.78–1.86	P_2-P_3
CD, $\mu\text{mol/l}$	0.86 0.73–0.89	1.30 0.99–1.37	0.86 0.78–0.88	P_1-P_2 P_2-P_3
KD-CT, standard units	0.34 0.31–0.37	0.66 0.56–0.68	0.46 0.44–0.52	P_1-P_2 P_1-P_3 P_2-P_3
TBARs, $\mu\text{mol/l}$	0.79 0.78–0.94	1.03 0.96–1.16	1.47 1.25–1.83	P_1-P_2 P_2-P_3 P_1-P_3
TAA, standard units	17.14 14.36–18.90	13.65 11.36–14.41	12.34 9.08–2.97	P_1-P_2 P_1-P_3
SOD activity, standard units	1.98 1.94–2.14	2.62 2.53–2.77	2.96 2.76–3.07	P_1-P_2 P_1-P_3
α -tocopherol, $\mu\text{mol/l}$	11.20 9.85–12.30	9.23 9.04–11.13	5.76 3.49–5.88	P_2-P_3 P_1-P_3
Retinol, $\mu\text{mol/l}$	0.51 0.48–0.65	0.95 0.86–1.08	0.52 0.51–0.53	P_1-P_2 P_2-P_3
GSH, mmol/l	1.98 1.89–2.09	2.35 2.27–2.41	2.26 2.11–2.28	P_1-P_2
GSSG, mmol/l	2.04 2.00–2.23	2.29 2.25–2.32	2.04 1.78–2.09	P_2-P_3

The ChS mode was characterized by a significant increase in the levels of KD-CT ($P<0.0001$) and TBARs ($P<0.0001$), in comparison with intact rats. The ChC mode was characterized by a lower level of TAA ($P=0.0003$), an increased SOD activity ($P<0.0001$), and a reduced level of α -tocopherol ($P<0.0001$), in comparison with the baseline level.

In addition, there were statistically significant differences in the studied indicators between stress modes. Thus, the DB level was characterized by the lowest values in AS ($P=0.0337$), while the content of CD and KD-CT was characterized by higher values in this mode, and the level of TBARs was reduced. Elevated levels of α -tocopherol ($P=0.0009$), retinol ($P<0.0001$), and GSSG ($P=0.0002$) were reported in the AOD system for AS compared to ChS.

Morphological changes in the dentition system tissues in Wistar rats by using the AS model related to an increase in the area of the periodontal blood vessels ($P<0.0001$), a decrease in the area of the periodontal CT ($P<0.0001$), increased area of the pulp blood vessels ($P<0.0001$), due to lower values of the

pulp CT ($P<0.0001$) in comparison with intact animals (Table 2). The wall thickness of the periodontal and pulp endothelium increased significantly under AS ($P<0.0001$ in both cases) (Table 2). The number of fibroblasts in the field of view of Wistar rats also increased in this mode ($P=0.0206$).

Table 2.

Morphological changes in the dentition system tissues in Wistar rats under various stress modes (M \pm SD, Me, IQR [P_{25} ; P_{75}])

Parameters	IA (1)	AS mode (2)	ChS mode (3)	Statistical significance of $P\leq 0.05$
Vessels, Periodontium, %	22.78 \pm 2.4 23.05 20.00–24.90	34.79 \pm 3.7 34.80 31.90–38.00	26.53 \pm 3.51 26.65 24.00–29.60	P_1-P_2 P_1-P_3 P_2-P_3
CT, Periodontium, %	77.22 \pm 2.4 76.95 75.10–80.00	65.21 \pm 3.7 65.20 62.00–68.10	73.47 \pm 3.51 73.35 70.40–76.00	P_1-P_2 P_2-P_3
Vessels, Pulp, %	23.12 \pm 2.2 22.40 21.20–25.20	44.16 \pm 7.2 43.20 40.00–49.60	27.28 \pm 3.90 26.40 24.40–29.60	P_1-P_2 P_1-P_3 P_2-P_3
CT, Pulp, %	76.88 \pm 2.2 77.00 74.80–78.80	55.84 \pm 7.2 56.80 50.40–60.00	72.72 \pm 3.90 73.60 72.00–75.60	P_1-P_2 P_1-P_3 P_2-P_3
VE, Periodontium, μm	1.17 \pm 0.42 1.19 0.82–1.28	4.41 \pm 0.74 4.45 3.91–5.16	2.23 \pm 0.45 2.22 1.86–2.28	P_1-P_2 P_1-P_3 P_2-P_3
VE, Pulp, μm	1.23 \pm 0.57 1.30 0.72–1.59	4.38 \pm 0.83 4.02 3.64–5.03	2.03 \pm 0.55 2.03 1.90–2.25	P_1-P_2 P_1-P_3 P_2-P_3
Fibroblasts, units in the field of view	32.94 \pm 4.6 31.40 31.30–36.30	38.92 \pm 5.82 37.10 36.80–40.60	72.98 \pm 9.85 73.30 68.30–81.90	P_1-P_2 P_1-P_3 P_2-P_3
Odontoblasts, μm	28.91 \pm 7.9 32.60 20.80–35.80	31.88 \pm 7.59 33.75 24.90–38.50	21.92 \pm 2.73 21.90 19.40–24.30	P_1-P_3 P_2-P_3

IA-Intact animals; VE- Vascular endothelium

Modeling the ChS mode in Wistar rats was accompanied by an increase in the vascular area of the periodontium ($P=0.012$) and pulp ($P=0.009$), and in the thickness of the periodontal ($P<0.0001$) and pulp ($P=0.0048$) endothelium walls, as well as by an increase in the number of fibroblasts in the visual field ($P<0.0001$) and a decrease in the level of odontoblasts ($P=0.0163$), compared to intact rats.

A comparative analysis of changes in the parameters of the dentition system in rats of this line in different stress modes showed a maximum increase in the values of the vascular area of the periodontium ($P<0.001$) and pulp ($P<0.0001$), with a decrease in the area of the periodontal ($P<0.0001$) and pulp CT ($P<0.0001$) in conditions of AS, compared with ChS. The endothelium of the periodontal vessels ($P<0.0001$) and the pulp ($P<0.0001$), as well as the odontoblast index ($P<0.001$), showed an increase in values, while the number of fibroblasts ($P<0.0003$) showed a decrease in AS, in relation to ChS data.

In our study, when implementing the AS mode in Wistar rats, an increase in primary, secondary and final LPO products was observed, which may be a natural phenomenon in conditions of stress factor exposure. In many ways, this may be due to participation of neurotransmitters of the sympathetic

and parasympathetic parts of the autonomic nervous system in increasing the LPO intensity under stress.⁽²⁴⁾ A likely consequence of these changes may be the development of prolonged and excessive LPO with further irreversible destructive changes in membranes and associated enzymes.⁽²⁵⁾ Our results are consistent with a number of studies that postulate the accumulation of LPO metabolites in AS in animals after immobilization was used as a stress factor.⁽²⁶⁾

The use of a different exposure mode (replacing a single exposure with a systematic one) in Wistar rats also led to an intensive accumulation of LPO products, in particular KD-CT and TBARs, in comparison with the pre-stress period. At the same time, we noted higher growth values of the final LPO products (TBARs), in contrast to the values registered during a single immobilization. Previously, it was shown that the mode of ChS exposure, as opposed to other types of exposure (e.g. with smaller hour intervals between effects) is characterized by a sharp decrease in resistance to acute hypoxia.⁽¹⁶⁾ It was also previously shown that this mode is characterized by more intensive activation of stress-implementing systems, which is manifested in an increase in the content of the circulating stress hormones corticosterone and epinephrine, and, accordingly, increased activity of metabolic reactions.⁽²⁷⁾ Hypermetabolism, which is necessary to ensure a new level of the body's functioning, requires mobilization of the body's energy resources, which can explain the strengthening of cascade LPO processes, accompanied by higher values of TBARs.⁽¹²⁾

When evaluating changes in the AOD system in Wistar rats, we obtained multidirectional results, depending on the stress mode. Thus, under AS in animals, there was a decrease in the level of TAA, the integral indicator, with a simultaneous increase in the average values of the AOD components: the SOD activity, retinol content and GSH. It is known that when this mode is implemented, all the body's reserves are directed to actively overcome the action of an extreme stimulus.⁽¹⁵⁾ At the same time, the effectiveness of protective reserves is manifested by maximizing functions, increasing the intensity of the main exchange and catabolic processes. Increasing the activity of antioxidant factors in this case can be considered as an adaptive response aimed at leveling the LPO processes. These components can act as biological membrane stabilizers and participate in the inactivation of free radicals; accordingly, they can hinder the development of chain free-radical oxidation processes of organic compounds, primarily unsaturated tissue lipids.⁽²⁵⁻²⁹⁾

The ChS mode, in comparison with intact animals, was accompanied by similar reactions to the previous mode—a decreased TTA level, an increased SOD activity and reduced content of α -tocopherol. The greatest number of differences in the studied components was recorded between stress modes. These differences related to low levels of α -tocopherol, retinol, and GSSG in ChS. The antioxidant role of fat-soluble vitamins is manifested at all levels from subcellular to organism. α -tocopherol reacts simultaneously with peroxide radicals of the main fatty acids of biomembranes and with singlet oxygen; it also inhibits amino acid radicals, protects cells and tissues from damage caused by NO-radicals, and prevents atherogenic

changes in low density lipoproteins.⁽³⁰⁾ Having conjugated DB in the molecule, retinol is able to interact with free oxygen radicals, which also makes it an effective antioxidant.⁽³¹⁾ As a result, we can talk about the lack of functioning of antioxidant factors when using this mode of exposure. We also registered a significant increase in the level of final TBARs in this stress mode, which, with a unidirectional decrease in AOD factors, can have an extremely negative effect.

Analysis of the characteristics of the dentition system's structural tissues in Wistar rats indicated an increase in vascular area and a decrease in the area of the periodontal CT under AS. The periodontium basis is CT with its main structural elements, collagen fibers; there are also various cellular elements (fibroblasts, osteoblasts).⁽³⁾ Based on the anatomical characteristics of the periodontium, we can conclude that in Wistar rats, when modeling the AS mode, there is an increase in the area of blood vessels, with a natural decrease in the main component—CT. A number of studies indicate the presence of numerous structural and functional disorders in periodontal tissues under stress.^(14,32) Stress factors cause blood flow disorders, cell hypoxia, and redox balance disorders.⁽³³⁾

Thus, we have shown a significant increase in the concentration of final LPO products in animals under AS, which can have a direct disordering effect on periodontal composition. We also found similar changes in the vessels and CT of the rats' tooth pulp. The tooth pulp is known to be a loose CT that fills the tooth cavity and contains a large number of nerve endings and blood and lymph vessels.⁽³⁾ Since the morphological features of the pulp are related to its functions, we can assume the development of dysregulatory changes in this component of the dentition system under the influence of a stress factor in experimental animals. Moreover, the area of the pulp CT is significantly reduced, which indicates serious morphological disorders that lead to excessive growth of the pulp's vascular component under AS.

Another important indicator of the presence of morphofunctional disorders under the stress factors is the size of the endothelium of the periodontal and pulp vessels. In Wistar rats with AS, there was a multiple increase in this indicator both in the periodontium and in the pulp. This can contribute to narrowing the lumen of blood vessels, reducing their hemodynamic properties and disrupting microcirculation. The cell elements that make up the periodontium and pulp are extremely diverse, represented by both sedentary and mobile cells, with the largest population of cells being fibroblasts, some of which can differentiate into stationary cell elements—fibrocytes—and the other part into myofibroblasts capable of contractile activity.^(3,33) In our study, we found an increase in the number of fibroblasts, which can be interpreted as activation of protective mechanisms due to the presence of morphological changes in tissues. The plastic function of the periodontium and pulp is also carried out due to the activity of odontoblasts, which, forming the peripheral layer, participate in the formation of dentin. The total number of odontoblasts under AS did not change and remained at the level of control (pre-stress) values.

Changing the mode of exposure to chronic caused an increase in the vascular area of periodontium and pulp with a natural decrease in the area of CT. At the same time, there

was a slightly reduced increase in the thickness of the vascular endothelium, against the background of a maximum increase in the number of fibroblasts and a decrease in the content of odontoblasts.

In the literature, there are data on the development of a number of morphological changes in the oral cavity in rats under conditions of chronic psychophysiological stress in the form of microcirculation disorders, local bleeding in tissues, changes in the directions of collagen fiber bundles and bundle deformation.⁽³³⁾ In addition, our study registered a more pronounced imbalance in the state of LPO processes in this mode, which may further contribute to chronization of inflammatory periodontal diseases.

Thus, in Wistar rats, the processes of LPO-AOD undergo changes depending on the mode of stress exposure. Thus, when modeling AS, there is an increase in LPO reactions at the stage of primary and secondary products with simultaneous activation of antioxidant factors. The ChS mode is characterized by a more pronounced imbalance during non-specific protective reactions; with insufficient reserve capacity of the antioxidant system, the ratio changes toward pro-oxidant factors. Depending on the stressor mode, the indicators of the dentition system also underwent changes with more intense morphofunctional changes in AS.

Competing Interests

The authors declare that they have no competing interests.

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*Corresponding author: Marina A. Darenskaya, PhD, ScD. Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk, the Russian Federation. E-mail: marina.darenskaya@inbox.ru

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Clinical and Bacteriological Considerations for Applying Diathermy in Treatment of Teeth with Partial Pulp Necrosis

Fatima Yu. Daurova, PhD, ScD¹; Diana I. Tomaeva¹; Timur V. Melkumyan, PhD, ScD^{2,1*}; Angela D. Dadamova²

¹RUDN University, Moscow, Russia

²Tashkent State Dental Institute, Tashkent, Uzbekistan

Abstract

Background: The aim of the present pilot study was to assess the bactericidal efficacy of a high frequency diathermy irradiation in treatment of teeth with diagnosed partial pulp necrosis.

Methods and Results: The study included 83 patients aged between 22 and 54 years (mean age of 39±10 years) with irreversible pulpitis and signs of partial pulp necrosis in multi-rooted teeth (n=83). All patients were randomized in two groups in accordance with conducting therapy modes: 1) a conventional root canal treatment (Group 1, n=40); 2) a conventional treatment protocol in conjunction with a high frequency diathermy irradiation (Group 2, n=43). The postoperative sensitivity of treated teeth was assessed with the help of the Verbal Rating Scale (VRS). The quality of a root canal disinfection was evaluated by the presence of a cultural growth. The periapical index scoring system (PAI) was used for evaluation of periapical changes in every tooth after endodontic therapy. The occurrence of postoperative pain in Groups 1 and 2 demonstrated a similar reduction in toothache dynamics at all time intervals when assessments were made, and analysis of tooth radiographs in both groups of patients did not reveal a significant difference. As for the evaluation of a cultural growth, the signs of turbidity were detected in 6 samples of Group 1 and in 4 samples of Group 2.

Conclusion: The possible antimicrobial efficacy of a high frequency diathermy in treatment of teeth with partial pulp necrosis was demonstrated. However, further studies should be made to confirm the results of this clinical trial. (**International Journal of Biomedicine. 2020;10(2):148-152.**)

Key Words: multi-rooted teeth • partial pulp necrosis • diathermy irradiation • antimicrobial efficacy

Introduction

Involvement of tooth pulp in an infectious inflammatory process inevitably leads to irreversible conditions, which usually may be resolved with an application of partial pulpotomy or pulpectomy.⁽¹⁻³⁾

According to the study of de Oliveira et al.,⁽⁴⁾ the prevalence of irreversible pulpitis from all the teeth with diagnosed pulp pathologies is up to 46%-47%, and with no significant difference between men and women.

Leakage of existing composite restorations is one of the main reasons for advanced pulp degradation, especially in deep class II caries lesions. The cervical part of proximal cavities is usually considered as one of the most susceptible to the formation of marginal gap because of resin polymerization shrinkage and poor bonding to caries-affected dentin.⁽⁵⁻⁸⁾

In addition, the character of bacterial flora of a resin-dentin interface, along with initial cytotoxicity of a filled resin material, may substantially contribute to the progression of pulp pathology, particularly on a background of inflammatory pulp changes.⁽⁹⁻¹²⁾

Eventually, due to unfavorable circumstances, irreversible pulpitis may degenerate into a necrobiotic state in a short period of time. The term «necrobiosis» was first introduced by L. Grossman to explain a tooth pulp condition. However, there are many dentists who from the practical standpoint usually to refer it as a «partial pulp necrosis» or «partially necrotic pulp.»^(1,13)

It must be underlined that the coronal portion of pulp in cases of partial pulp necrosis is usually presented by necrotic tissue and contains many pathogens and their toxic byproducts, while the radicular segment is vital despite the inflammatory reaction.⁽¹⁴⁾ Therefore, the presence of vital tissue in the apical part of a root canal expands the opportunity to perform the generally acceptable treatment principle of a partial pulpectomy, which has a more promising prognosis for a tooth

*Corresponding author: Prof. Timur Melkumyan, PhD, ScD.
Tashkent State Dental Institute. Tashkent, Uzbekistan.
E-mail: t.dadamov@gmail.com

and its periradicular tissues than a total pulpectomy.^(15,16)

Nevertheless, the critical role of microorganisms at the beginning of pulpal and periapical disease should not be underestimated, because the primary endodontic cases are typically presented by the polymicrobial mix of approximately equal proportions of Gram-negative and Gram-positive species, in which the latter ones are most responsible for a failed outcome of a root canal therapy.⁽¹⁷⁻²⁰⁾

That is why, in order to increase the success rate of treatment of teeth with advanced pulp pathologies many studies have been aimed to find the way to fight infection more efficiently and have emphasized application of aggressive disinfectant solutions.^(21,22)

However, it has been found that injudicious use of aggressive solvents and bactericidal agents in an effort to kill all opportunistic pathogens in a root canal system might be the cause of a more depleting effect on residual vital tissues of the periapical zone.^(23,24)

Therapeutic principles for pulpectomy, as well for pulpotomy and pulp capping, are the same and assert that proper treatment of a pulp wound is of paramount concern.^(2,14)

There are few studies indicating the efficacy of electrocautery in rehabilitation of teeth with chronically inflamed pulp, but most of them were aimed to increase the treatment efficacy of pulp lesions in primary teeth. The obtained clinical data and results of radiographic examinations revealed that in cases of established antiseptic conditions the use of monopolar diathermy might be a good alternative to a standard therapy, which means an application of aggressive irrigating solutions and medications.^(25,28)

The potency of diathermy to stop bleeding from a pulp stump is undisputable. In addition, such treatment may defend the pulp wound from possible invasion of pathogens into the bloodstream and create comfortable conditions for sealer setting because of the low risk of oozing from the apical side, and may cause a positive bactericidal effect in a lumen of a root canal due to the short-term rise in temperature.⁽²⁹⁻³²⁾

Experimental studies by Fish and MacLean might serve as the first example of a bactericidal effect of high temperatures in dentistry, when they cauterized gingival tissue in order to prove the sterility of tissues in a periradicular zone.⁽³⁾

Nowadays, there are many studies demonstrating the beneficial antimicrobial effect of lasers caused by thermal irradiation alone or in conjunction with irrigating solutions. However, there are no available data that might describe or evaluate a diathermy in terms of bactericidal abilities.⁽³³⁻³⁶⁾

The aim of the present pilot study was to assess the bactericidal efficacy of a high frequency diathermy irradiation in treatment of teeth with diagnosed partial pulp necrosis.

Materials and Methods

The study included 83 patients aged between 22 and 54 years (mean age of 39±10 years). They were diagnosed with irreversible pulpitis and signs of partial pulp necrosis in multi-rooted teeth. The total number of multi-rooted teeth in the study was 83. Written informed consent was obtained from all participants.

Exclusion criteria were cases of total pulp necrosis in at least one of the roots of a tooth, and teeth with an x-ray confirmation of apical periodontitis. All cases were done by one operator and assigned for a single-visit treatment protocol. All patients were randomized in two groups in accordance with conducting therapy modes: 1) a conventional root canal treatment; 2) a conventional treatment protocol in conjunction with a high frequency diathermy irradiation. It should be noted that principles of cavity preparation and root canal chemomechanical instrumentation, as well as the technique of permanent obturation for all teeth in the study, were similar and performed with a consideration of aseptic rules.

However, the following differences in treatment protocols were made. In Group 1 (n=40), a radicular pulp tissue was severed with a sterile hand instrument; in Group 2 (n=43), a pulp wound was cauterized at first and was followed by severing pulp with a sterile instrument. Cauterization was performed with the help of a «ДК-35MC» electrocautery unit in a pulse mode.

In addition, there were distinctions in treatment protocols of both groups caused by culture sampling. In Group 1, before root canal sealing the following steps were undertaken: (1) disinfecting the root canal obturation with a warm 0.5% sodium hypochlorite solution; (2) drying of the canal with paper points; (3) irrigating the canal with a saline solution and sampling dentin from the cervical third of a root canal with a sterile hand reamer of appropriate size; (4) transferring the reamer with a dentine mash into a tube with 5 mL of Brain Heart Infusion (BHI) broth; (5) irrigating the canal with a saline solution; (6) drying the canal with paper points.

In Group 2, steps (1) and (2) were the same; at step 3, root dentin was exposed to high frequency electromagnetic radiation. The following steps were as in Group 1: (4) taking a culture; (5) irrigating with saline and (6) eliminating moisture.

Radicular dentin was irradiated at a power level of 4.1W and radiofrequency of alternating current cycles at 2640kHz («ДК-35MC» electrocautery unit). Radiation was performed by insertion of the endo tip into a cervical third of the root canal for 3 sec. During manipulation, the main consideration was to avoid direct contact of an activated endo tip with dentine.

The quality of a root canal disinfection was evaluated by the presence of a cultural growth. Results of microbial tests were assessed after four days of incubation, and the appearance of turbidity indicated the presence of a residual microflora.

The postoperative sensitivity of treated teeth was assessed on the basis of patient complaints and registered with the help of the Verbal Rating Scale (VRS) at 24h, 3 days, 7 days and 14 days. Every patient was asked to evaluate causal tooth sensitivity on a scale from 0 to 3, taking into account a previous toothache experience.

Radiographs were taken before, during, and right after the treatment to assure a proper filling. Recall x-ray examinations were taken in the interval of about 6 months, 1 year and 2 years, and at other times in cases of emergency. The periapical status of all teeth was examined using the periapical index scoring system (PAI). Scores of 1 or 2 assumed that periapical changes were absent and that teeth with values from

3 to 5 were pointing to the presence of apical periodontitis. For multi-rooted teeth, the root with the highest PAI score was recorded.

Statistical analysis was performed using StatSoft Statistica v7.0. The mean (M) and standard deviation (SD) were calculated. Student's unpaired and paired t-tests were used to compare average values for data with normal distribution. Group comparisons with respect to categorical variables are performed using chi-square tests with Yates correction or, alternatively, Fisher's exact test when expected cell counts were less than 5. A probability value of $P < 0.05$ was considered statistically significant.

Results and Discussion

The obtained results of the present clinical trial revealed that the mean values describing the occurrence of postoperative pain in both groups of patients were almost the same, with a similar reduction in toothache dynamics at all time intervals that assessments were made (Table 1). There were two cases of flare-ups in Group 1 and one case in Group 2, which were successfully managed with periosteal releasing incisions and had no influence on the total outcomes of provided therapies.

Analysis of tooth radiographs in both groups of patients did not reveal a significant difference. There were two cases of radiographically detected periapical bone changes in Group 1 and two cases in Group 2, with no clinical manifestations that would confirm the presence of obvious pathology (Tables 2, 3).

When we examined the tubes with culture mediums for growth after the assigned 4-day period of incubation, there were 6 samples in Group 1 with signs of turbidity; in Group 2, four cases of cultural growth were detected ($P=0.510$).

Successful root canal treatment with a long-term prognosis depends on many factors and the most important of them are high infection control, proper instrumentation and quality canal obturation.⁽³⁷⁾

The number of clinical trials associates the occurrence of endodontic failures mainly with insufficient antiseptic protocols and passage of resistant microorganisms down through the root canal into its periapical zone.⁽³⁸⁾

In addition, it has been reported that a few germs, such as *E. Faecalis* and *C. albicans*, may survive root canal chemomechanical instrumentation and resist high concentrations of antimicrobial agents with different pH values due to formation of biofilms within dentinal tubules.^(39,40)

In relation to this, the results of several studies indicate that a thorough root canal obturation is necessary in order to entomb the residual pathogenic microflora within the walls of root canal dentin.^(41,42)

The application of laser thermal energy to disinfect a dry root canal and activation of hypochlorite solutions have been debated in many *in vitro* studies and also in limited clinical trials. However, despite real benefits of laser usage in conjunction with irrigating solutions, further comprehensive studies must be undertaken to reveal whether lasers should be used as a unique treatment modality.^(34,36)

Table 1.
Severity of postoperative pain after root canal therapy in groups

Pain severity	Day 1		Day 3		Week 1		Week 2	
	Group 1 n (%)	Group 2 n (%)	Group 1 n (%)	Group 2 n (%)	Group 1 n (%)	Group 2 n (%)	Group 1 n (%)	Group 2 n (%)
No pain (0)	5 (12.5)	7 (16.3)	9 (22.5)	7 (16.3)	18 (45)	20 (46.5)	29 (72.5)	32 (74.4)
Mild (1)	14 (35)	15 (34.9)	21 (52.5)	22 (51.2)	19 (47.5)	19 (44.2)	11 (27.5)	11 (25.6)
Moderate (2)	19 (47.5)	19 (44.2)	10 (25)	14 (32.5)	2 (5)	4 (9.3)	-	-
Severe (3)	2 (5)	2 (4.7)	-	-	1 (2.5)	-	-	-
Mean±SD	1.45±0.78	1.37±0.81	1.03±0.7	1.16±0.69	0.65±0.7	0.67±0.68	0.28±0.45	0.26±0.44
<i>P</i>	>0.05		>0.05		>0.05		>0.05	

Table 2.
PAI in Groups 1 and 2

Groups	Group 1					Group 2				
	1	2	3	4	5	1	2	3	4	5
PAI score										
Right after filling	39	1	-	-	-	41	2	-	-	-
After 6 months	29	11	-	-	-	34	9	-	-	-
After 1 year	20	20	-	-	-	23	20	-	-	-
After 2 years	10	28	2	-	-	12	30	1	-	-

Table 3.
Descriptive analysis for PAI in Groups 1 and 2

Interval	Groups	Min	Max	Mean	SD	<i>P</i>
Right after filling	Group 1	1	2	1.03	0.15	>0.05
	Group 2	1	2	1.05	0.21	
After 6 months	Group 1	1	2	1.28	0.45	>0.05
	Group 2	1	2	1.2	0.41	
After 1 year	Group 1	1	2	1.5	0.5	>0.05
	Group 2	1	2	1.46	0.5	
After 2 years	Group 1	1	3	1.88	0.52	>0.05
	Group 2	1	3	1.74	0.49	

One of the earliest attempts to use diathermy for pulpal disinfection was made by Ferranty in order to bring back a single-visit treatment modality into endodontic practice. However, he mainly emphasized proper shaping and cleaning of canals to achieve better therapeutic results.⁽⁴³⁾

In the present pilot study, it was hypothesized that applying a high frequency diathermy irradiation in addition to cauterizing a pulp wound may serve as an additional thermosterapeutic effect against root canal pathogens in treatment of teeth with partial pulp necrosis.

Thus, the results of cultural growth demonstrated that a conventional antimicrobial therapy was effective in 85% of cases. However, after diathermy irradiation, a bactericidal efficacy was detected in approximately 91% of samples. In addition, it was noted that the presence of a low difference between the groups in values of residual microflora was not associated with the outcomes of radiographic examinations.

In conclusion, the occurrence of postoperative pain in Groups 1 and 2 demonstrated a similar reduction in toothache dynamics at all time intervals when assessments were made, and analysis of tooth radiographs in both groups of patients did not reveal a significant difference. As for the evaluation of a cultural growth, the signs of turbidity were detected in 6 samples of Group 1 and in 4 samples of Group 2.

Therefore, the possible antimicrobial efficacy of a high frequency diathermy in treatment of teeth with partial pulp necrosis was demonstrated. However, taking into account the low number of clinical results and a short period of observations, further studies should be made to confirm the results of this clinical trial.

Competing Interests

The authors declare that they have no competing interests.

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Effectiveness of Short-Term Heart Rate Variability Biofeedback Training and the Risk of Internet Addiction in Adolescents 15-16 Years of Age

Liliya V. Poskotinova, PhD, ScD*; Olga V. Krivonogova, PhD; Oleg S. Zaborsky

*N. Laverov Federal Center for Integrated Arctic Research
Arkhangelsk, the Russian Federation*

Abstract

Background: Adolescents with an Internet overuse problem and risk of Internet addiction (IA) have a disturbed autonomic nervous system balance. The aim of the study was to determine the effectiveness of short-term heart rate variability biofeedback (HRV-BF) training to increase the total power (TP) of HRV spectrum in adolescents 15-16 years of age with different risks of IA development.

Methods and Results: The study involved 20 healthy youths (15-16 years of age) of Arkhangelsk secondary school. The survey was conducted using the Chen Internet Addiction Scale (CIAS) in the Russian version of Malygin et al.(2011). SBP (systolic blood pressure), DBP (diastolic blood pressure) and HRV indicators (HR [heart rate], TP of the HRV spectrum, and SI [Stress Index]) were recorded in relaxation (3 min) and during the HRV-BF training session (3 min). According to the CIAS score, 2 groups were identified: Group 1 (n=9) with minimal IA risk (CIAS score <47) and Group 2 (n=11) with significant IA risk (CIAS score ≥47 points). Group 1, after HRV-BF training, showed a significant increase in TP compared to the initial value, on average by 2.3 times ($P=0.036$). At the same time, SI decreased significantly ($P=0.025$). In Group 2, after HRV-BF training we did not find significant change in TP and SI, compared to the initial data. Moreover, HR became statistically higher ($P=0.021$). TP level after HRV-BF training in Group I was significantly higher than in Group 2 ($P=0.043$). SBP and DBP did not statistically change during the training in both groups. Correlation analysis performed on the total sample (n=20) revealed a significant negative correlation between high TP levels during HRV-BF training and low CIAS scores on the Wit-scale ($r_s = -0.46$, $P=0.048$).

Conclusion: A significant risk of IA developing in puberty may be accompanied by a decrease in the autonomic nervous reactivity during the HRV-BF session. The greatest influence on reduction of HRV-BF efficiency during short-term training has withdrawal symptoms associated with excessive Internet use. (**International Journal of Biomedicine. 2020;10(2):153-156.**)

Key Words: Internet addiction • adolescents • heart rate variability biofeedback

Abbreviations

HR, heart rate; **SBP**, systolic blood pressure; **DBP**, diastolic blood pressure; **HRV**, heart rate variability; **IA**, Internet addiction; **TP**, total power; **SI**, Stress Index; **BMI**, body mass index.

Introduction

In the adolescent period, the autonomic regulation of cardiac activity remains imperfect due to an unstable sympathovagal balance. At the same time, young people become highly involved in Internet communication due to the educational purposes and because of the high need for

positioning ourselves in the media space. Because of this involvement, adolescents may develop signs of Internet addiction, exhibiting also the symptoms of deteriorating health. Cerniglia et al. identify IA as a non-chemical behavioral addiction that includes the interaction of a person with a machine as an “instrumental” form of social interaction (i.e. a form of addiction mediated by machines).⁽¹⁾ Chen and coauthors proposed psycho-diagnostic tools that can determine the level of risk of developing IA, including in adolescents.^(2,3) There is evidence of reduced background overall HRV in individuals with problematic Internet use, and in individuals with Internet gaming addiction,⁽⁴⁾ which indicates a reduced

*Corresponding author: Liliya V. Poskotinova, PhD, ScD.
Department of Biorhythmology of N. Laverov Federal Center
for Integrated Arctic Research, Arkhangelsk, Russia. E-mail:
liliya200572@mail.ru

autonomic flexibility.⁽⁵⁾ HRV-BF training helps to optimize the mechanisms of baroreflex, an increase in TP of HRV and vagal effects on HR.⁽⁶⁾ Short-term HRV-BF training can be considered as a kind of cognitive test, when in a short period of time people are able to implement a strategy to purposefully change their visceral functions.⁽⁷⁾ The issue of the effectiveness of HRV-BF training in individuals with IA, or the risk of its development, remains open.

The aim of the study was to determine the effectiveness of short-term heart rate variability biofeedback (HRV-BF) training to increase the total power (TP) of HRV spectrum in adolescents 15-16 years of age with different risks of IA development.

Materials and Methods

The study involved 20 healthy youths (15-16 years of age) of Arkhangelsk secondary school. The study was conducted in January 2020. The study was approved by the Ethics Committee of the N. Laverov Federal Center for Integrated Arctic Research, RAS (Protocol №3 dated 12.02.2020). Written informed consent was obtained from all participants.

Affiliation to healthy persons was established according to the rules formulated by Order No. 514n of the MHRF dated 08.10.17.⁽⁸⁾ Height and BMI were between the third and the 97th percentile, according to growth charts for height-for-age (for boys) and BMI-for-age (for boys).⁽⁹⁾ The survey was conducted using the Chen Internet Addiction Scale (CIAS)⁽²⁾ in the Russian version of Malygin et al.⁽¹⁰⁾ The CIAS is a self-report measurement consisting of 26 items on a 4-point Likert scale—from 1 point (does not match my experience) up to 4 points (definitely matches my experience). Thus, the minimum CIAS score was 26 points, and the maximum was 104 points. Respondents with a CIAS score above 64 points were considered persons with symptoms of Internet-dependent behavior.^(10,11) A score of 43 to 64 points was associated with a risk of developing Internet-dependent behavior, and less than 43 points indicated a minimal risk of such behavior.⁽¹⁰⁾ The total CIAS score was determined, as well as scores for Compulsive Use (Com), Withdrawal Symptoms (Wit), Tolerance (Tol), Interpersonal and Health-Related Problems (IH), and Time Management Problems (TM). The integral indicators reflecting the core symptoms of Internet overuse (IA-Sym=Com+Wit+Tol) and problems associated with IA (IA-RP=IH+TM) were also highlighted.

HRV indicators were recorded in the sitting position using the Ramena Varicard device (Russia). We determined HR (bpm), TP of the HRV spectrum (ms²), and Stress Index (SI) (units), reflecting the level of sympathetic effects on the heart rhythm. SI was calculated by the formula $[SI = \text{Amo}50/2 \times \text{VAR} \times \text{Mo}]$, where Mo(ms) is the cardiointerval value dividing the cardio-interval-gram series in half, VAR – variation range between the minimum and maximum values in the cardio-interval-gram series, and Amo50,% – amplitude of mode – number of R-R intervals]. HRV indicators were recorded in relaxation (3 min) and during the HRV-BF training session (3 min), as well as SBP and DBP using an A&D monitor (Japan). All participants

had a 3-minute preliminary training. During HRV-BF training, the participant had to maintain a state of calmness and muscle relaxation, and breathe with a deep calm inhalation and a smooth, slow exhalation. The subject performed visual control of TP on a computer monitor, which should have increased with effective HRV-BF training.⁽¹²⁾

Statistical analysis was performed using the statistical software «Statistica». (v. 13.0, StatSoft, USA). Median values are presented with interquartile (IQ) ranges (IQR; 25th to 75th percentiles). The Mann-Whitney test was used to compare median values. The frequencies of categorical variables were compared using Pearson χ^2 . The Spearman correlation coefficient (r_s) was used to assess the relationship between variables. A probability value of $P < 0.05$ was considered statistically significant.

Results

It was found that only 2 people had a stable pattern of Internet-dependent behavior (CIAS score >64 points). In the total sample, the median CIAS score was 47 points with a range of Min-Max of 32-72 points (Table 1). According to the CIAS score, 2 groups were identified: Group 1 (n=9) with minimal IA risk (CIAS score <47) and Group 2 (n=11) with significant IA risk (CIAS score ≥ 47 points). Group 2 had a significantly higher score on all CIAS scales, excluding Com.

Table 1.

CIAS score in the participants of both groups

CIAS scales	Me (n=20)	Min-Max (n=20)	Group 1	Group 2	P
			Me (P ₂₅ ; P ₇₅)	Me (P ₂₅ ; P ₇₅)	
CIAS score	47	32-72	39 (35.5; 43.5)	51 (48; 57)	<0.001
IA-Sym	27	19-39	22 (20; 26.5)	28 (27; 33)	0.004
IA-RP	20	13-33	16.5 (14; 18)	24 (20; 24)	<0.001
Com	10	5-14	8.5 (7.5; 9.5)	10 (8; 13)	0.104
Wit	9	6-15	8 (7; 10)	11 (9; 11)	0.015
Tol	7	5-14	6 (5.5; 6.5)	9 (7; 10)	0.013
IH	11	7-16	9 (8; 11)	13 (11; 15)	0.012
TM	8	5-17	6.5 (6; 8)	10 (8; 13)	0.003

Initial values of HRV and blood pressure in the participants of both groups were statistically identical. Group 1, after HRV-BF training, showed a significant increase in TP compared to the initial value, on average by 2.3 times ($P=0.036$) (Table 2). At the same time, SI decreased significantly ($P=0.025$). Such results indicate that short-term HRV-BF training is effective, resulting in an increase in vagal influences and a decrease in sympathetic activity in relation to the autonomic regulation of heart rhythm.

In Group 2, after HRV-BF training we did not find significant change in TP and SI, compared to the initial data, which indicates the level of sympathetic activity was preserved, despite attempts by participants to find the optimal respiratory

rate in order to increase the control indicator. Moreover, HR became statistically higher ($P=0.021$). SBP and DBP did not statistically change during the training in both groups.

Table 2.

Cardiovascular parameters after HRV-BD training, Me ($P_{25};P_{75}$)

Parameter	Baseline	After HRV-BF training	P
Group 1 (CIAS <47)			
SBP, mmHg	115 (105; 119)	118.5 (117; 120.5)	0.051
DBP, mmHg	71 (66; 74)	71.5 (66.5; 76.5)	0.612
HR, bpm	76 (60; 91)	78.5 (67.5; 90)	0.500
TPx1000, ms ²	2.88 (1.99; 6.74)	6.55 (4.04; 10.29)*	0.036
SI, units	84.5 (38; 237)	36.5 (31; 141)	0.025
Group 2 (CIAS ≥ 47)			
SBP, mmHg	116 (113; 128)	114.5 (113; 123)	0.374
DBP, mmHg	69 (63; 76)	71.5 (67; 76)	0.386
HR, bpm	75 (70; 83)	79.5 (75; 81)	0.021
TPx1000, ms ²	3.03 (2.52; 4.65)	2.98 (2.45; 3.36)*	0.213
SI, units	92.5 (69; 116)	98.5 (57; 143)	0.423

* - $P=0.043$ between Groups 1 and 2 groups after HRV-BF training

Correlation analysis performed on the total sample ($n=20$) revealed a significant negative correlation between high TP levels during HRV-BF training and low CIAS scores on the Wit-scale ($r_s = -0.46$, $P=0.048$). After the exclusion of one person with a high TP ($>11,000$ ms²), this correlation became more pronounced ($r = -0.68$, $P=0.003$). Thus, a significant risk of IA developing in puberty may be accompanied by a decrease in the autonomic nervous reactivity during the HRV-BF session.

Discussion

The selection of short-term training with biofeedback is required to restore autonomic nervous system balance in adolescents during the intense training process, when there is not enough time to implement a long-term training in a course with biofeedback procedures. Our previous studies involving adolescents slightly younger than the examined group (14-15 years of age) showed a more pronounced effectiveness of the short-term HRV-BF training (3min) after performing a physical load of a speed-strength nature than the control group that did not use this training.⁽¹³⁾ Consequently, in healthy adolescents without pathology of the cardiovascular system, baroreflex mechanisms should be sufficiently formed to implement self-regulation in the management of the cardiorespiratory system's own functions, even for such a short time period. However, the problems of the psycho-emotional sphere and behaviors in adolescents with Internet overuse were frequently associated with a reduction in overall HRV, with disturbances in vagal regulation of the heart rhythm.^(4,5)

The results of the present study showed that the baseline HRV indicators in adolescents with a minimal risk and those with a pronounced risk of Internet-dependent behavior were identical, without signs of a pronounced decrease in overall HRV. However, the implementation of a short-term HRV-BF session in individuals with a pronounced risk of IA was obviously difficult. It can be assumed that against the background of neurobiological disorders that cause the risk of dysthymia, affective and social-anxiety disorders, and depression in Internet-dependent adolescents,⁽¹⁾ disturbances in the sensations of signals from their own body also develops and, therefore, the effectiveness of biological feedback decreases. Nevertheless, it should be noted that a similar low HRV-BF efficiency was not found in individuals with high CIAS score, but with an initially high TP of HRV (i.e. in persons with pronounced vagal reserves of cardiac activity). The leading role of the CIAS Wit-score for Internet overuse in reducing the effectiveness of HRV-BF requires further study of adolescents with a wider age range. Thus, even the presence of a moderately pronounced risk of developing Internet-dependent behavior at the stage when age-related autonomic regulation of cardiac activity is formed in adolescents may be accompanied by a decrease in the ability to self-regulate. The low effectiveness of HRV-BF shows the difficulty monitoring bodily functions in individuals with predominantly withdrawal symptoms (i.e. with a deterioration in well-being when access to Internet resources is impossible). It is assumed that the long-term HRV-BF training can provide the optimization of cortico-visceral connections and reduce the risk of psychosomatic disorders in persons with Internet-dependent behavior.

Competing Interests

The authors declare that they have no competing interests.

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Auditory Evoked Potential P300 and Risk of Internet Addiction in Young People

Elena V. Krivonogova, PhD; Liliya V. Poskotinova, PhD, ScD*; Olga V. Krivonogova, PhD

*N. Laverov Federal Center for Integrated Arctic Research
Arkhangelsk, the Russian Federation*

Abstract

Background: Young people spend a lot of time using Internet resources; therefore, they are most susceptible to Internet addiction (IA), in which the parameters of speed and accuracy of information processing by brain structures can change. The aim of the work was to evaluate the parameters of the auditory evoked potential P300 in young people aged 16-17 years with different risk levels of IA.

Methods and Results: A total of 46 healthy young people (14 boys and 32 girls, aged 16-17 years) living in Nadym city (Russia) took part in the study. Signs of IA were determined using the Chen Internet Addiction Scale (CIAS) in the Russian version of Malygin et al.(2011). The parameters of the auditory evoked potential P300 were evaluated using an electroencephalograph (Neuron-Spectrum-4/EPM, Russia) using a standard auditory oddball paradigm. According to CIAS scores, two groups were identified: Group 1 (n=11/23.9% [6 boys, 5 girls]) with minimal IA risk, Group 2 (n=29/63% [8 boys, 21 girls]) with a moderate IA risk, and Group 3 (n=6/13.1% [all girls]) with a pronounced and stable IA pattern. Group 3 showed the lowest P300 latency in the temporal leads (F8, T4) on the right. Correlation analysis showed that the P300 latency in the F8 lead negatively correlates with the overall CIAS score ($r_s = -0.36$, $P = 0.01$). The persons of Group 2 had significantly higher P300 amplitude compared to Group 1 in the C3 ($P_{1-2} = 0.010$) and C4 ($P_{1-2} = 0.013$) leads. In Group 3, the P300 amplitude was significantly lower than in Groups 1 and 2 in the anterior temporal (F7 F8) and in frontal (F4) leads on the right ($P_{3-1,2} = 0.010$). Inverse correlations between the total CIAS score and the P300 amplitude were revealed in the F7 ($r_s = -0.60$, $P = 0.003$), F8 ($r_s = -0.70$, $P = 0.001$), F4 ($r_s = -0.71$, $P = 0.001$), and F3 ($r_s = -0.50$, $P = 0.018$) leads.

Conclusion: In young people, the severity of Internet-dependent behavior is associated with a decrease in the P300 latency in the right temporal lobe of the brain. Individuals with a moderate risk of IA showed a maximum amplitude of P300 in the central parts of the brain whereas individuals with signs of IA showed a minimal amplitude of P300 in the anterior temporal (F7 F8) and frontal (F4) brain regions on the right. The data obtained allow us to consider the revealed changes in the amplitude-time characteristics of the P300 in the temporal lobe of the brain as neurobiological markers of the risk of developing Internet addiction. (*International Journal of Biomedicine. 2020;10(2):157-160.*)

Key Words: young people • Internet addiction • auditory evoked potential P300 • Internet addiction

Introduction

The diversification of digital technologies into everyday life significantly changes the composition of life values of the modern young generation. The significance of this or that information and written word, training and spending free time undergo permanent transformation in modern society. Neurophysiological mechanisms and cognitive characteristics in people with excessive use of Internet resources (Internet

overuse) are currently being actively studied and are not fully disclosed. The literature has demonstrated that as a result of interaction with the environment, as well as the assimilation of new information and new experience, physiological changes occur in the brain as a result of its neuroplasticity.^(1,2) In most cases, attention is paid to the features of brain activity with the excessive use of video games and social networks,⁽³⁾ but there is no answer to the question of how much an involvement in the use of Internet resources can have a damaging or optimizing effect on brain activity. Particular attention is paid to the developing brain of adolescents, since many cognitive functions are actively formed during this period and are strongly influenced by environmental factors.⁽⁴⁾ Therefore, the search for neurobiological markers of IA in the form of

*Corresponding author: Liliya V. Poskotinova, PhD, ScD.
Department of Biorhythmology of N. Laverov Federal Center
for Integrated Arctic Research, Arkhangelsk, Russia. E-mail:
liliya200572@mail.ru

local changes in the function of brain structures responsible for cognitive functions is relevant.

The aim of the work was to evaluate the parameters of the auditory evoked potential P300 in young people aged 16-17 years with different risk levels of IA. An assessment of the P300 component, or cognitive potential, was used for assessing neurophysiological processes associated with recognition, decision making, directed attention and random access memory.⁽⁵⁾ The P300 component is a measurable direct reaction of the brain to a certain sensory, cognitive or mechanical stimulus. It belongs to ERP (event related potentials), which are stereotypical electrophysical answers to stimulants.⁽⁶⁾ P300 components are most easily provoked by a simple discriminating task. The examinee is presented with two randomly repeating stimuli so that one of them is repeating proportionally rarely. In the auditory version of this test (auditory evoked potentials) two different, repeating tones are used, where the targeted stimulus (tone) appears fewer times than the non-targeted stimulus (tone). The examinee must count tones (i.e. classify the frequency of the targeted tone).^(7,8)

Materials and Methods

A total of 46 healthy young people (14 boys and 32 girls, aged 16-17 years) living in Nadym city (Yamal-Nenets Autonomous Okrug of Russia) took part in the study in March 2020. The study was approved by the Ethics Committee of the N. Laverov Federal Center for Integrated Arctic Research, RAS (Protocol №3 dated 12.02.2020). Written informed consent was obtained from all participants.

The exclusion criteria at the time of the examination were infectious diseases, cardiovascular diseases, and neurological diseases. Signs of IA were determined using the Chen Internet Addiction Scale (CIAS)⁽⁹⁾ in the Russian version of Malygin et al.⁽¹⁰⁾ The CIAS is a self-report measurement consisting of 26 items on a 4-point Likert scale—from 1 point (does not match my experience) up to 4 points (definitely matches my experience). Thus, the minimum CIAS score was 26 points, and the maximum was 104 points. The subjects with CIAS scores 27-42 had a minimal risk of IA, 43-64 - a moderate risk of IA, and ≥ 65 - a pronounced and stable IA pattern.

The parameters of the auditory evoked potential P300 were evaluated using an electroencephalograph (Neuron-Spectrum-4/EPM, Russia) using a standard auditory oddball paradigm. The signal was filtered with a band pass filter with a 1.5 Hz–30 Hz range. The study was carried out according to the standard method in a situation of a randomly occurring event in response to auditory non-verbal stimulation at the touch of a button. Binaural stimulation was performed with a stimulus duration of 50ms, an intensity of 80dB, a period between stimuli of 1sec, a tone frequency of 2000 Hz (for a target stimulus, probability of 30%) and 1000 Hz (for a non-target stimulus, probability of 70%). The number of averagings for targeted stimulus was 25-30. The amplitude-time parameters of the response were estimated: the peak-to-peak amplitude (mcV) of the N250-P300 wave and the P300 latency (ms). The amplitude and latency of the P300 was estimated in accordance with the International 10-20 system of applying

Electroencephalogram leads using a monopolar ear reference electrode. The studied brain regions included F3, F4 (frontal), C3, C4 (central), P3, P4 (parietal), F7, F8 (anterior temporal), and T3, T4 (mid-temporal) leads, with even leads on the right and odd leads on the left.

Statistical analysis was performed using the statistical software «Statistica» (v. 13.0, StatSoft, USA). Median values are presented with interquartile (IQ) ranges (IQR; 25th to 75th percentiles). A non-parametric Kruskal-Wallis test was used for comparisons of median values among three groups ($P < 0.05$), followed by post-hoc testing using un-paired Mann-Whitney U tests ($P < 0.017$). The Spearman correlation coefficient (r_s) was used to assess the relationship between variables ($P < 0.05$).

Results

According to CIAS scores, three groups were identified: Group 1 (n=11/23.9% [6 boys, 5 girls]) with minimal IA risk, Group 2 (n=29/63% [8 boys, 21 girls]) with a moderate IA risk, and Group 3 (n=6/13.1% [all girls]) with a pronounced and stable IA pattern. The latency and amplitude values of P300 in all recorded leads in girls and boys in Groups 1 and 2 did not statistically differ, which made it possible to consider P300 parameters in the whole group. Group 3 showed the lowest P300 latency in the temporal leads (F8, T4) on the right (Table 1).

Table 1.

P300 latency (ms) in young people, Me (P₂₅;P₇₅)

Parameter	Group 1	Group 2	Group 3	P
F4	307(292;335)	302(297;323)	298(292;312)	0.572
F3	314(299;324)	305(290;325)	308(284;312)	0.223
C4	296(292;335)	299(284;322)	298(292;308)	0.923
C3	308(284;326)	299(278;325)	302(286;308)	0.212
P4	294(280;313)	296(278;320)	298(286;304)	0.684
P3	296(271;310)	297(272;319)	298(284;304)	0.883
F8	327(311;335)	304(290;322)	300(286;308)	0.001
F7	318(296;338)	314(294;330)	300(284;316)	0.343
T4	313(294;322)	300(282;313)	296(292;296)	0.024
T3	302(284;313)	302(287;322)	298(291;307)	0.921

Correlation analysis showed that the P300 latency in the F8 lead negatively correlates with the overall CIAS score ($r_s = -0.36$, $P = 0.01$), which also indicates a shortening of the processing time of sensory information by the structures of the temporal part of the brain in individuals with pronounced signs of Internet-dependent behavior.

The P300 amplitudes were different in the groups, according to the Kruskal-Wallis criterion, in most brain leads, and the largest P300 amplitude was detected in individuals in Group 2 (i.e. in people with a moderate risk of IA) (Table 2). Nevertheless, in pairwise comparisons among the three groups

at the required level of significance (Mann–Whitney U-test, $P < 0.017$), the persons of Group 2 had significantly higher P300 amplitude compared to Group 1 in the C3 ($P_{1,2} = 0.01$) and C4 ($P_{1,2} = 0.013$) leads. In Group 3, the P300 amplitude was significantly lower than in Groups 1 and 2 in the anterior temporal (F7, F8) and in frontal (F4) leads on the right ($P_{3,1;3,2} = 0.01$).

Table 2.

Amplitude P300 (mcV) in young people, Me ($P_{25}; P_{75}$)

Parameter	Group 1	Group 2	Group 3	P
F4	16.6(15.2;19.1)	20.6(13.6;24.7)	11.5(10.7;12.9)	0.023
F3	14.3(10.3;18.4)	19.2(14.2;23.5)	12.5(11.5;13.3)	0.034
C4	13.3(10.9;16.4)	20.8(13.4;27.3)	14.1(13.1;15.1)	0.040
C3	11.5(6.6;16.7)	19.6(12.2;25.9)	12.8(12.1;15.9)	0.031
P4	11.6(8.7;13.7)	16.8(10.4;22.9)	13.1(10.7;15.9)	0.032
P3	9.6(6.6;15.2)	16.2(9.2;19.1)	14.7(8.7;17.6)	0.503
F8	10.8(7.7;16.2)	12.4(7.2;16.3)	6.6(5.8;7.3)	0.021
F7	11.0(8.8;13.6)	11.3(7.4;12.7)	6.1(5.2;6.6)	0.014
T4	9.8(8.4;14.3)	13.8(9.3;17.1)	11.6(8.4;15.2)	0.244
T3	10.7(5.6;12.2)	11.9(9.7;14.7)	9.7(7.8;11.7)	0.121

Inverse correlations between the total CIAS score and the P300 amplitude were revealed in the F7 ($r_s = -0.60$, $P = 0.003$), F8 ($r_s = -0.70$, $P = 0.001$), F4 ($r_s = -0.71$, $P = 0.001$), and F3 ($r_s = -0.50$, $P = 0.018$) leads. These data confirm the pattern in the form of a decrease in the P300 amplitude in individuals with pronounced Internet-dependent behavior.

Discussion

The distribution of the average latency and amplitude of the P300 was gender-independent in Groups 1 and 2, that is, the differences obtained in the P300 parameters were due precisely to the severity of the Internet-dependent behavior. The greater participation of girls in the study and their exclusive presence in Group 3 can be explained by the fact that girls are more sincere in discussing psychological and social problems than boys.⁽¹¹⁾ This may affect the CIAS score. The main areas of the brain that are associated with the generation of brain activity during signal detection include the temporoparietal, medial temporal lobes, and lateral prefrontal cortex.⁽¹²⁾

It is assumed⁽¹³⁾ that the temporal lobe is most involved in the processing of audiovisual information, and is also among the key areas involved in the integration of auditory and visual signals, as well as in the emotional perception of information. Ezzyat et al.⁽¹⁴⁾ showed that electrical stimulation of the temporal lobes of cerebral hemispheres improves memory and subsequent reproduction of information. It is likely that a decrease in P300 latency and an increase in P300 amplitude in individuals with a moderate risk of IA can be associated with an increase in the functional load on cortical processing centers of various types of sensory stimulation while working on the

Internet. Nevertheless, at a certain stage, signs of IA are formed, which are accompanied by a pronounced decrease in the P300 amplitude. According to other authors, the P300 amplitude is a very sensitive indicator, the variance of which depends on genetic⁽¹⁵⁾ and environmental factors, emotional state, and anxiety level.⁽¹⁶⁾ The reduced P300 amplitude has been found in people suffering from other types of addiction (alcoholism, substance abuse).^(17,18) It is assumed that the low P300 amplitude reflects a violation of the functions of inhibitory control of the central nervous system associated with the frontal lobe of the brain.⁽¹⁹⁾ A decrease in the P300 amplitude is associated with a change in the activity of neurotransmitter systems that are involved in generating the P300.⁽²⁰⁾ People with symptoms of IA have shown a decrease in the susceptibility of dopamine D2 receptors in the striatum.⁽²¹⁾ Pogarell et al.⁽²²⁾ found that the susceptibility of D2/D3 receptors to dopamine in the striatum positively correlated with the P300 amplitude in patients with depression. The increasing need for alternating multiple windows and hyperlinks in the browser of Internet resources, the desire to open new hyperlinks and the increase in search queries on the Internet can be caused by the easy receipt of information rewards related to the stimulation of the dopaminergic system of the brain.⁽²³⁾

Thus, in young people, a relationship was found between the P300 parameters and the severity of Internet-dependent behavior. The severity of Internet-dependent behavior is associated with a decrease in the P300 latency in the right temporal lobe of the brain. Individuals with a moderate risk of IA showed a maximum amplitude of P300 in the central parts of the brain whereas individuals with signs of IA showed a minimal amplitude of P300 in the anterior temporal (F7, F8) and frontal (F4) regions on the right. The data obtained allow us to consider the revealed changes in the amplitude-time characteristics of the P300 in the temporal lobe of the brain as neurobiological markers of the risk of developing Internet addiction.

Competing Interests

The authors declare that they have no competing interests.

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Polysomnographic Pattern of Melatonin Therapy in Perimenopausal Women

Irina Madaeva, PhD, ScD*; Natalya Semenova, PhD; Radzhana M. Zhambalova; Lyubov I. Kolesnikova, Academician of the RAS; Sergey I. Kolesnikov, Academician of the RAS

*Scientific Centre for Family Health and Human Reproduction Problems
Irkutsk, Russia*

Abstract

Background: Earlier, we described our own study results of the chronobiological circadian rhythm of salivary fluid melatonin secretion in menopausal women, which allowed us pathogenetically to substantiate the use of melatonin drugs for insomnia in the perimenopausal period. The aim of this research was to evaluate the sleep quality using polysomnographic monitoring in perimenopausal women with sleep disorders (SDs) before and after 3 months of melatonin therapy.

Methods and Results: The study involved 21 perimenopausal women with complaints of SDs. All women completed a questionnaire to assess the subjective severity of insomnia (Insomnia Severity Index, ISI) and underwent a clinical-anamnestic and gynecological examination. Polysomnography (PSG) was performed before and after 3 months melatonin therapy in a daily dose of 3 mg. After melatonin intake, PSG demonstrated improvement in sleep latency, overall sleep efficiency, and an increase of REM sleep. A statistically significant number of EEG activation reactions indicate a decrease in sleep fragmentation and an improvement in sleep segmental structure.

Conclusions: The use of melatonin in a dose of 3 mg/day for 3 months is one of the main methods for treatment of SDs in age-related estrogen-deficient situations. The main clinical effect, which significantly improves the quality of life, is associated with the elimination of pre- and intrasomnic disorders. (*International Journal of Biomedicine. 2020;10(2):161-164.*)

Key Words: polysomnography • melatonin • perimenopausal women • sleep • insomnia

Abbreviations

AHI, the apnea-hypopnea index; **EOG**, electrooculography; **EMG**, Electromyography; **ISI**, the insomnia severity index; **PSG**, polysomnography; **SDs**, sleep disorders; **REM**, rapid-eye-movement; **SWS**, slow-wave sleep; **WASO**, wake after sleep onset

Introduction

The onset of menopause is one of the critical periods in a woman's life. According to a number of studies, from 25% to 50% of women in the menopausal period report sleep-related problems, compared with 15% of women of fertile age.⁽¹⁾ It has been shown that sleep efficiency indicators decrease with age and sleep latency, wakefulness time during

sleep (WASO) increases, and REM sleep time significantly decreases.⁽²⁾ However, the famous Wisconsin Sleep Cohort Study,⁽³⁾ which included 589 premenopausal, perimenopausal, and postmenopausal women, showed that menopausal status was moderately related to self-reported dissatisfaction with sleep but was not consistently associated with symptoms of insomnia or sleepiness. Symptoms and signs of sleep abnormalities in midlife women should not be attributed primarily to menopause before ruling out underlying sleep disorders.

It is well known that women are more likely to suffer from insomnia than men are.⁽⁴⁾ This fact has been noted since the menarche period, and with the development of age-related

*Corresponding author: Irina M. Madaeva, PhD, ScD. The Chief of Somnological Center, Scientific Centre for Family Health and Human Reproduction Problems. Irkutsk, Russia. E-mail: nightchild@mail.ru

estrogen deficiency, gender differences in the frequency of insomnia disorders become more and more significant. Most scientists attribute this to hormonal and metabolic changes that occur in a woman's body at the onset of menopause. The regulatory role over circadian biorhythms is assigned to be produced by the epiphysis melatonin hormone.⁽⁵⁻⁷⁾ Age-related features are not only a decrease in the level of melatonin secretion, but also a change in the daily time curve of this hormone production.^(8,9) All these data made it possible to substantiate pathogenetically the use of melatonin in the treatment of SDs in various pathologies. There is a scientific rationale for proposing melatonin-agonists as an adjunctive treatment of mood stabilizers in treating SDs in bipolar disorders.⁽¹⁰⁾ Thus, the effectiveness of melatonin has been repeatedly shown in SDs in cerebral vascular insufficiency.⁽¹¹⁾ The use of melatonin in gynecology showed a decrease in the intensity of autonomic disorders in menopausal syndrome.⁽¹²⁾

Earlier, we described our own study results of the chronobiological circadian rhythm of salivary fluid melatonin secretion in menopausal women, which allowed us pathogenetically to substantiate the use of melatonin drugs for insomnia in the perimenopausal period.^(13,14) The need to objectify the effectiveness of melatonin therapy using instrumental methods determined the purpose of the study: Comparative assessment of sleep quality using polysomnographic monitoring in perimenopausal women with SDs before and after 3 months of melatonin therapy.

Materials and Methods

The study involved 21 perimenopausal women with complaints of SDs who were referred by a gynecologist-endocrinologist to the Somnology Center of the Scientific Center for Family Health and Human Reproduction Problems (Irkutsk, the Russian Federation) from 2016 to 2018. Patients complained of SDs for 6 months or more, repeated at least 4 or more nights per week in the form of difficulty falling asleep (more than 20 minutes from the time the lights turned off) and frequent night awakenings (at least 2-3 episodes per night). All women completed a questionnaire to assess the subjective severity of insomnia (Insomnia Severity Index, ISI)⁽¹⁵⁾ and underwent a clinical-anamnestic and gynecological examination. After the examination, melatonin therapy was administered for 3 months in a dose of 3 mg once daily, 30 minutes before bedtime. All women completed the study, and the effectiveness of melatonin therapy was evaluated by PSG.

Inclusion criteria were age 45-55; oligomenorrhoea or amenorrhoea during last 12 months; a basal level of follicle-stimulating hormone (FSH) > 20 IU/ml; ultrasounds criteria: 1) endometrial dysfunction: mismatch of structure and thickness corresponding to the first and the second phases of the menstrual cycle; 2) ovarian follicle reserve depletion

Exclusion criteria were hormone replacement therapy and the use of drugs affecting melatonin secretion; decompensation of cardiovascular, mental, neurological, and endocrine diseases; an exacerbation of chronic diseases; presence of chronic SDs in the past; hypnotics administration during the last two weeks.

The polysomnographic monitoring was carried out in a specially equipped laboratory, using the GRASS-TELEFACTOR Twin PSG (Comet) system with an As 40 amplifier with the SPM-1 integrated sleep module (USA), according to the standard methodology. During sleep, the EEG was recorded from central (C3-A2, C4-A1) and occipital (O1-A2, O2-A1) derivations; the EOG was recorded from electrodes placed lateral to the outer canthus of each eye, slightly above (right eye) and below (left eye) the bi-canthal plane, and referenced to the contralateral mastoid electrode (A1 or A2); the EMG was recorded with electrodes placed on the chin and submentally. The ECG was recorded using one standard lead. The oro-nasal breathing airflow was recorded using a thermocouple generating an electrical signal in response to fluctuations in the air temperature during breathing. The thoracic and abdominal breathing efforts were recorded through piezoelectric sensors generating an electrical signal in response to stretching of the elastic fixation belt. The degree of blood oxygen saturation (pulse oximetry) was determined by applying a special sensor to the finger of the subject. Sensors were also applied to record snoring episodes and the patient's body position during sleep.

Statistical analysis was performed using STATISTICA 6.1 software (Stat-Soft Inc., USA). For descriptive analysis, results are presented as mean ± standard deviation (SD). Categorical variables were analyzed using the Chi square test. The Wilcoxon criterion was used to compare the differences between the paired samples. Group comparisons with respect to categorical variables were performed using the Chi square test or, alternatively, Fisher's exact test when expected cell counts were less than 5. A probability value of $P < 0.05$ was considered statistically significant.

The study was carried out in compliance with Ethical Principles for Medical Research Involving Human Subjects, Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the 64th WMA General Assembly, Fortaleza, Brazil, October 2013. The study was approved by the Ethics Committee of the Scientific Center for Family Health and Human Reproduction Problems. Written informed consent was obtained from each patient.

Results

Table 1 presents comparative data from an anamnestic study before and after 3 months of using 3mg of melatonin 30-40 minutes before bedtime. The results of the clinical and anamnestic examination demonstrate a high percentage of a combination of pre-, intra- and postsomnic disorders. Moreover, only one patient complained only of difficulty falling asleep. Taking into account the data on the assessment of the subjective severity of insomnia, the mean value of ISI was determined. Thus, before treatment, the ISI value was 21.3 ± 0.54 , which corresponds to SDs of moderate severity. It is noteworthy that the subjective perception of one's sleep after taking the melatonin preparation was noticeably changed. Thus, complaints of presomnic disorders persisted only in 2 women who perceived the proper period of falling asleep for 5-7 minutes. Complaints of a combination of pre-

and intrasomnic disorders persisted in only 2 women. Thus, the objectification of the overall picture of sleep using a diagnostic study in the sleep laboratory was the next step in evaluating the effectiveness of melatonin.

Table 1.

Data from an anamnestic study before and after 3 months of melatonin therapy

Variable	Before melatonin therapy	After melatonin therapy
Age, yrs	51.2±4.7	52.1±5.1
BMI, kg/m ²	27.09±1.56	26.3±1.2
Incidence of major SDs (%): - difficulty falling asleep - night awakenings (more than 2 episodes per night) - difficulty morning awakening - combination of 2 and/or 3 manifestations of insomnia	10-58.8 9-52.9	2- 11.7 3-17.6
	13-76.4 16- 94.1	5-29.4 2-11.7
ISI	21.3±0.54	10.1±2.1*

*- $P < 0.05$

According to the PSG study, the total study time in all patients was 7-8 hours of nighttime sleep. An analysis of the information obtained made it possible to determine the duration of Stages 1 and 2 of SWS, Stage 3 of SWS, and the phase of REM sleep in minutes. Attention was also paid to indicators of overall sleep efficiency, estimated as the ratio of total sleep time to total recording time, expressed in percentage, latency to sleep, and WASO indicators. AHI, EEG arousal reactions, and percentage oxygen saturation of the blood (SaO₂) during night sleep were evaluated. The results of the PSG study are presented in Table2.

Table 2.

PSG indicators before and after 3 months of melatonin therapy

Variable	Before melatonin therapy	P-value	After melatonin therapy
Total study time, min	450.3±32.3	>0.05	432.5±21.5
Total sleep time, min	318.5±19.5	<0.05	413.9±12.1
Sleep latency	39.45±6.71	<0.05	23.25±3.71
Overall sleep efficiency, %	70.74±9.43	<0.05	95.7±5.09
WASO, min	41.57±6.22	>0.05	36.2±4.8
SWS Stages 1 and 2, min	177.9±64.21	>0.05	189.22±16.78
SWS Stage 3, min	91.92±36.62	>0.05	113.77±33.25
REMS, min	94.72±21.77	<0.05	135.63±22.76
EEG activation reactions, n	34.2±6.7	<0.05	24.2±7.1
AHI, e/h	3.31±2.74	>0.05	4.36±3.45
SaO ₂ , %	98.16±0.94	>0.05	97.23±1.6

PSG demonstrated improvement in sleep latency, overall sleep efficiency, and an increase of REM sleep. A statistically significant number of EEG activation reactions indicate a decrease in sleep fragmentation and an improvement in sleep segmental structure. The observed tendency toward a decrease in wakefulness time during sleep is not statistically significant; this tendency is associated with the preservation of nocturnal awakenings, although their number decreased to one episode in patients with complaints of 2 or more episodes of awakenings. The tendency toward an increase in superficial sleep can be explained by an increase in the total sleep time in patients taking melatonin. A slight increase in AHI was detected, although these indicators are within the reference values, which, quite possibly, is explained by a more general relaxation of smooth muscles due to an increase in the total duration of REM sleep. The current PSG study showed an improvement of some sleep parameters in perimenopausal women with insomnia after melatonin intake in a dose of 3mg/day for 3 months.

Discussion

Our results are consistent with the previously described effects of the use of melatonin drugs in the treatment of SDs. The efficacy of melatonin administration in patients with chronic cerebral insufficiency with SDs has been shown quite convincingly during a multicenter open observational non-comparable study of its effectiveness and safety.⁽¹¹⁾ However, this large-scale study evaluated only the subjective characteristics of sleep. It should be noted that the use of melatonin as an adaptogen that significantly improves the quality of life in monotherapy for menopausal disorders and premenstrual syndrome has been found previously.⁽¹⁶⁾ Our results are in good agreement with the results of other researchers,⁽¹⁷⁾ who found a decrease in latency to sleep and a decrease in microactivation with the use of melatonin. Our study also demonstrates a change in the structure of sleep. Analyzing the results obtained, it should be noted that there is an increase in the presence of REM sleep after melatonin use, which is the main factor in improving quality of sleep and, accordingly, normalizing the “sleep-wake” continuum. It can be considered that an increase in the presence of REM sleep is one of the manifestations of the compensatory-adaptive reaction of the sleep homeostasis system. These findings are consistent with the conclusions about the role of REM in human mental life.⁽¹⁸⁻²⁰⁾ However, we believe that the effect of taking melatonin-based drugs is based not only on their chronobiological effect, but also on their antioxidant potential ability to correct pro-oxidant/antioxidant discoordination, and increasing free radical lipid oxidation due to estrogen deficiency.⁽²¹⁾

Summarizing all the above, relying on our own data and the research results of other authors, it can be stated that the use of melatonin in a dose of 3mg/day for 3 months is one of the main methods for treatment of SDs in age-related estrogen-deficient situations. The main clinical effect, which significantly improves the quality of life, is associated with the elimination of pre- and intrasomnic disorders. We agree with

the authors⁽¹²⁾ that melatonin acts as a systemic adaptogen in the disorders of the female reproductive system.

However, it is of great interest to change the structure of sleep by the combined therapy of melatonin and menopausal hormonal drugs in more severe manifestations of insomnia disorders with a tendency toward an increase in systemic disorders associated with estrogen and melatonin deficiency. However, it will be the goal of further scientific research.

Competing Interests

The authors declare that they have no competing interests.

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Cervical Dysplasia and Human Papillomavirus in Women of the Republic of Sakha (Yakutia)

Maria P. Kirillina, PhD^{1,2*}; Irina V. Kononova, PhD¹; Sargylana I. Sofronova, PhD¹; Matrena N. Mikhailova¹; Varvara A. Vorontsova³; Elena L. Lushnikova, PhD, ScD⁴

¹*Yakut Science Centre of Complex Medical Problems, Yakutsk, Russia*

²*M. K. Ammosov North-Eastern Federal University, Yakutsk, Russia*

³*OOO "MALEX", Yakutsk, Russia*

⁴*Federal Research Center of Fundamental and Translational Medicine, Novosibirsk, Russia*

Abstract

The purpose of this study was to investigate the relation between the presence of cervical dysplasia and HPV infection in women of the Republic of Sakha (Yakutia) (RS(Y)), to analyze cytological samples for detecting the presence of pathology and the degree of cervical dysplasia, and to detect the level of HPV infection, and establish the dependence of HPV-test positivity on the presence of dysplasia and the woman's age.

Methods and Results: Cytological material was taken from cervix uteri and cervical canal scraping from 100 patients aged between 22 and 60 years (mean age of 38.9±9.2 years). The sample from every patient was obtained by traditional cytology and liquid-based cytology (LBC). The results of LBC were interpreted under the terminology system of the Bethesda System (2015). HPV detection and typing were performed by real-time PCR using commercial test systems according to their instructions.

The analysis of cytological samples by the LBC method in women of RS(Y) revealed a high prevalence of NILM in the samples to compare with intraepithelial lesions of the cervix. Among intraepithelial lesions, LSIL was significantly more common. Positive HPV tests were observed in less than half of the cases. The vast majority of positive HPV tests were high-risk HPV types, and 16 and 51 types marked the most frequent. The positive tests for high-risk HPV types in women with NILM and intraepithelial lesions (LSIL, HSIL, ASCUS) were approximately equal. The frequency of positive tests for high-risk HPV types in women under 45 was higher than in women 46 years and older.

Conclusion: The combined use of LBC and HPV testing for high-risk HPV infection improves the effectiveness of diagnostics by reducing the amount of uninformative material and allows detecting pathological changes at an earlier stage. (*International Journal of Biomedicine. 2020;10(2):165-168.*)

Key Words: human papillomavirus • cervical cancer • liquid-based cytology • squamous intraepithelial lesion

Abbreviations

ASCUS, atypical squamous cells of undetermined significance; CC, cervical cancer; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; hrHPV, high-risk HPV; HSIL, high-grade SIL; LBC, liquid-based cytology; NILM, negative for intraepithelial lesion or malignancy; PCR, polymerase chain reaction; SIL, squamous intraepithelial lesion; LSIL, low-grade SIL

Introduction

HPV is one of the most common causes of sexually transmitted diseases. It is believed that more than 50% of

sexually active women have encountered not only one but several more types of HPV.⁽¹⁾ Epidemiological and molecular-biological data indicate an important role of HPV in the occurrence of cervical intraepithelial neoplasia (CIN) and cervical cancer (CC). Currently, it is generally accepted that HPV is one of the major factors in the development of CC. According to Wheeler et al., 3 years after HPV infection, CIN2-3 develops one in four women.⁽²⁾

*Corresponding author: Maria P. Kirillina, PhD. Yakut Science Centre of Complex Medical Problems, Yakutsk, Russia.
E-mail: kirillinamp@mail.ru

HPVs can infect basal epithelial cells of the skin or inner lining of tissues and are categorized as cutaneous types or mucosal types. HPVs contain a 7.9-kb circular double-stranded DNA genome that consists of four parts: an early region (E1, 2, 4–7 genes), a late region (L1, 2 genes), a long control region (LCR), and a small, highly variable, non-coding region (NCR) between E5 and L2.⁽³⁾ To date, more than 200 types of HPVs have been well characterized,⁽⁴⁾ more than 40 of which can affect the mucous membranes of the genital organs.⁽⁵⁾

Based on their association with cervical cancer and precursor lesions, HPVs can also be grouped into high-risk (16, 18, 31, 33, 35, 39, 45, 46, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82) and low-risk (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 82) HPV types.⁽⁶⁻⁹⁾ Low-risk HPV types generally cause benign lesions of the cervix and condyloma.⁽¹⁰⁾

HPV affects the basal layer of the epithelium, where cell division is most active. Koilocytes are the predominant cellular features of infection with HPV. Later, under the influence of the immune system, the virus can be eliminated from the body. In the case of long-term persistence, HPV-DNA fits into the cell genome with subsequent step-by-step transformation of the epithelium up to the formation of cancer cells.⁽¹¹⁾

The incidence of HPV infection is the highest in the age group of 15-19 years: About 40% of positive tests of this group indicate the presence of HPV-DNA in the material taken from the cervix. The greater the age of women surveyed, the greater the decrease in the percentage of positive results: in the age group of 20-24 year-olds, there are more than 30% with positive results; in the group of 25-29 year-olds, there are fewer than 30% positive. In the group of women who have reached the 30-year mark, there is a further decrease in the frequency of HPV infections to 15%-17%. In women after menopause, HPV infection is very rare but has an important prognostic value due to the risk of developing malignant cervical pathology. In young women, HPV infection is mostly transient, while infection in postmenopausal women usually has the character of a long-term persistent infection. This is very dangerous for molecular changes that lead to the initiation of the process of carcinogenesis.⁽¹²⁾

Given the high prevalence of CC, which is ranked the third most prevalent cancer in the world among women of reproductive age,⁽²⁾ the diagnosis of precancerous cervical pathology at the stage of intraepithelial neoplasia is an urgent global need. Currently, the development of methods and systems for early diagnosis of CIN is a top priority for healthcare. For the early diagnosis of CIN, the cytological method and molecular methods for detecting HPV-DNA are widely used.^(13,14) Molecular biology studies are direct methods for detecting HPV infection and have a significantly higher sensitivity than indirect method-cytological research. Currently, the highest sensitivity for detecting HPV infection belongs to the PCR method, in which the theoretical limit of recognition is a genomic copy per 100,000 cells.⁽¹⁵⁾ The combination of the HPV test and the cytological method has a higher predictive value for detecting CIN than a single HPV test.⁽¹⁶⁻¹⁸⁾

The purpose of this study was to investigate the relation between the presence of cervical dysplasia and HPV infection in women of the Republic of Sakha (Yakutia) (RS(Y)), to

analyze cytological samples for detecting the presence of pathology and the degree of cervical dysplasia, and to detect the level of HPV infection, and establish the dependence of HPV-test positivity on the presence of dysplasia and the woman's age.

Materials and Methods

The study was conducted in the laboratory of pathomorphology, histology and cytology of the NEFU Medical Institute. Cytological material was taken from cervix uteri and cervical canal scraping from 100 patients aged between 22 and 60 years (mean age of 38.9±9.2 years) after examination and extended colposcopy in the "Malex+" clinic (Yakutsk, Russia). The sample from every patient was obtained by traditional cytology and LBC on the automated system CellPrep Plus (Korea). The Romanovskiy-Gimza stain method was used. The results of LBC were interpreted under the terminology system of the Bethesda System,⁽¹⁹⁾ which includes NILM, LSIL (encompassing: HPV/mild dysplasia/CIN 1), and HSIL (encompassing: moderate and severe dysplasia, CIS; CIN 2 and CIN 3).

HPV detection and typing (types 6, 11, 44, 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82) were performed by real-time PCR using commercial test systems according to their instructions. The study data—the incidence of intraepithelial lesions of the cervix in the samples and the level of HPV infection among the examined women—are presented in the form of proportions (percent).

The study was carried out in compliance with Ethical Principles for Medical Research Involving Human Subjects, Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the 59th WMA General Assembly, Seoul, Republic of Korea, October 2008. The study was approved by the Ethics Committee of the Yakut Science Center of Complex Medical Problems. Written informed consent was obtained from all participants.

Statistical analysis was performed using the statistical software «Statistica» (v. 13.0, StatSoft, USA). The frequencies of categorical variables were compared using the Chi-square test. A probability value of $P < 0.05$ was considered statistically significant.

Results and Discussion

Assessing the levels of background and precancerous pathology by the LBC method, the absence of intracellular lesions were detected in 66/66% cases, of which 26% of patients had no pathology and 40% had reactive changes—squamous metaplasia, inflammation, moderate hyperplasia (no significant differences were obtained). In general, including reactive changes, cervical pathology during LBC was detected in 74/74% cases. LSIL and HSIL were detected in 34/34% cases. LSIL was detected in 27 cases (79.4% of all intraepithelial lesions), of which CIN1 in 20 cases (58.8% of all intraepithelial lesions) cases and CIN1 with koilocytes in 7 cases (20.6% of all intraepithelial lesions) ($P=0.006$). HSIL was recognized in 6 cases and accounted for 17.6% of the total

number of dysplasias in the study group. Differences were statistically significant between the frequency of occurrence of LSIL and HSIL ($P=0.006$). In 1(2.9%) case, ASC-US was diagnosed (Fig.1).

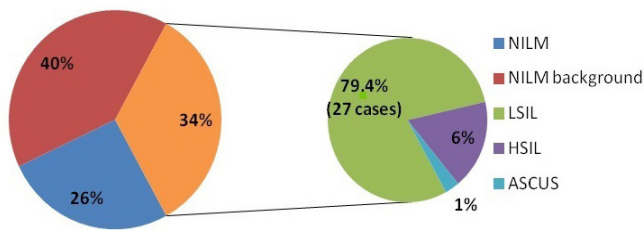


Fig.1. Structure of detected precancerous lesions of the cervix by LBC.

Forty-seven (47%) women were tested for HPV, of which 19(40.4%) were confirmed to have HPV. The presence of high-risk HPV types was significantly more common in 17 women (89.47% of all positive HPV tests) ($P<0.001$). Of these, 8 women were diagnosed with NILM (47% of all positive tests for high-risk HPV types) and 9 with intraepithelial lesions (53% of all positive tests for high-risk HPV types) ($P>0.72$). In turn, the distribution of positivity for high-risk HPV types in women with intraepithelial lesions was carried out as follows: with the diagnosis of LSIL in 5 women (29.4% of all positive tests for high-risk HPV types), HSIL in 3 women (17.6% of all positive tests for high-risk HPV types), and ASCUS in 1 woman (6% of all positive tests for high-risk HPV types). The positivity for high-risk HPV types in women with NILM and intraepithelial lesions (LSIL, HSIL, and ASCUS) was approximately equal.

The analysis of the presence of positive tests for hrHPV types in women with dysplasia, depending on the LBC diagnosis, found that positive and negative results occur with approximately the same frequency. In women with LSIL, there were 5 with positive results and 5 with negative results. In women with HIS, there were 3 with positive and 2 with negative results. Analysis of the distribution of HPV positivity for hrHPV types showed that HPV type 16 was the most common, which was found in 7 patients (36.8 of all positive HPV tests). The next most common type was type 51, found in 4 women (21% of all positive HPV tests). Two types of viruses were detected in 6 women (31.5% of all positive HPV tests). These women had combinations the following types: 16 and 51, 16 and 39, 68 and 39, 31 and 53, 51 and 73, and 31 and 58. The frequency of distribution of other types (39, 68, 31, 52, 73, 58, and 18) varied from 10.5% to 5.2% (Fig.2).

The frequency of HPV detection depending on age showed that the maximum incidence of HPV in samples was significantly more frequent at the age of 25-45 years—15 cases (78.9% of all positive HPV tests) ($P<0.001$). It characterizes the immune status of this age group, and in most cases, it is transient. Approximately 70% of young women have HPV infection that disappears 12 months after detection. Long-term preservation of HPV is mainly associated with hrHPV types

(mainly, types 16 and 18).⁽²⁰⁾ In women 46 years and older, signs of HPV infection were less common and accounted for 4 cases (21%).

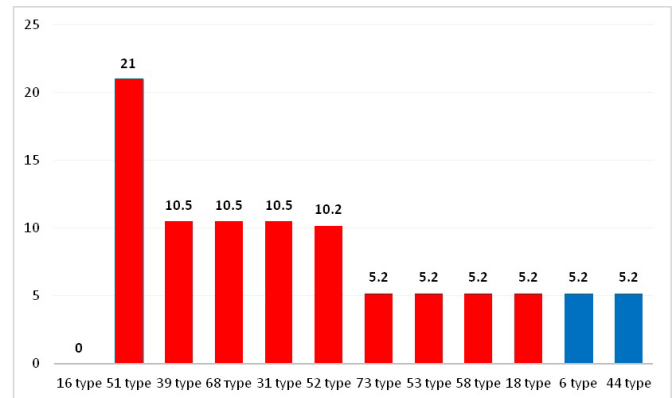


Fig.2. Frequency of distribution of HPV types (%)

Thus, the analysis of cytological samples by the LBC method in women of RS(Y) revealed a high prevalence of NILM in the samples to compare with intraepithelial lesions of the cervix. Among intraepithelial lesions, LSIL was significantly more common.

Positive HPV tests were observed in less than half of the cases. The vast majority of positive HPV tests were hrHPV types, and 16 and 51 types marked the most frequent. The positive tests for hrHPV types in women with NILM and intraepithelial lesions (LSIL, HSIL, and ASCUS) were approximately equal. The frequency of positive tests for hrHPV types in women under 45 was higher than in women 46 years and older.

In conclusion, since each stage of morphological research has not only some capabilities, but also limitations, it dictates the need to use methods of complex diagnostics. The combined use of LBC and HPV testing for hrHPV infection improves the effectiveness of diagnostics by reducing the amount of uninformative material and allows detecting pathological changes at an earlier stage. Such a combined approach significantly increases the value of diagnostic measures and will expand the capabilities of the cytologist in diagnosing and giving recommendations for clinical practice.

Competing Interests

The authors declare that they have no competing interests.

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Trends in Mortality from Preventable Causes in the Sakha Republic (Yakutia)

Albina A. Ivanova, PhD, ScD*; Aleksandr F. Potapov, PhD, ScD;
Leonid F. Timofeev, PhD, ScD; Tatiana S. Makarova, PhD; Alkviad V. Bulatov, PhD;
Lena V. Ignateva, PhD

*M. K. Ammosov North-Eastern Federal University
Yakutsk, Sakha Republic (Yakutia), the Russian Federation*

Abstract

A retrospective analysis of the official statistics for the period 1990-2019 was performed in order to study the rates and structure of mortality of the population in the Sakha Republic (Yakutia) (SR(Y)). It has been established that over the past 30 years, the medical and demographic situation in the SR(Y) was characterized by a high birth rate and high mortality of the population in young age groups from preventable causes, primarily external causes. In the structure of the external causes of death, violent deaths ranked first over the entire study period. In the total number of all deaths, people of working age accounted for more than 40%. In the structure of the causes of working-age population mortality, external causes (injuries and poisoning) ranked first over the entire study period, followed by circulatory system diseases and neoplasms. A relatively high mortality rate in children aged 0-17 years from external causes remained, exceeding the average indicator of the Russian Federation by 36% (2018). This fact has an adverse effect on reproduction in the population, as well as the age and sex composition and the formation of the labor force in Yakutia. (**International Journal of Biomedicine. 2020;10(2):169-173.**)

Key Words: premature mortality • external causes • Yakutia • retrospective analysis

Introduction

With a low density (0.3 people per 1 km²), the population of the Sakha Republic (Yakutia) SR(Y), as of 01.01.2019, totaled 967,000 people. Young age groups dominated in population structure: under working age – 24.7%, working age – 57.7%, over working age – 17.6%. Under the rough division of the constituent entities of the Russian Federation (RF) by the state of health of the population, the SR(Y) falls into the category of those with a positive natural increase and a young population structure, along with such ethnic regions as Yamalo-Nenets and Khanty-Mansi Autonomous Okrugs, and the Republics of Altai and Tyva.⁽¹⁾ Compared with 2010, the average age of the SR(Y) population increased by almost 2 years in 2018, rising to 34.6 years: 33.5 for men and 36.1 for women.

For several decades (1990-2017), the medical and demographic situation in Yakutia was considered more

favorable than other regions of the Far Eastern Federal District (FEFD) due to the maintained natural increase of the population. However, Yakutia's situation was also characterized by high mortality from preventable causes in young age groups, primarily injuries and poisoning, against the background of a high birth rate.⁽²⁾ In 1990-2003, due to mortality and external migration, the population declined by 162.9 thousand people (14.7%). In the subsequent years (2004-2019), the population increased by 17,000 people (1.8%) (Fig. 1). The annual migration from Yakutia decline in that period made by 5,500 people.

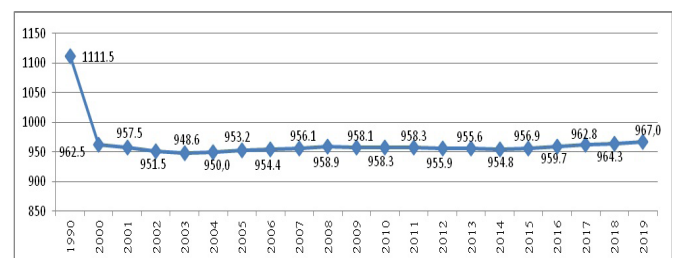


Fig. 1. Population trends in the SR(Y) (as of the beginning of the years, thousand people).

*Corresponding author: Prof. Albina A. Ivanova, PhD, ScD.
M.K. Ammosov North-Eastern Federal University, Yakutsk, Russia.
E-mail: iaa_60@mail.ru

Over the entire study period, the birth rate in the republic greatly exceeded the same indicators of the RF and of the FEFD, of which Yakutia is a part (Table 1). The high birth rate contributed to maintaining the natural population increase in the region, despite the high mortality rate (MR) and external migration. However, in 1990-2018, the birth rate decreased by 30%, which, subsequently, triggered a decreased natural increase of the population by 53.5% (from 12.7 to 5.9 per 1,000 population). The total fertility rate decreased from 2.0 to 1.85 per 1,000 population. By 2018, the number of women of reproductive age had decreased by almost 10%, compared with 2010.⁽³⁾

Table 1.

Birth and mortality rates in the SR(Y), FEFD, and RF in 1990-2019

Territory	1990	1995	2000	2005	2010	2015	2016	2017	2018	2019*
Total birth rate (number of births per 1,000 population)										
SR(Y)	19.6	15.3	13.7	14.3	16.8	17.0	16.0	14.5	13.7	13.2
FEFD	15.4	10.4	9.7	11.5	13.2	13.9	13.3	12.1	11.9	11.1
RF	13.4	9.3	8.7	10.2	12.5	13.3	12.9	11.5	10.9	10.1
Total MR (number of deaths per 1,000 population)										
SR(Y)	6.7	9.8	9.7	10.2	9.8	8.5	8.4	8.1	7.8	7.8
FEFD	8.2	12.8	13.2	15.3	13.8	12.6	12.5	12.1	12.0	12.2
RF	11.2	15.0	15.3	16.1	14.2	13.0	12.9	12.4	12.5	12.3

*- preliminary data

The working age category dominated the age structure of the republic's population (57.7% in 2019). Over the study period, the number of people over working age increased by more than twofold (from 70.3 to 170.1 thousand), due to a decrease in the number of working-age people by 16.3%, children and adolescents – by 32.8% (Table 2). Still, the number of people in this category was almost 1.5 times less than the number of people under working age. The challenge of the global population aging—that is, those over working age making up a higher percentage of the population—is observed primarily in developed countries with low birth rates and high life expectancy. In Yakutia, the aging process is considerably slower due to the high premature mortality rate, especially in the male population.

Table 2.

The population structure changes in the SR(Y) (abs. number in thousand people and share as of the beginning of the years)

Age categories	1990	2000	2005	2010	2015	2019	Increase (decline)
Under working age	356.2 32.6%	271.2 28.2%	236.5 24.9%	220.9 23.3%	233.2 24.4%	239.4 24.7%	-116.8 -32.8%
Working age	667.4 60.9%	597.0 62.0%	616.7 64.9%	609.0 64.1%	579.2 60.5%	557.5 57.7%	-109.9 -16.3%
Over working age	70.3 6.7%	94.3 9.8%	97.5 10.2%	119.5 12.6%	144.5 15.1%	170.1 17.6%	+ 99.8 142.0%
Total	1111.6	962.5	950.7	949.3	956.9	967.0	-144.6 -13%

There is evidence that the aging process has begun in Yakutia: The share of children and adolescents decreased from 32.6 to 24.7%; the share of people of working age decreased from 60.9 to 57.7%; there was redistribution of working-age people toward older ages; and the share of people over working age significantly increased (from 6.7 to 17.6%).

Mortality is a sufficiently sensitive indicator of socio-economic living conditions and healthcare quality. Compared with fertility, mortality is more susceptible to external influences; it can be reduced in a shorter time (within 3-5 years) than fertility can be increased (10 or more years). In addition, high mortality contributes greatly to depopulation (65%); therefore, influencing mortality, one can obtain more remarkable results and significantly reduce the progress of depopulation, although this will not solve the population demographic problems completely.⁽⁴⁾ Preventable mortality is an integral indicator of the performance of the health system. Not being a precise parameter, it gives a certain idea of the quality and effectiveness of medical services and public health policies in a country, and raises the possibility of certain problems in the health system.⁽⁵⁾

The demographic history of Yakutia witnessed two periods of increasing mortality rates in the republic: first – the years 1990-1995 (addition rate of 46%, from 6.7 to 9.8 ‰), second – the years 2000-2005 (addition rate of 5%, from 9.7 to 10.2 ‰).⁽⁶⁾ Next, after a period when the indicator stabilized at the level of 9.3‰–9.8‰, since 2013 it decreased to 7.8‰ in 2019. Despite the positive trend, over the study period mortality was the most pressing issue in the population policy of the republic. In this respect, a further study of mortality remains relevant.

The aim of our research was to study the current trend in mortality from preventable causes in the SR(Y) and its influence on the medical and demographic situation in the region.

Materials and Methods

The following information was used in the study: data from the Territorial Authority of the Federal State Statistics Service in the SR(Y), medical death certificates (form 106/u-02), and forensic medical examination reports. A retrospective analysis of statistical data was performed with statistical, analytical, and mathematical methods, as well as comparative analysis methods.

Results and Discussion

A retrospective analysis of the main causes of mortality in Yakutia in 1990-2018 revealed that at the beginning of the study period, the top three positions were taken by circulatory system diseases (CSD), external causes, and neoplasms; in 2015-2018, external causes moved to the third position after neoplasms (Table 3).

The three leading causes of death (CSD, neoplasms, and external causes) accounted for almost 79% of total deaths in 2018(67.0% in 1990). The analysis of the changes in mortality rates from these causes in 1990-2018 revealed the following (Table 4):

As of 2018, in comparison with the starting indicators of the study (1990), we observed:

1) An increase in the MR from neoplasms by 14.6% (from 122.0 to 139.8 per 100,000 population). The mortality of men from this class of diseases (161.3 per 100,000 population) was 26% higher than in women (119.5 per 100,000 population).

2) An increase in the MR from CSD by 54.7%, with maximum values observed in the period 2005-2010. The present period showed a positive trend: in 2014-2018, the mortality from CSD decreased by 12.9%. There were certain gender differences: the mortality of men from this class of diseases (412.7 per 100,000 population) was almost 1.5 times higher than in women (298.6 per 100,000 population)

3) A significant reduction in the mortality from external causes by 25.5% (from 164.6 to 122.6 per 100,000 population). The MR of men from injuries, poisoning and other consequences of external causes (202.5 per 100,000 population) was 4 times higher than in women (47.3 per 100,000 population); however, the rate of decrease in men's mortality amounted to 24.8% (from 269.2 to 202.5 per 100,000 population), whereas in women – 19.1% (from 58.5 to 47.3 per 100,000 population)

Table 3.
Mortality ratios in the SR(Y) by some main causes (per 100,000 population)

Causes by classes	Years					
	1990	2000	2005	2010	2014	2018
Some infectious and parasitic diseases	14.0	15.2	15.3	11.4	10.6	13.6
Neoplasms	122.0	132.6	125.8	120.7	128.2	139.8
Circulatory system diseases	228.9	381.7	465.1	469.5	406.3	354.0
Respiratory diseases	40.8	43.3	36.3	34.9	27.9	28.5
Digestive system diseases	26.1	45.8	46.1	55.7	37.1	38.7
Injuries, poisoning, and some other consequences of the impact of external causes	164.6	243.9	229.2	195.3	155.0	122.6

Table 4.
Trends in MR from neoplasms, CSD and external causes (1990-2018) (per 100,000 population)

Years	RF			FEFD			SR(Y)		
	Neo-plasms	CSD	EC	Neo-plasms	CSD	EC	Neo-plasms	CSD	EC
1990	194.4	618.7	134.0	122.0	228.9	164.6
2000	203.2	840.0	219.0	172.9	655.7	260.5	132.6	381.7	243.9
2005	201.2	908.0	220.7	182.1	786.7	281.8	126.3	466.8	230.0
2010	205.1	806.4	151.7	191.0	735.8	202.5	120.7	469.5	195.4
2014	202.0	659.5	129.4	190.4	638.5	165.8	128.2	406.3	155.0
2018	203.0	583.1	98.5	201.0	525.4	141.5	139.8	354.0	122.6

EC- External causes

Among 11 regions of FEFD, the SR(Y) has the lowest MR, including those from neoplasms, CSD, and respiratory and digestive diseases (Table 5).

Table 5.
Population mortality in the SR(Y), FEFD, and RF from main causes in 2018 (per 100,000 population)

Territory	Total deaths	including from:					
		Some infectious and PD	Neo-plasms	CSD	RD	DSD	EC
RF	1,245.6	23.6	203.0	583.1	41.6	65.0	98.5
FEFD	1,203.1	23.1	201.0	525.4	53.2	74.2	141.5
Republic of Buryatia	1,074.3	21.9	191.1	445.9	61.3	62.5	147.7
SR(Y)	784.1	13.6	139.8	354.0	28.5	38.7	122.6
Zabaikalsky Krai	1,229.4	20.2	202.0	510.8	85.7	57.4	166.5
Kamchatka Krai	1,126.2	14.0	163.4	562.6	47.9	71.7	124.7
Primorsky Krai	1,345.0	35.9	235.9	640.7	52.3	84.9	116.3
Khabarovsk Krai	1,283.5	24.2	191.7	617.0	43.9	82.8	136.5
Amur Oblast	1,338.3	17.0	204.6	460.0	44.7	83.4	177.3
Magadan Oblast	1,136.2	7.0	195.6	497.0	63.1	82.7	133.9
Sakhalin Oblast	1,265.7	15.1	241.3	374.8	56.1	123.3	168.0
Jewish Autonomous Oblast	1,366.8	28.0	234.2	779.1	63.4	72.7	141.6
Chukotka Autonomous Okrug	1,104.9	44.4	167.7	440.4	40.4	78.8	252.5

RD - Respiratory diseases; DSD - Digestive system diseases; EC- External causes; PD - parasitic diseases.

Compared with the average data for the RF over the study period, the MR from external causes in the RS(Y) was higher (according to the 2018 data – by 19.7%). As for the structure of external causes of mortality, Yakutia had high mortality rates from accidental alcohol poisoning, accidental drowning, suicides and homicides. In 2018, these values exceeded the respective figures of both the RF as a whole and the FEFD (Table 6).

Table 6.
MR from some external causes in 2018 (per 100,000 population)

Territory	Total deaths	including from:				
		All kinds of traffic injuries	Accidental alcohol poisoning	Accidental drowning	Suicides	Homicides
RF	98.5	13.0	7.5	3.3	12.4	5.4
FEFD	141.5	16.5	6.8	5.4	20.8	11.4
SR(Y)	122.6	12.1	10.1	12.8	23.9	14.3

The preliminary 2019 data show a certain positive trend: decreased mortality from traffic accidents from 12.1 to 8.6 per 100,000 population, from suicides – from 23.9 to 22.8, homicides – from 14.3 to 13.6.

External causes are certainly preventable causes of premature mortality. In terms of preventability, first come the causes of death depending on lifestyle and prevention of risk factors (82% for men and 67% for women). Injuries and poisoning accounted for 54% of all preventable death losses in Russia. More than half (52.6%) of all deaths from external causes, including 72.2% of homicides and 42.1% of suicides, were alcohol-related.⁽⁷⁾ According to the Ministry of Internal Affairs of Yakutia, alcohol-related homicides and suicides in the region made up 90% and 42%, respectively. In 2019, the MR from accidental alcohol poisoning in the republic amounted to 15.1 per 100,000 population (cf. the figure for the RF at 7.9 per 100,000 population, for FEFD - 6.7). In general, a number of studies have shown that preventable deaths account for 40% of the total, with their share in the working age reaching 70%.⁽⁶⁾

The comparison of the mortality rates of working-age people from the main classes of causes in Yakutia in 2019 established a positive trend. Since 1996, the MR of the working-age population in the republic consistently exceeded the figures for the RF. The situation in 2019 appears to be more favorable, except for the mortality from external causes (Table 7).

Table 7

Mortality of working-age population in the SR(Y), FEFD, and RF from main causes in 2019* (per 100,000 population)

Territory	From all causes	including from:					
		Some infectious and PD	Neo-plasms	CSD	RD	DSD	EC
RF	466.9	32.8	70.7	140.4	16.9	45.3	108.1
FEFD	588.0	28.7	78.7	171.8	28.2	59.5	167.5
SR(Y)	457.6	17.0	58.5	151.6	13.6	38.7	144.6

*preliminary data; RD - Respiratory diseases; DSD - Digestive system diseases; EC- External causes; PD - parasitic diseases.

By individual factors of external causes, the MRs of the working-age population in Yakutia from accidental alcohol poisoning (15.1 per 100,000 population) and suicides (33.9) were almost twice as high as the average in Russia (7.9 and 14.8, respectively), from homicides (21.0) – almost 3 times as high. These figures also significantly exceeded the same indicators of the FEFD (Table 8).

By gender, the mortality of working-age men in Yakutia from accidental alcohol poisoning (22.4 per 100,000 population) was more than 1.5 times higher than the Russian average (12.8), from suicides (54.2 against 24.9) – 2 times as high, from homicides (34.6 versus 11.0) – 3 times as high. The MR of working-age women in Yakutia from the above reasons also exceeded the same indicators for Russia as a whole: from

accidental alcohol poisoning (6.9 per 100,000 population against 2.4) by 2.8 times, from suicides (11.0 against 3.6) by 3 times, from homicides (5.7 against 2.9) by 2 times.

Table 8.

The structure of external causes of mortality of the working-age population in the SR(Y) in 2019* (per 100,000 population)

Territory	Mortality from external causes	including from:			
		All kinds of traffic injuries	Accidental alcohol poisoning	Suicides	Homicides
RF	108.1	16.0	7.9	14.8	7.2
FEFD	167.5	21.1	6.7	25.5	17.0
SR(Y)	144.6	12.2	15.1	33.9	21.0

*preliminary data

The constantly unfavorable situation with child mortality is one of the serious problems of the demographic situation in the SR(Y). In retrospect, there was an increase in the standardized MR for adolescents aged 15-17 years in 1999-2008 by 30 times (from 0.04 per 1,000 children aged 15-17 years in 1999 to 1.2 % children of the corresponding age in 2008).⁽⁸⁾ In 2014, the mortality in the age of 0-17 years for both genders amounted to 108.6 per 100,000 population of the corresponding age and exceeded the figure of the RF by 20.8% (86.0). In the structure of causes of death in children aged 1-14 years, external causes (65%) ranked first, followed by congenital anomalies (6.8%), and nervous system diseases and CSD (4.9% each). At the age of 15-17 years, 90.6% of all deaths occurred in external causes, 6.3% - from CSD, 3.1% - from nervous system diseases. In the structure of deaths from external causes in this age group, suicides were the main cause (42.7% in 2008, 46.9% in 2012, and 34.1% in 2014); the following ranking places were occupied by homicides (13.3%), and traffic injuries(6.7%). The figure of suicidal deaths at the age of 0-17 years in 2014 was 9.0 per 100,000 population of the corresponding age and 3 times higher than the average indicator of the RF (in 2013 – 7.1, in 2012 – 9.9). The standardized MR from suicides in this age group (19.8 per 100,000 people) was by 8.1% higher than the Russian level.

The recent data on the child MR at the age of 0-17 years in Yakutia still showed higher values than the average for the RF; according to the 2018 data, it amounted to 23.9 per 100,000 population of the corresponding age, which was 36% higher than the same indicator for the entire country (Table 9).

Table 9.

Child mortality in the ages 0-17 from external causes in 2012-2018 (per 100,000 population of the respective age)

Territory	2012	2013	2014	2015	2016	2017	2018
RF	23.2	21.3	21.1	18.2	16.6	15.3	15.3
SR(Y)	41.2	34.6	35.1	32.1	19.9	20.1	23.9

At the same time, there was a clearly negative trend in child mortality; in the period 2012-2018 it decreased by 42% (throughout the RF by 34%).

In conclusion, the study of the trends in the mortality rate suggests that a high share of losses from external causes in young age groups remains a specific feature of the population mortality in Yakutia. Certainly, there are some positive changes in the demographic situation; yet, the high rate of violent deaths is still pressing, being the most negative and acute problem; it is also a marker of the existing social disadvantage in the region.

The main trends that have a negative impact on the demographic situation and formation of the labor force in the republic are as follows:

- High rates mortality among the working-age population of both genders;
- The dominance of preventable external causes of death (accidents, poisoning, injuries) in the mortality structure of the working-age and child population;
- The decline in the labor force due to a consistent child MR (in the 1990s generation, small-numbered as it is, both in the urban and rural areas) with an increased share of retired people and a subsequently greater demographic load on the working-age population.

Competing Interests

The authors declare that they have no competing interests.

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CASE REPORT

Drug-Induced Parkinsonism and Neuroleptic Malignant Syndrome: A Case Report

Alexey A. Tappakhov, PhD^{1,2*}; Tatiana E. Popova, PhD, ScD^{1,2}; Yulia I. Khabarova, PhD²; Maria V. Yakovleva, PhD²; Alina E. Adamova²; Tatiana G. Govorova¹; Michil E. Andreev¹

¹M.K. Ammosov North-Eastern Federal University

²Yakut Science Center of Complex Medical Problems

Yakutsk, the Republic of Sakha (Yakutia), Russia

Abstract

The article presents a clinical case of neuroleptic malignant syndrome (NMS) and drug-induced parkinsonism after a single dose of thiorizadine, and discusses the issues of differential diagnosis and treatment methods. A 57-year-old patient with long-term remission of schizophrenia due to insomnia was prescribed thiorizadine. After a single dose, symptoms of parkinsonism in the form of hypokinesia, muscle rigidity and bradyphrenia developed and began to progress. Three weeks later NMS developed, and treatment was carried out in the intensive care unit. When signs of parkinsonism persisted, she was hospitalized in the neurological department. Regression of symptoms occurred by her taking amantadine sulfate. (**International Journal of Biomedicine**. 2020;10(2):174-177.)

Key Words: neuroleptic malignant syndrome • drug-induced parkinsonism • antipsychotic drugs • Parkinson's disease

Abbreviations

DIP, drug-induced parkinsonism; **NMS**, neuroleptic malignant syndrome; **MoCA**, Montreal Cognitive Assessment; **MMSE**, Mini-Mental State Examination

Introduction

The synthesis of antipsychotics was a cardinal event of the 20th century and became a new stage in the development of psychopharmacotherapy and the treatment of mental disorders.⁽¹⁾ However, it was soon discovered that these drugs could have unfortunate side effects. In 1954, two articles were published that described the development of drug-induced parkinsonism (DIP) in patients treated with reserpine and chlorpromazine.^(2,3) Currently, DIP is observed in 10%-25% of patients who are treated with antipsychotics, and is one of the common causes of secondary parkinsonism.⁽⁴⁾ Rarer causes of DIP include prokinetics (domperidone, metoclopramide), calcium channel blockers (cinnarizine, diltiazem, verapamil), central sympatholytics (a-methyldopa), drugs that deplete

monoamine reserves (reserpine, tetrabenazine) serotonergic drugs and antidepressants (amitriptyline, imipramine).^(5,6)

The Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-V), defines DIP as the presence of a resting tremor, muscular rigidity, akinesia, or bradykinesia, developing within a few weeks of starting or raising the dosage of a medication (typically a neuroleptic) or after reducing the dosage of an antiparkinsonian agent.⁽⁷⁾ Therefore, in contrast to the diagnostic criteria for Parkinson's disease, slowness and impoverishment of movements are not obligatory signs for verifying DIP. Most cases with antipsychotics develop within 3 months, but in the case of calcium channel blockers, the drug exposure can be up to 12 months.⁽⁸⁾ Despite the lack of consensus on the time of regression of parkinsonism symptoms with a dose reduction or complete withdrawal of the causative drug, most often this time is 6 months.⁽⁵⁾ In one study, the improvement time ranged from 2 to 19 months (average 10 months).⁽⁹⁾

A rarer complication of antipsychotic drug therapy is neuroleptic malignant syndrome (NMS), which is manifested

*Corresponding author: Alexey A. Tappakhov, PhD, Ass. Professor of the Department of Neurology and Psychiatry of Medical Institute, M.K. Ammosov North-Eastern Federal University, Yakutsk, Russia. E-mail: dralex89@mail.ru

by muscle rigidity, impaired consciousness, hyperpyrexia, and autonomic dysfunction.⁽¹⁰⁾ NMS is an urgent condition, accompanied by an impairment of vital functions, and can lead to death.⁽¹¹⁾ Typical antipsychotics such as haloperidol and chlorpromazine are most commonly associated with NMS; however, in some cases atypical antipsychotics, neuroleptics, prokinetics, and lithium drugs have been described.⁽¹²⁾

In this article, we present our own clinical observation of a patient with prolonged remission of schizophrenia, who, after a single dose of thiorizadine, developed DIP and NMS.

Case presentation

A 57-year-old woman, an economist, with a prolonged remission of schizophrenia. At the age of 32, she developed insomnia, anxiety and hypothyria. It seemed to her that her colleagues were accusing her of the economic crisis in Russia. Then she began to hallucinate and heard “voices” of the accusatory content, as a result of which she was hospitalized in a psychiatric hospital. In the clinical picture of the psychotic state, depressive-paranoid symptoms and catatonia were detected. She was treated with antipsychotics and insulin coma sessions. A diagnosis of schizophrenia was established. There were no more exacerbations of the disease.

At the age of 46, the patient developed an ischemic stroke with motor aphasia and right-sided hemiparesis with full recovery. Three months before contacting us, she developed insomnia, and also stopped eating. Relatives associated these symptoms with stress. After 4 days, due to continued insomnia, the doctor prescribed thiorizadine and amitriptyline. The patient took the medicine. Her relatives reported that she could take several tablets of thiorizadine. The next morning, she became inhibited, could not stand up, talk, or look “at one point.” She was hospitalized in the therapeutic department. On examination, symptoms of parkinsonism were identified: slowness of movements, muscle rigidity, microbasia, “mask-face,” and hypophony. On Day 6 of hospitalization, in the daytime, she began to talk better and motor activity increased. However, in the evening her condition deteriorated.

On Day 8 of treatment, slowness of movements and muscle rigidity significantly increased, she began to speak beside the point, and a sub-febrile temperature appeared. Cerebrospinal fluid was without pathology. Sinus tachycardia was detected by an ECG. On Day 15 of treatment, hyperthermia (>40°C), severe muscle rigidity, paroxysmal tachycardia, and stupor developed. A blood test revealed an increase in the levels of urea (15.1 mmol/l), creatinine (144.9 mmol/l), creatine kinase (3466 U/l), and leukocytes ($14.8 \times 10^9/l$). An MRI of the brain showed no signs of stroke or inflammation. We conducted detoxification therapy and a single session of plasmapheresis. During treatment, consciousness recovered, muscle rigidity decreased, and autonomic disorders were stopped; creatinine kinase decreased to 394U/l. However, symptoms of parkinsonism persisted.

She was discharged from the hospital on Day 40 from the time of hospitalization.

The patient retained slowness of movement, muscle rigidity, stoop, slowness of thinking, and impaired walking

(she could only move with support). Relatives were also worried about the lack of emotions and motivation (“she refuses everything, does not want to do anything”). As relatives noted, the patient periodically experienced short-term episodes of some improvement, during which physical activity increased, she could independently move around the apartment, and more fully and clearly answer questions.

Neurological examination revealed a slowing of the eye saccades, hypomimia, bradyllalia, muscle rigidity (the “lead tube” phenomenon), axial muscle rigidity, no paresis and no tremor, severe symmetrical hypokinesia, microbasia, and a need for support to walk. On 3 parts of the UPDRS scale - 46 points.

Qualitative examination of cognitive functions was not possible. The patient revealed bradyphrenia, was confused about the anamnestic information, and could confuse dates and events; but she correctly orientated herself and recognized her relatives.

The patient was hospitalized in the neurological department, where treatment with amantadine sulfate (50 mg 3 times a day) was started. The dose of the medicine was increased to 100 mg 3 times a day. During the week, symptoms of parkinsonism significantly decreased (3 part of UPDRS - 19 points). However, the patient developed anxiety, hypotension, bradyphrenia, apathy, and memory problems. The MoCA and MMSE points were 21/20. The geriatric depression scale revealed deep depression (26 points). Venlafaxine was added at a dose of 37.5 mg 2 times a day with further titration. The patient was referred to a psychiatrist for observation.

Discussion

The development of extrapyramidal neuroleptic syndromes, including DIP and NMS, is associated with inhibition of dopaminergic transmission.⁽¹³⁾ The likelihood of parkinsonism developing in antipsychotic therapy is directly proportional to the drugs’ ability to block postsynaptic D2-dopamine receptors and inversely proportional to their ability to block M-cholinergic receptors and serotonin 5-HT₂ receptors.⁽⁴⁾ Therefore, typical antipsychotics (for example, haloperidol, chlorpromazine, thiorizadine, etc.) have a high risk of developing DIP, because they have a high ability to inhibit dopaminergic activity but have a weak ability to reduce cholinergic and serotonergic systems. Atypical antipsychotics have a lower risk; the main effect is due to the blockade of the D₄-dopamine receptors in the limbic system and serotonin receptors in the striatum (clozapine, quetiapine, olanzapine, etc.).⁽⁵⁾ It has been established that the development of parkinsonism requires the blockade of more than 75% of dopamine receptors, while the antipsychotic effect is achieved when 50%-75% of the receptors are blocked.⁽⁶⁾

The risk factors for DIP include old age, because as the person ages, the amount of dopamine decreases and sensitivity to antipsychotics increases.^(8,9) In addition, patients with cognitive impairment are more prone to side effects of antipsychotics, which is associated with initially low cholinergic activity.⁽¹⁴⁾ Other risk factors include female gender, the presence of extrapyramidal disorders, organic

brain damage and brain atrophy, HIV infection, and a family history of parkinsonism.^(4-6,13)

A feature of drug parkinsonism is that motor disorders are potentially reversible (usually within 6 months). However, symptoms can persist and even worsen in 30% of cases after discontinuation or dose adjustment of the causative drug.⁽¹⁵⁾ A large study involving 2991 patients showed a 3.2-fold increase in the risk of Parkinson's disease with antipsychotics, and in 30% of cases signs of the disease developed during antipsychotic therapy.⁽¹⁶⁾ Symmetry of symptoms, the predominance of postural tremor, the presence of oromandibular dyskinesias, and a variable (often negative) response to levodopa are associated with DIP.^(5,17) Parkinson's disease, in contrast, is indicated by previous hyposmia and rapid eye movement (REM) sleep behavior disorder.^(6,18) Positron emission tomography can help in the differential diagnosis of Parkinson's disease and DIP.⁽¹⁹⁾

The treatment of DIP involves the abolition of the causative drug. If there is a need to continue antipsychotic therapy, atypical antipsychotics (usually clozapine or quetiapine) can be prescribed. Another group of drugs is anticholinergics, the mechanism of action of which is associated with the selective blocking of M1-cholinergic receptors in the basal ganglia. NMDA-receptors antagonists (amantadines) reduce the level of glutamate and acetylcholine in the striatum, as well as increase dopamine synthesis in presynaptic terminals, and inhibit the reuptake of dopamine from the synaptic cleft.^(4,5,13,20)

The development of NMS is associated with a blockade of D2-dopamine receptors, not only in the basal ganglia, but also in the hypothalamus.⁽¹⁰⁾ However, according to the pharmacological monitoring in Canada, the number of reports of NMS with atypical antipsychotics (in particular, clozapine) is higher than NMS with typical antipsychotics.⁽²¹⁾ Similar results were obtained when analyzing the database of the Australian Advisory Committee on Drug Side Effects.⁽²²⁾ Therefore, the leading role in NMS cannot belong to dopamine mechanisms but may be associated with dysfunction of the serotonergic and cholinergic systems.⁽²³⁾ This conclusion is also supported by the clinical similarity of NMS to serotonin syndrome.⁽¹²⁾

NMS often develops in the initial stage of treatment with antipsychotics or with an increase in their dose. The risk increases with polypharmacy, especially while taking antidepressants and antiparkinsonian drugs.⁽²⁴⁾ Other factors associated with the risk of NMS are immobility, dehydration, high fever, intercurrent infections, and a family history of catatonia.^(21,25)

The differential diagnosis of NMS is presented in Table 1.

NMS is an urgent condition. Of primary importance is the cessation of the causal antipsychotics. Supportive therapy includes antipyretic methods, correction of metabolic disorders, prevention of respiratory and cardiovascular failure. Recent reviews recommend the administration of a dopamine receptor agonist, bromocriptine. Benzodiazepines are recommended to reduce muscle stiffness and reduce rhabdomyolysis. Electroconvulsive therapy can be used in refractory cases.^(11,13,21)

Table 1.

The differential diagnosis of NMS

Urgent conditions	Key diagnostic features
NMS	Hyperthermia over 38°C Muscle rigidity Change in consciousness Autonomic disorders Increased creatine kinase in blood Increased white blood cell count
Serotonin syndrome	The acute onset Change in consciousness Signs of central nervous system hyper-excitability: motor disorders (tremor, myoclonus, akathisia), hyperreflexia, clonus, convulsions Autonomic disorders (often mydriasis, tachycardia) Mild or moderate muscle rigidity without increased creatine kinase in blood
Febrile schizophrenia ("lethal catatonia")	Symptoms mimics NMS! No causal relationship with antipsychotics Oneiroid syndrome
Akinetic crisis	Symptoms mimics NMS! It develops in patients with Parkinson's disease with intercurrent infections, exacerbation of chronic diseases, or abrupt cancellation of antiparkinsonian therapy

In the clinical case described here, the development of NMS as a result of a single dose of the typical antipsychotic thioridazine is an occurrence of interest; it is probably associated with the mechanisms of drug idiosyncrasy.⁽²³⁾ The absence of a previous history of parkinsonism reduces the likelihood of a significant decrease in dopamine-synthesizing neurons, which could predispose the patient to a high sensitivity to antipsychotics. A history of a catatonic state in schizophrenia may indicate a genetic "hyper" sensitivity to antipsychotics, although we cannot be completely sure which drug from this group was used. It is likely that there are genetic characteristics that affect the metabolism of antipsychotics and can predispose a patient to the development of NMS. For example, under discussion is the clinical significance of the genetic polymorphism of cytochrome P450 (CYP) 2D6 in the metabolism of haloperidol and its effect on the risk of NMS developing.⁽²⁶⁾

In the present case, the persistence of parkinsonism symptoms after NMS, and the presence of fluctuations with episodes of improvement, could suggest the onset of Parkinson's disease or dementia with Levi bodies. However, the absence of any previous non-motor symptoms, the symmetry of symptoms, and a positive response to amantadine indicate DIP.

Competing Interests

The authors declare that they have no competing interests.

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