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Irina Litvinenko, Editorial Secretary, Telephone: +37332205877

Address of the Editorial Office
192, Stefan cel Mare Avenue, Chisinau, MD-2004, the Republic of Moldova
Phone: +37322 205209. Mobile: +37379429274
www.moldmedjournal.md
editor@moldmedjournal.md
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ORIGINAL RESEARCH

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Translation and validation of the Russian version of the personality inventory for DSM-5 (PID-5)

*[1]**Svetlana Lozovanu,** MD, PhD, Associate Professor; [1,2]**Ion Moldovanu,** MD, PhD, Professor;
[1]**Victor Vovc,** MD, PhD, Professor; [1]**Andrei Ganenco,** MD, Assistant Professor;
[1]**Andrei Blajevschi,** MD; [1]**Tudor Besleaga,** MD, PhD, Associate Professor

[1]Department of Physiology and Biophysics, Nicolae Testemitsanu State University of Medicine and Pharmacy
[2]Department of Headache and Autonomic Disorders, Institute of Neurology and Neurosurgery
Chisinau, the Republic of Moldova

*Corresponding author: svetlana.lozovanu@usmf.md
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Abstract

Background: The 5th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) has included major and radical changes in the personality disorder (PD) diagnosis method, from categorical to dimensional one. It includes Alternative Model for Personality Disorders (AMPD). This model explains that personality disorders are characterized by impairment in personality functioning and presence of pathological personality traits. The current study consists in the validation and cultural adaptation of the Russian version of the Personality Inventory for DSM-5 (PID-5), respecting the stages of intercultural adaptation specific to the medical, sociological and psychological fields.

Material and methods: The PID-5 questionnaire translated into Russian was used by 30 Russian-speaking subjects living in the Republic of Moldova that use English in the specialized activity. After a 30 minute break, all of these subjects were asked to fill out the original questionnaire in English.

Results: After comparing the answers to the 220 items, we obtained the following results: 26 persons, representing 86.7% of the total number of participants, responded identically to all 220 items, one person (3.3%) admitted only one difference in test responses, 3 persons (10.0%) admitted a different response in 3-4 items.

Conclusions: The result of the presented work is the Russian-language version of the PID-5 questionnaire, which proposes a methodical evaluation of the Russian speaking people with a mental health problem, the residents of the Republic of Moldova.

Key words: DSM-5, Alternative Model DSM-5 for Personality Disorders, PID-5, Russian version of PID-5.

Introduction

The Diagnostic and Statistical Manual of Mental Disorders (DSM) was published by the American Psychiatric Association (APA) to help the psychologist and psychiatrist in diagnosing people with mental health problems. The 5th edition of the DSM (DSM-5) is the latest version of this manual, and has included major and radical changes in the personality disorder (PD) diagnosis method, from categorical to dimensional one. The traditional categorical paradigm of PD described in the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV, 2000) or in the tenth edition of the International Classification of Diseases (ICD-10) have been thoroughly criticized both conceptually and psychometrically [1,2].

In the context of the DSM-5 research plan, experts from the American Psychiatric Association (APA) and the National Institute of Mental Health (NIMH) have set up working groups for research planning and drafting recommendations for future DSM editions. The Gaps Work Group analyzed 18 alternative proposals for a dimensional classification of Personality Disorder (PD). The conclusion was that most of these proposals have a common hierarchical structure with 4 to 5 top-level domains and 15 to 30 lower-level dimensions [3]. The authors argue that both normal personality and pathological personality could be integrated into a hierarchical model with two higher-order domains of internalizing and externalizing behaviors which corresponds to the general psychopathology model [4,5]. Finally, several authors analyzed the hierarchical structure of the traits using the method proposed by Goldberg, which is based on the estimation of a series of models of factors from a smaller number to an increasing number of factors [5], and the cross-model correlation is then used to estimate relationships between hierarchy levels. At the level of the two factors, Internalization and Externalization were expressed. At the level of the three factors, the Externalization behavior replicated while the Internalization behavior split into Detachment and Negative Affectivity. The fourth level was characterized by dividing the Externalization behavior into Disinhibition and Antagonism. Finally, at the fifth level, Detachment split into Detachment and Psychoticism [3,6,7,8].

Thus, the 5th edition of the Diagnostic and Statistical Manual of Mental Disorders includes this DSM-5 Alter-

native Model for Personality Disorder (AMPD) in Section III (Emerging Measures and Models) of DSM-5 [9]. This model explains that personality disorders are characterized by impairment in personality functioning and presence of pathological personality traits. This approach can also diagnose features specific for a personality disorder, which can be done when the personality disorder appears to be present but does not meet all of the above-mentioned criteria for personality disorder. All of these changes in the AMPD are still being evaluated by experts, and the model is called a dimensional-categorical hybrid model of personality disorders [10]. The dimensional approach could not fully replace the categorical approach, but an integration of them was attempted. The categorical approach of personality disorders determines the clinician to decide whether the disorder is present or absent. On the other hand, the dimensional approach allows the clinician to examine the severity of the disorder, and not to focus only on the threshold that indicates the presence of the disorder. Therefore, the dimensional approach can help the clinician to explain the disorders in a more comprehensive way.

This model, published in Section III (Emerging Measures and Models) DSM-5, includes a tool of dimensional assessment for maladaptive personality traits – Personality Inventory for DSM-5 (PID-5) [11].

The personality taxonomy in DSM-5 involves five high-order domains that are specified by the twenty-five lower-order facets described in DSM-5 [12,13]. These five areas are Negative Affectivity (frequent and intense experiences of negative emotions that manifest themselves in either behavioral or interpersonal relationships), Detachment (the tendency to avoid socio-emotional experience, including withdrawal from interpersonal interactions and restriction of experience and affective expression), Antagonism (behaviors that put the individual in disagreement with others), Disinhibition (tendency towards immediate satisfaction), and Psychoticism (presenting a wide range of strange, eccentric or unusual cultural behaviors and cognitions).

Review by Al-Dajani et al., published in 2016, confirms that many studies use this questionnaire, namely over 30 papers in 3 years after the publication of DSM-5 [9]. The psychometric properties of PID-5 have been illustrated in a series of studies associating the model of personality traits in DSM-5 with other well-known instruments in clinical practice, such as general characteristics of personality [14,15,16], alternative conceptions of maladaptive personality traits [17], pathological beliefs [18] and psychopathy [19,20].

Because the Krueger's study from 2012 was made on a sample of respondents with therapeutic interventions and psychiatric patients, several authors published evidence of PID-5 factor structure in both students and the general population [7,13,14]. It can be assumed that the distribution of PID-5 personality traits is different for patients and the general population in the prevalence, form and severity of psychopathology of personality [21].

It is very important that the hierarchical structure of the PID-5 inventory, which measures the pathology of the personality based on the Big Five, has been preserved in the translations into Indonesian, Italian, German, Danish, French, Czech, Spanish, Brazilian and Portuguese [6,10,20,22, 23,24,25,26,27,28]. Moreover, an approximately identical structure was found in a 100-item version and a short version of 25 items of Danish PID-5 [6]. Such international studies are important as it universalizes and generalizes the model of pathological personality traits.

As one of the attempts to develop the dimensional approach of personality disorder included in DSM-5 in the Republic of Moldova, the adaptation and validation of PID-5 in the Romanian version was carried out (article in printing). Since it is an important clinical tool that helps physicians to diagnose patients with personality disorder, it can be deduced that further testing of the validity of this tool is also required for Russian-speaking residents of the Republic of Moldova. Moreover, the widespread availability of this medical questionnaire in both languages would stimulate clinicians to use it.

The most recently postulated test of validity in psychometry was proposed by Messick [29]. It has been argued that all components of the validity methods can be explained by the validity of the construct. Validity is an evolving property of an instrument and validation is a dynamic process in progress [29,30,31]. It is therefore important for physicians to always ensure that the tools they have used are valid enough, since the interpretation of the tests will be based on the obtained scores and the diagnosis based on these interpretations has a direct impact on people's lives.

Material and methods

The current study consists in the validation and cultural adaptation of the Russian version of PID-5, respecting the stages of intercultural adaptation specific to the medical, sociological and psychological fields. The research methodology is presented in the next section [29,32,33].

Stage I: Initial translation

The first step in adaptation is translation in the perspective.

Two bilingual translators, whose mother tongue is the target language (Russian), produced the two independent translations. Translators have been professional, certified translators, as well as specialists with experience in mental health care and treatment (psychologist with training in assessment and psychodiagnosis and psychiatrist, both with psychotherapy training). Each one produced a written report that included some comments and suggestions. Additional comments were needed to highlight provocative phrases or uncertainties. Their conclusions were also summarized in a written report. The content element, answering options and instructions have all been translated in this way.

Stage II. Synthesis of translations

The translation of the questionnaire from source language into the target language was done, taking into account

the criterion of the degree of conceptual overlap between the source culture and the target culture. Conceptual overlap is given by the extent to which a concept has the same meaning in both languages [29,32,34]. The two translators, included in a Discussion Group, synthesized the translation results. Respecting the original protocol, a discussion group consisted of the two translators, plus other mental health specialists (neurologist, clinical psychologist, physiologist) with experience in translating from English. The two versions were confronted, and the differences were discussed in the group, so the first Russian version was completed.

The issues on which the discussions focused were related to both the content and the applicability of the contents of the items in the Russian socio-cultural context, as well as the language formulas [29, 32].

Stage III. Performing the retroversion

Retroversion is a process to assess validation that highlights gross expressions or conceptual errors in translation, necessary to ensure that the translated version reflects the same content element as the original version. This step often excludes unclear wording in translations. Retroversion was carried out by two authorized professional translators who translated the combined version (stage II) back to English, and then the authors checked whether there were differences of interpretation and discrepancies between the two variants. Subsequent changes were made with the agreement of both parties. Comparison of the retroversion with the original version led to a second revision of the material and the list of problematic items. However, the similarity between the retroversion and the original version does not guarantee a satisfactory translation; it simply provides a consistent translation [32]. Retroversion is only a type of assessment of validation by increasing the probability of "highlighting the imperfections" [33].

Results

Stage IV. Validity testing

To determine the fidelity indicators of the questionnaire, the internal consistency of the inventory was analyzed. We have calculated the internal coefficient Cronbach's alpha, which measures the extent to which the indices that make up a scale are intercorrelated. For a proper correlation of indices, a value of at least 0.7 of C-alpha (5) is required. Table 1 shows the results of internal consistency for the Russian version of PID-5. Following these results, namely – 0.931 in men and 0.928 in women – we can conclude that the scale is true. Cronbach's alpha is dependent on the number of inventory items, and in this case we have a very high coefficient.

Table 1

Cronbach's alpha for PID-5

	Cronbach's alpha	Nr. of items
Men	0.931	220
Women	0.928	

Subjects. The development of the study implied the application of the PID-5 questionnaire translated into Russian to a number of 30 Russian-speaking subjects (tab. 2) living in the Republic of Moldova, that use English in the specialized activity (clinical context – interviewing the patient, training programs in English), as well as in creating and editing materials in English (research papers, financing projects). After a 30 minute break, all of these subjects were asked to fill out the original questionnaire in English.

Table 2

Demographic data of the participants

Age, years				
	Nr.	Min	Max	Mean ± SD
Women	21	28	57	22.61±0.55
Men	9	32	51	22.34±0.92

After comparing the answers to the 220 items, we obtained the following results: 26 persons, representing 86.7% of the total number of participants, responded identically to all 220 items, one person (3.3%) admitted only one difference in test responses, 3 persons (10.0%) admitted a different response in 3-4 items. In the next step, each item which had different answer in the test /repeated test was analyzed separately and difference (in points) was calculated.

Conclusions

The procedure described in this article included translation, retroversion, validation and the cultural adaptation of the Russian-language version of the PID-5 questionnaire. The result of the presented work is the Russian-language version of the PID-5 questionnaire, which proposes a methodical evaluation of the Russian speaking people with a mental health problem, the residents of the Republic of Moldova.

Performing translation, with the assurance of conceptual overlap, is the phase which precedes the stability test which is calculation of Cronbach' alpha of internal consistency (inter-items correlation). The obtained results demonstrate that this translation provides sufficient consistency and validity to be used in future studies, also it makes possible to reliably use the translated tool to evaluate the individual differences and personality traits. The results also impose to continue studies in more representative groups, focusing on the clinical cases where personality disorders prevail.

References

1. Ryder AG, Costa PT, Bagby M. Evaluation of the SCID II Personality Disorder Traits for DSM-IV: coherence, discrimination, relations with general personality traits, and functional impairment. J Pers Disord. 2007;21:626-37.
2. Trull T, Durrett CA. Categorical and dimensional models of personality disorder. Ann Rev Clin Psychol. 2005;1:355-80.
3. Widiger TA, Simonsen E. Alternative dimensional models of personality disorder: finding a common ground. J Pers Disord. 2005;19(2):110-30.
4. Widiger TA, Costa PT. Integrating normal and abnormal personality structure: the Five'Factor Model. J Pers. 2012;80(6):1471-1506.

5. Goldberg LR. Doing it all Bass-Ackwards: the development of hierarchical factor structures from the top down. J Res Pers. 2006;40:347-58.
6. Bo S, Bach B, Mortensen EL, Simonsen E. Reliability and hierarchical structure of DSM-5 pathological traits in a Danish mixed sample. J Pers Disord. 2016;30(1):112-29.
7. Wright AGC, Thomas KM, Hopwood CJ, Markon KE, Pincus AL, Krueger RF. The hierarchical structure of DSM-5 pathological personality traits. J Abnorm Psychol. 2012;121(4):951-7.
8. Krueger RF. The structure of common mental disorders. Arch Gen Psychiatry. 1999;56(10):921-6.
9. Al-Dajani N, Gralnick TM, Bagby RM. A psychometric review of the Personality Inventory for DSM-5 (PID-5): current status and future directions. J Pers Assess. 2016;98(1):62-81.
10. Adhiatma W, Hendrianti J. The convergent validity of Indonesian version of personality inventory for DSM-5 (PID-5). J Psikologi (Indonesia). 2018;17(2):97-106.
11. Krueger RF, Derringer J, Markon KE, Watson D, Skodol AE. Initial construction of a maladaptive personality trait model and inventory for DSM-5. Psychol Med. 2012;42:1879-1890.
12. Krueger RF, Eaton NR, Clark LA, Watson D, Markon KE, Derringer J, et al. Deriving an empirical structure of personality pathology for DSM-5. J Pers Disord. 2011;25:170-191.
13. Fossati A, Krueger RF, Markon KE, Borroni S, Maffei C. Reliability and validity of the personality inventory for DSM-5 (PID-5): predicting DSM-IV personality disorders and psychopathy in community-dwelling Italian adults. Assessment. 2013;20(6):689-708.
14. Gore WL, Widiger TA. The DSM-5 dimensional trait model and five-factor models of general personality. J Abnorm Psychol. 2013;122:816-821.
15. Hopwood CJ, Thomas KM, Markon KE, Wright AGC, Krueger RF. DSM-5 personality traits and DSM-IV personality disorders. J Abnorm Psychol. 2012;121:424-432.
16. De Fruyt F, De Clercq B, De Bolle M, Wille B, Markon K, Krueger RF. General and maladaptive traits in a five-factor framework for DSM-5 in a university student sample. Assessment. 2013;20(3):295-307.
17. Watson D, Stasik SM, Ro E, Clark LA. Integrating normal and pathological personality: relating the DSM-5 trait-dimensional model to general traits of personality. Assessment. 2013;20(3):312-326.
18. Hopwood CJ, Wright AGC, Krueger RF, Schade N, Markon KE, MoreyLC. DSM-5 pathological personality traits and the Personality Assessment Inventory. Assessment. 2013;20(3):269-285.
19. Strickland CM, Drislane LE, Lucy M, Krueger RF, Patrick CJ. Characterizing psychopathy using DSM-5 personality traits. Assessment. 2013;20(3):327-338.
20. Riegel KD, Ksinan AJ, Samankova D, Preiss M, Harsa P, Krueger RF. Unidimensionality of the Personality Inventory for DSM-5 facets: evidence from two Czech-speaking samples. Personal Ment Health. 2018;12(4):281-297.
21. Bastiaens T, Claes L, Smits D, De Clercq B, De Fruyt F, Rossi G, et al. The construct validity of the Dutch Personality inventory for DSM-5 personality disorders (PID-5) in a clinical sample. Assessment. 2015;23(1):42-51.
22. Lugo V, de Oliveira SES, Hessel CR, Monteiro RT, Pasche NL, Pavan G, et al. Evaluation of DSM-5 and ICD-11 personality traits using the Personality Inventory for DSM-5 (PID-5) in a Brazilian sample of psychiatric inpatients. Personal Ment Health. 2019;13(1):24-39.
23. Roskam I, Galdiolo S, Hansenne M, Massoudi K, Rossier J, Gicquel L, et al. The psychometric properties of the French version of the Personality Inventory for DSM- 5. PLoS ONE. 2015;20;10(7):e0133413.
24. Zimmermann J, Altenstein D, Krieger T, Holtforth MG, Pretsch J, Alexopoulos J. The structure and correlates of self-reported DSM-5 maladaptive personality traits: findings from two German-speaking samples. J Pers Disord. 2014;28(4):518-40.
25. Riegel K. [Personality inventory for DSM-5: PID-5]. Prague: Hogrefe-Testcentrum. 2015; p. 12-14. Czech.
26. Thimm JC, Jordan S, Bach B. The Personality Inventory for DSM-5 Short Form (PID-5-SF): psychometric properties and association with big five traits and pathological beliefs in a Norwegian population. BMC Psychol. 2016;4(1):61.
27. Pires R, Sousa Ferreira A, Guedes D, Gonçalves B, Henriques-Calado J. [A study of the psychometric qualities of the Portuguese version of the Personality Inventory for DSM-5 (PID-5): full version, reduced form and brief form]. Revista Iberoamericana de Diagnostico y Evaluacion Psicologica. 2018;47(2):197-212. Portuguese.
28. Aluja A, García LF, Cuevas L, Lucas I. Dimensional pathological personality predicting personality disorders: comparison of the DAPP-BQ and PID-5 shortened versions in a Spanish community sample. J Psychopathol Behav Assess. 2019;41(1):160-173. Epub 2018 November 9.
29. Brown T. Construct validity: a unitary concept for occupational therapy assessment and measurement. Hong Kong J Occup Theory. 2010;20(10):30-42.
30. Cronbach LJ. Construct validation after thirty years. In: Linn RL, editor. Intelligence: Measurement, theory, and public policy: Proceedings of a symposium in honor of Lloyd G. Humphreys. Urbana, IL: University of Illinois Press; 1989. p. 147-171.
31. Messick S. Validity of psychological assessment: validation of inferences from persons' and performances as scientific inquiry into score meaning. Am Psychol.1995;50(9):741-749.
32. Beaton DE, Bombardier C, Guillemin F, Ferraz MB. Guidelines for the process of cross-cultural adaptation of self-report measures. Spine. 2000;25(24):3186-3191.
33. Leplege A, Verdier A. The adaptation of health status measures. A discussionof certain methodological aspects of the translation procedure. In: Shumaker S, Berzon R, editors. The international assessment of health-related quality of life: theory, translation, measurement and analysis. Oxford, UK: Rapid Communication; 1995. p. 93-101.
34. Knudsen HC, Vázquez-Barquero JL, Welcher B, Gaite L, Becker T, Chisholm D, et al. Translation and cross-cultural adaptation of outcome measurements for schizophrenia. EPSILON Study 2. European Psychiatric Services: Inputs Linked to Outcome Domains and Needs. Br J Psychiatry. 2000;177(Suppl 39):s8-14.

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Distortion products of otoacoustic emissions and their role in assessing hearing loss in young children

Doina Chiaburu-Chiosa, MD, PhD Applicant

Department of Otorhinolaryngology, Nicolae Testemitsanu State University of Medicine and Pharmacy Chisinau, the Republic of Moldova

*Corresponding author: doinachiaburu@yahoo.com
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Abstract

Background: Hearing loss in children is far beyond the scope of otology, since audition is the basis of developing speech and cognitive abilities, as well as the child's personality. Due to its incidence and severe consequences that often lead to disability, hearing loss remains an acute issue for scholars and specialists of various fields.

Material and methods: There have been studied distortion products otoacoustic emissions in children from two groups: the control group included 30 children, aged between 1-36 months, with normal hearing; whereas the study group consisted of 110 children aged between 1-36 months with sensorineural deafness.

Rezults: We studied auditory distortion products (ADP) in the control group of children, where prior impedance had excluded any middle ear pathology, whereas the medical history data regarding the functional development of the auditory system and behavioral audiometry showed normal hearing. We studied ADP at frequencies of 500; 750; 1000; 1500; 2000; 3000; 4000 and 6000 Hz. The analysis of the obtained results revealed some particularities of the ADPs according to the tested frequencies. Thus, ADPs recording that explores 1000 Hz; 1500Hz; 2000Hz; 3000Hz; 4000Hz and 6000Hz frequencies showed no difficulty, being 100% recorded in all children within the control group. The background noise varied from – 10 dB SPL to – 20 dB SPL. Low frequencies were difficult to assess due to a significant environmental noise which in some cases was higher than the ADP amplitude.

Conclusions: As a result of ADP recording carried out in examined children, we conclude: the ADP recording, namely the "ADP audiogram" test, is an objective method with high sensitivity, which can be used in hearing screening in early childhood. The criterion for impaired hearing based on the "ADP audiogram" is the spectral interruption for frequencies higher than 1000 Hz.

Key words: distortion products, otoacoustic emissions, hearing loss, children.

Introduction

Hearing loss in children is far beyond the scope of otology, since audition is the basis of developing speech and cognitive abilities, as well as the child's personality. Due to its incidence and severe consequences that often lead to disability, hearing loss remains an acute issue for scholars and specialists of various fields. According to worldwide-specialized literature, the occurrence of this disorder remains quite common and differs from one source to another. Statistical data provided by the National Institute of Deafness and Other Communication Disorders (NIDaOCD) show that deafness occurs in 1-3 cases per 1,000 healthy newborns and in 2-4 cases per 100 newborns admitted to Neonatal Intensive Care Unit.

Distortion product otoacoustic emissions (DPOAEs) or auditory distortion products (ADP) reflect outer hair cell integrity and cochlear function. When used appropriately in the audiology clinic, they are an effective diagnostic tool and can detect hearing loss with accuracy. DPOAEs are easily and rapidly recorded in newborns and children, and provide basic hearing screening information as well as detailed diagnostic information in cases of suspected hearing loss. In the past decade, solid guidelines have been established to select the most effective recording parameters, thereby optimizing the DPOAE's diagnostic potential [1,2,3,4].

DPOAEs can be used effectively to diagnose or detect hearing loss in infants and children. Although they are technically not a measure of "hearing," they are correlated with hearing. As such, they are useful in the audiology clinic. Under good-to-excellent test conditions, the correlation is fairly straightforward: When DPOAEs are present and normal in amplitude and configuration, their presence indicates that the cochlear amplifier is normally functional. In the absence of neurological or isolated inner hair cell dysfunction, this result is consistent with normal hearing. When DPOAEs are absent, their absence indicates that there is some dysfunction in the cochlea, though the level of dysfunction and, thus, degree of the hearing loss is not clear. This reliable correlation between DPOAEs and hearing allows for the effective clinical application of this easily recorded response [5,6].

Purpose of the study is aimed at studying major characteristics of acoustic distortion products in children with normal hearing, as well as the diagnostic values of this method in assessing the hearing function in young children.

Material and methods

There have been studied distortion products otoacoustic emissions in children from two groups: the control group included 30 children, aged between 1-36 months, with normal hearing; whereas the study group consisted of 110 children aged between 1-36 months with sensorineural deafness (tab. 1).

Table 1

Distribution of patients by groups

The study group	The control group
110 children	30 children
With sensorineural hearing loss	With normal hearing
Age 1-36 months	Age 1-36 months

The distribution of patients by gender: 59.3% boys and 40.7% girls (tab. 2). Distortion products otoacoustic emissions have been studied on the following frequencies: 500; 750; 1000; 1500; 2000; 3000; 4000 and 6000 Hz.

Table 2

Distribution of patients by gender and age

Patients	%
Boys	59.3%
Girls	40.7%
Age (years)	1-36 months

Results

Our task was to highlight two main aspects: 1) basic properties of acoustic distortion products in children with normal hearing and "normal hearing" criteria based on ADPs; 2) diagnostic value of this method in assessment of hearing function in early childhood.

First, we studied ADPs in the control group of children, where prior impedance had excluded any middle ear pathology, whereas the medical history data regarding the functional development of the auditory system and behavioral audiometry showed normal hearing. We studied ADPs at frequencies of 500; 750; 1000; 1500; 2000; 3000; 4000 and 6000 Hz. The analysis of the obtained results revealed some particularities of the ADPs according to the tested frequencies (tab. 3).

Thus, ADPs recording that explores 1000 Hz; 1500Hz; 2000Hz; 3000Hz; 4000Hz and 6000Hz frequencies showed no difficulty, being 100% recorded in all children within the control group. The background noise varied from – 10 dB SPL to – 20 dB SPL. Low frequencies were difficult to assess due to a significant environmental noise which in some cases was higher than the ADP amplitude. This explains ADP recording that explores 500 Hz frequencies in only 29% and at 750 Hz in 70% of testing. ADPs that explore 500 Hz frequencies exhibited both a significant background noise and a lack of ADP (negative amplitude). The obtained results correspond to the data from P. Bonfils [7], which claim that ADP low frequency testing is not feasible due to a high background noise.

Table 3

Recording acoustic distortion products in tested children

DP 2 $F_1 - F_2$	Geometric mean of primary frequencies Hz	Incidence %		X^2	P
		Control Lot n=60	Basic Lot n=220		
353	500	20.00	23.64	0.354	> 0.05
529	750	70.00	47.73	9.378	<0.05
1058	1000	100.00	3.,64	238.075	<0.001
1413	1500	100.00	0.00	274.087	<0.001
1779	2000	100.00	0.00	274.087	<0.001
2116	3000	100.00	0.00	274.087	<0.001
2824	4000	100.00	0.00	280.000	<0.001
4232	6000	100.00	0.00	274.087	<0.001

The result analysis of the ADP amplitude indicates some of its properties depending on the tested frequencies (tab. 4). Thus, for 500 Hz; 750 Hz 4000 Hz; 6000 Hz frequencies, the PDA's amplitude was higher, and decrease to 1000 Hz showing the lowest value. Frequencies of 1500 Hz; 2000 Hz and 3000 Hz are close, due to their amplitude values.

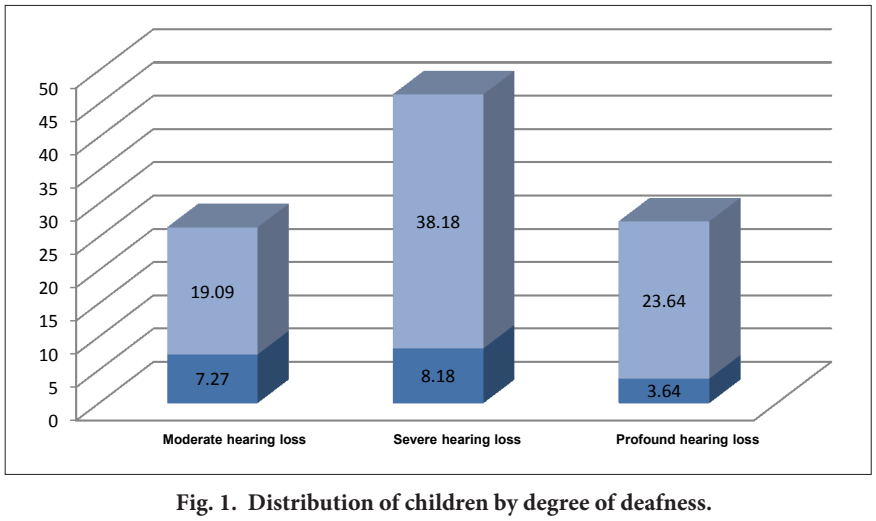

Fig. 1. Distribution of children by degree of deafness.

Table 4

Amplitude of acoustic distortion products in children with normal hearing

N/o	DP $2F_1 - F_2$	Geometric mean of F1 and F2 Hz	M	±m
1	353	500	15.83	1.63
2	529	750	10.48	0.55
3	1058	1000	6.93	0.48
4	1413	1500	9.93	0.48
5	1779	2000	9.27	0.46
6	2116	3000	8.16	0.52
7	2824	4000	11.91	0.51
8	4232	6000	14.61	0.65

Therefore, an "ADP audiogram" with positive amplitude values at frequencies of 750; 1000; 1500; 2000; 3000; 4000 and 6000 Hz indicates a normal hearing. The predominant background noise over the ADPs at frequencies of 500Hz and, in some cases of 750Hz, is not a criterion for impaired hearing. In our opinion, the lack of ADPs at low frequencies, if they are present on the frequency route of 1000; 1500; 2000; 3000; 4000 and 6000 Hz, is more related to some technical difficulties, since the ADPs cannot be selected from the environmental noise.

The ADP's amplitude revealed higher values at 500 Hz, 750 Hz, 4000 Hz and 6000 Hz frequencies, and a decrease up to 1000 Hz, showing minor significance. 1500 Hz; 2000 Hz and 3000 Hz frequencies remain almost the same in their amplitude values. An ADP "audiogram" with positive amplitude values at 750, 1000; 1500; 2000; 3000; 4000 and 6000 Hz frequencies indicates normal hearing.

ADP recording results in children from the control group reveal an ADP absence at frequencies above 1000 Hz, which graphically appears as an amplitude spectrum within the ADP "audiogram" (negative values of amplitude) – scotoma (fig. 2).

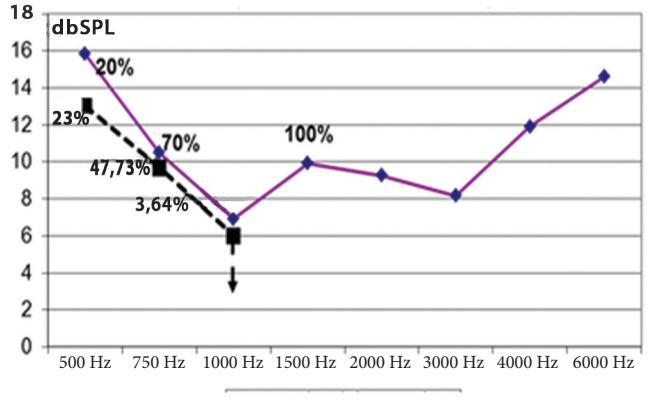

Fig. 2. The PD-gram (the mean amplitude and incidence recording).

The obtained data showed 100% absence of ADP at 1500; 2000; 3000; 4000 and 6000 Hz frequencies. ADPs which assess 750 Hz frequencies were present in 47.73% of cases; and 500 Hz in 23.67% of cases. ADP was even detected in assessing 1000 Hz frequencies in 3.64% of minimum cases. Therefore, the analysis of the obtained results in the ADP "audiograms" recording in the control group reveals lack of ADP in all children with sensorineural deafness.

Thus, ADP recording can be used to test hearing function in children since the early days of their lives. According to our research, sensitivity of ADP recording method is 81.9% and specificity is 99.5%.

Once the ADP properties and the basic criteria for the normal hearing based on the "ADP audiogram" recording had been determined in children from the control group, the ADP study was carried out in deaf children. The results of ADP recording in children from the basic group show the lack of ADPs at higher frequencies of 1000 Hz. The "DP audiogram" is displayed graphically as a spectral interruption (negative amplitude values) – scotoma.

According to the obtained data, ADPs at 1500; 2000; 3000; 4000 and 6000 Hz frequencies were absent in 100% (220 ears). However, it is worth mentioning that low frequency testing was possible in some deaf children. Thus, ADPs exploring at 750 Hz frequencies were present in 47.73% (105 ears), at 500 Hz in 23.67% (52 ears), and even at frequencies of 1000 Hz in 3, 64% (8 ears).

This could be explained by a better hearing condition at frequencies where ADPs are present, as in our cases – at the lowest frequencies, which is normal for sensorineural deafness. Therefore, the obtained result analysis based on the "ADP audiogram" recording in the basic group shows lack of ADPs in all children with sensorineural hearing loss.

Our research data confirm the existing literature findings that acoustic distortion products are present in all cases of normal hearing and absent in cases of sensorineural deafness [8,9,10,11,12,13].

Therefore, based on the ADP recording, we can identify if the child hears or not, even from the first months of life. According to our data, sensitivity of the ADP recording method is 81.9% and specificity is 99.5%.

Conclusions

As a result of ADP recording carried out in examined children, we conclude that the ADP recording, namely the "ADP audiogram" test, is an objective method with high sensitivity, which can be used in hearing screening in early childhood. The criterion for impaired hearing based on the "ADP audiogram" is the spectral interruption for frequencies higher than 1000 Hz.

Acoustic distortion products are present in all cases of normal hearing, whereas the "ADP audiogram" is being marked at frequencies of 750; 1000; 1500; 2000; 3000; 4000; 6000 Hz. In case of hearing loss, the "ADP audiogram" shows a high-frequency scotoma. As an objective method

with a 81.9% sensitivity and 99.5% specificity, the "ADP audiogram" recording can be used in pediatric audiology as a screening method.

References

1. Abdala C. Distortion product otoacoustic emissions: a tool for hearing assessment and scientific study. Volta Rev. 2001 Spring;103(4):281-302. PubMed PMID: 23559685.
2. Abdala C. A longitudinal study of DPOAE ipsilateral suppression and input/output characteristics in human neonates. J Acoust Soc Am. 2003;114(6 Pt 1):3239-50. PubMed PMID: 14714805.
3. Abdala C, Fitzgerald T. Ipsilateral distortion product otoacoustic emission (2f1-f2) suppression in children with sensorineural hearing loss. J Acoust Soc Am. 2003;114(2):919-931. PubMed PMID: 12942973.
4. Brown A, McDowell B, Forge A. Effects of chronic gentamicin treatment on hair cells can be monitored using acoustic distortion products. Hear Res. 1989;42(2-3):143-156. PubMed PMID: 2606800.
5. Abdala C, Chatterjee M. Maturation of cochlear nonlinearity as measured by DPOAE suppression growth in humans. J Acoust Soc Am. 2003;114(2):932-943. PubMed PMID: 12942974.
6. Lasky R. Distortion product otoacoustic emissions in human newborns and adults. I. Frequency effects. J Acoust Soc Am. 1998;103(2):981-991. PubMed PMID: 9479751.
7. Bonfils P, Dumont A, Marie P, Francois M, Narcy P. Evoked otoacoustic emissions in newborn hearing screening. Laryngoscope. 1990;100(2 Pt 1):186-9. PubMed PMID: 2299961.
8. Howard M, Stagner B, Lonsbury-Martin B, Martin G. Effects of reversible noise exposure on the suppression tuning of rabbit distortion-product otoacoustic emissions. J Acoust Soc Am. 2002;111(1 Pt 1):285-296. PubMed PMID: 11831802.
9. Lasky R. Distortion product otoacoustic emissions in human newborns and adults. II. Frequency effects. J Acoust Soc Am. 1998;103(2):992-1000. PubMed PMID: 9479752.
10. Norton S, Gorga M, Widen J, Folsom R, Sininger Y, Cone-Wesson B, et al. Identification of neonatal hearing impairment: evaluation of transient evoked otoacoustic emission, distortion product otoacoustic emission, and auditory brain stem response test performance. Ear Hear. 2000;21(5):508-528. PubMed PMID: 11059707.
11. Abdala C, Visser-Dumont L. Cochlear function in older infants. Hear Rev. 2003;10:16-22.
12. Brown A, Kemp D. Suppressibility of the 2fl-f2 stimulated acoustic emissions in gerbil and man. Hear Res. 1984;13(1):29-37. PubMed PMID: 6706860.
13. Gorga M, Neely S, Dorn P. Distortion product otoacoustic emission test performance for a prior criteria and for multifrequency audiometric standards. Ear Hear. 1999;20(4):345-62. PubMed PMID: 10466570.

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Prevalence of primary headaches in adolescents

Tatiana Lozan, MD, MPH, PhD Applicant

Functional Neurology Scientific Laboratory, Institute of Neurology and Neurosurgery
Chisinau, the Republic of Moldova

Corresponding author: lozan.tatiana@gmail.com
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Abstract

Background: The aim of this study was to estimate overall prevalence of primary headaches and prevalence of migraine (MG) and tension-type headache (TTH) among adolescents in the Republic of Moldova.
Metetial and methods: In total there were 3389 adolescents whose age ranged from 10 to 19 years, recruited from urban and rural areas of the country. This school-based study was conducted during the academic year 2015-2016. The information was collected with the use of self-administered questionnaire based on the criteria of International Classification of Headache Disorders: ICHD-2 (2004) and ICHD-3 (2013). Primary headaches were classified, according to the type of headache and after the frequency of headache attacks within the month.
Results: The overall prevalence of primary headaches in Moldovan adolescents is 38.75% (girls – 49.7%, boys – 27.8%), and it is higher in urban area (48.23%) than in rural (30.05%). The prevalence of MG is 19.7%. The prevalence of MG is higher in girls (27.5%) than in boys (12.1%). The prevalence of migraine is higher in urban adolescents (27.1%) compared to rural ones (13.0%). The prevalence of TTH is 7.9%. The prevalence of TTH is almost equal in both sexes (8.0% in girls and 7.7% in boys). The prevalence of TTH in urban adolescents is 10.2% and it is more than 1.7 times higher compared to the recorded level in rural areas – 5.8%.
Conclusions: The present study is the first Moldovan survey on epidemiology of headaches in adolescents. It is very important to continue developing different aspects of epidemiology of adolescents' headaches in the Republic of Moldova.

Key words: prevalence, headache, adolescents, migraine, tension-type headache.

Introduction

Epidemiology of primary headaches among adolescents is an important scientific subject. The number of epidemiological studies in primary headaches in adolescents increased considerably in the last years. Estimation of overall prevalence of primary headaches and prevalence for every type of migraine (MG) and tension-type headache (TTH) is important for measurement of their impact in adolescence. Overall prevalence of primary headaches in adolescence ranges from 21% to 91% [1, 5]. Reported range of migraine prevalence in adolescents is 6.3% – 21.3% [1, 2]. Reported range for tension-type headache prevalence is 5.1% – 25.9% [10, 12]. Epidemiology of primary headaches at the national level in adolescents in the Republic of Moldova was studied for the first time. The study of epidemiological particularities of primary headaches in adolescents was performed for estimating the general prevalence of headaches, specific prevalence for each type (migraine and tension-type headache) and prevalence for each subtype of MG and TTH (infrequent episodic migraine, frequent episodic migraine, chronic migraine, rare episodic tension-type headache, frequent episodic tension-type headache, and chronic tension-type headache) by age, gender, geographical area, and residence area.

Material and methods

Methods of selecting and designing the study sample

Current scientific research is a descriptive epidemiological study. The sample for this research was composed of 3389 teenagers aged 10–19 years and is representative for the adolescent population in the Republic of Moldova. The group of respondents was stratified as it is shown in table 1. Urban area was represented by two big cities Chisinau and Balti. Rural area was divided into the North, Center and South and was represented by small towns from each area.

The study sample was divided into the clusters. As a cluster served a medium size school class (30 students). The total number of clusters constituted 112. The clusters have been randomly selected from the list of educational institutions. Taking into account the probable variations in the

Table 1

Distribution of the sample by demographic criteria

Urban (N = 1706)				Rural (N = 1683)			
Males (N = 785)		Females (N = 920)		Males (N = 728)		Females (N = 955)	
10-14 y	15-19y	10-14y	15-19y	10-14y	15-19y	10-14y	15-19y
(N = 408)	(N = 375)	(N = 447)	(N = 473)	(N = 424)	(N = 294)	(N = 426)	(N = 525)

number of adolescents in the classes, 3600 questionnaires were originally distributed, out of which 3389 were validated, which constituted the volume of the research sample.

Methods of collecting information

This school-based study was conducted during the academic year 2015–2016, avoiding the period of tests, theses or examinations. Data for the research was collected using a self-administered, complex, structured questionnaire, based on the International Classification of Headache Disorders (ICHD). ICHD–II (2004) and ICHD–III (2013) beta criteria, comprised 7 chapters, with a total of 56 questions [2, 4, 7]. Clinical issues of headache were evaluated in Chapter III of the questionnaire titled "Headache in Adolescents", which contains questions that reflect the criteria for the diagnosis of MG and TTH and their forms in strict accordance with ICHD-2 (2004). Headache was assessed using the ICHD-2 (2004) and ICHD-3 (2013) beta versions, so that the minimum acceptable duration was 1–2 hours for adolescents aged up to 18 years and 4 hours for those of 19 years old [2, 4, 8]. At the time the students filled in the self-administered questionnaire, they were supervised by the school psychologist and / or the supervisor. This procedure has ruled out the induction of answers. After completing, 3389 questionnaires were verified and validated. At the end of the "General Data" section, adolescents were asked to answer the question "Have you had headaches in the last half year that were not related to influenza or head trauma", which was an exclusion criterion in survey for respondents, who answered "NO" (N = 1903). "YES" questionnaires (N = 1486) were divided, according to diagnosis, into 7 groups: PM (N=393), IEM (N=329), FEM (N=321), CM (N=115), ITTH (N=239), FTTH (N=69), CTTH (N=12) (fig. 1).

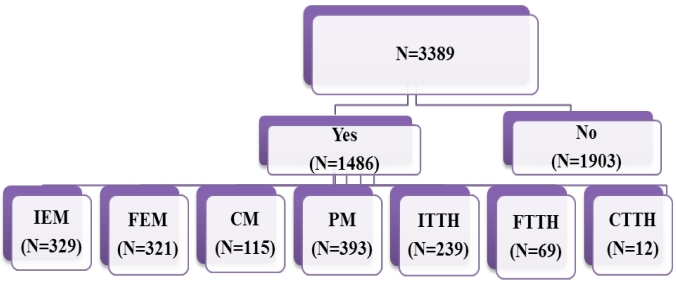

Fig. 1. Distribution of validated questionnaires.

The analysis of the descriptive data was performed by calculating averages and standard deviations in percent, with the 95% confidence interval for the continuous quantitative variables and ordinal variables. For the examination of associations between the category variables, the X^2-test was applied. The statistical analysis was performed by applying IBM SPSS Statistics 22.

Results and discussion

Overall and specific prevalence of primary headaches in adolescents

The general prevalence of primary headaches in adolescents in the Republic of Moldova was estimated 38.75% and it is comparable to the values presented in the literature – from 21% to 91% [1]. The general prevalence of primary headache is significantly higher (p<0.001) in girls than in boys (fig. 2).

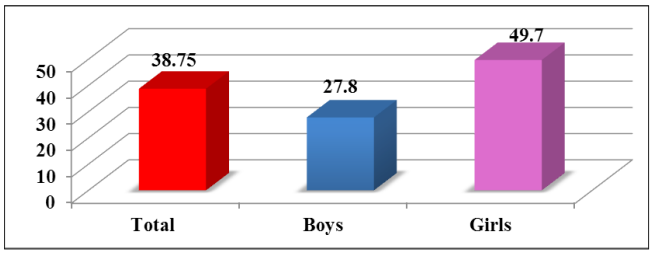

Fig. 2. General prevalence of primary headaches in adolescents by gender (%).

Primary headache prevalence values increased from 31.2% at 10 years to 61.3% at 19 years with a significant difference in values (p <0.001).

Analysis of the prevalence according to the residence environment indicates that the rural adolescents suffer from headaches 1.6 times less compared to urban adolescents (p<0.001), (fig. 3).

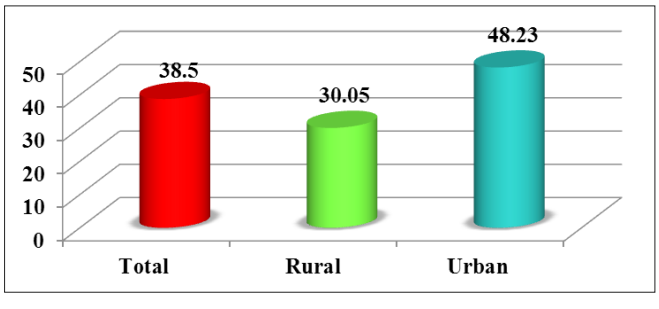

Fig. 3. General prevalence of primary headaches in adolescents depending on the residence environment (%).

Considering the geographical areas of the Republic of Moldova – North, South and Center, there was not detected significant difference in the prevalence values of primary headache in adolescents (p> 0.05%).

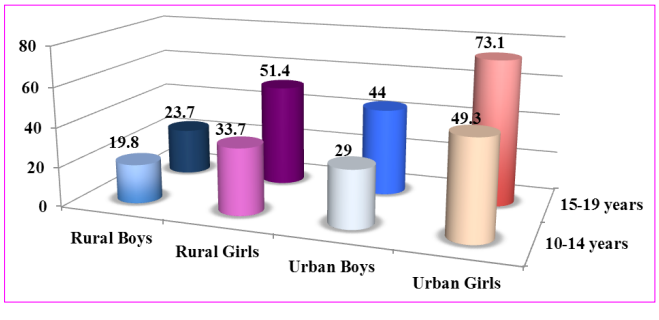

Fig. 4. Primary prevalence of primary headaches in adolescents by residence area, age groups and gender (%).

In both rural and urban areas, by gender and age groups, the overall prevalence of primary headaches is lower at the age of early adolescence (10-14 years) compared to late ado-

lescence (15-19 years) in both sexes (p<0.001). During the adolescence period, the prevalence of primary headaches increases 1.5 times in both sexes, both in rural and urban areas, with higher values after 14 years of age. The lowest prevalence was 19.8% in 10-14 year old male adolescents in the rural area, and the overall prevalence of primary headaches of 73.1% was estimated in urban female adolescents aged between 15 and 19 years (fig. 4).

Structure of morbidity through primary headaches in adolescents

Primary headaches were classified, according to the type of headache, in MG and TTH. After the frequency of headache attacks within the month, in the form: infrequent – up to 4 days / month, frequent 5 – 14 days / month and chronic – more than 15 days per month. As a result, migraine was classified as infrequent episodic migraine (IEM), frequent episodic migraine (FEM), chronic migraine (CM). Episodic migraine was not classified in migraine with aura and migraine without aura, both forms being analyzed simultaneously. Probable migraine (PM) was defined as headache, which corresponds to all diagnostic criteria, except one for migraine with or without aura, stipulated in ICHD–III (2013) beta [4]. TTH was classified as infrequent episodic tension-type headache (ITTH), frequent episodic tension-type headache (FTTH), and chronic tension-type headache (CTTH).

The MG is most common type of headache in the adolescents in the Republic of Moldova with a prevalence of 19.7% (PM is 11.1%) and is higher than the median (8%) reported in the previous epidemiological studies [1, 10, 13]. The prevalence of TTH is 7.9% and it tends to be lower comparing to reported range 5.3% –25.9% in other research [5, 6, 9], (fig. 5).

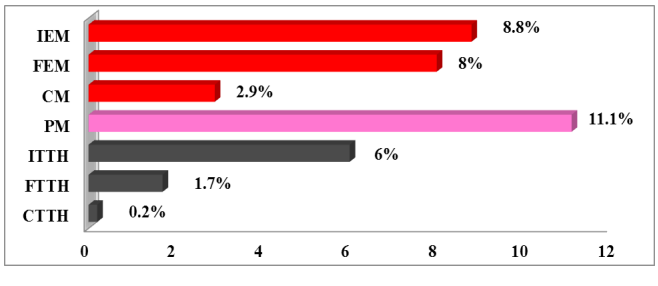

Fig. 5. Prevalence of primary headaches among adolescents in the Republic of Moldova.

In boys, MG prevalence is 12.1%, with a higher rate compared to the prevalence of TTH– 7.7%. In girls, the prevalence of MG is 27.5% and it is higher than TTH prevalence of 8.0%. Migraine, being the predominant type of primary headache in adolescents in our country, affects girls more frequently, having the 2.3 – fold higher prevalence compared to the prevalence of MG in boys. This difference is significant (p <0.001) for all forms of MG, both episodic and chronic. PM in adolescent girls also is higher than in boys. The prevalence of TTH is almost equal in both sexes (fig. 6).

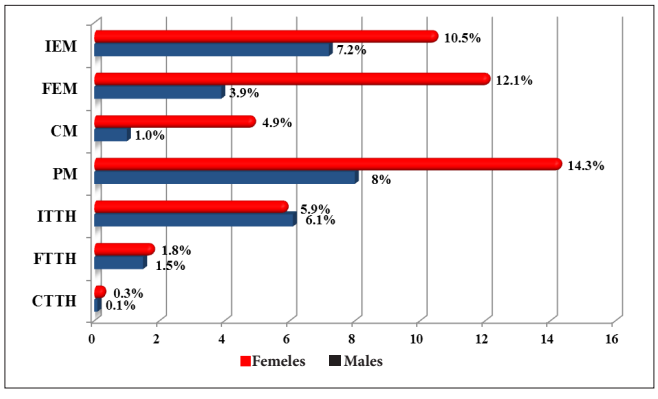

Fig. 6. Gender difference in prevalence of types and subtypes of headache in the RM.

The prevalence of migraine increases with age in both sexes, from 14.8% in early adolescence (10–14 years) to 28.2% in late adolescence (15–19 years). In girls up to 14 years, migraine prevalence is 20.2% (PM – 13.4%), and it is 38.5% (PM – 15.5%) in late adolescence. The prevalence of migraine in boys increases from 9.7% (PM – 7.9%) at the age of early adolescence, up to 16.2% (PM – 8.3%) in late adolescence. During the early adolescence, the infrequent form of episodic migraine affects both boys and girls equally, while frequent and chronic forms are found predominantly among girls. During late adolescence migraine in all forms of its manifestation affects girls more frequently, the gender difference being significant (p <0.001).

At the age of 10–14, the prevalence of tension-type headache for both sexes is 6.2%, which increases to the age of 15–19 to 10.8%. This increases in boys from 5.9% at early adolescent age to 11.2% in late adolescence. In girls, the prevalence of tension-type headache is 6.6% at 10–14 years of age and it increases in late adolescence to 10.4%. The level of tension-type headache among both genders is similar in both age groups, with no statistically significant difference between prevalence values. By recording low prevalence rates, both in early adolescence and in late adolescence, this form of headache is more characteristic of the adult age (tab. 2).

Depending on the residence environment, the prevalence of MG is 2 times higher in urban adolescents – 27.1% compared to rural ones –13.0%. The prevalence of TTH in urban adolescents is 10.2% and is more than 1.7 times the recorded level in rural areas – 5.8%. Overall, the prevalence of tensional headache results in lower values compared to migraine prevalence (fig. 7).

The prevalence of MG in urban adolescents increases with age from 19.8% in early adolescence, up to 35.7% in late adolescence. In rural adolescents, there is also an increase in the prevalence of migraine, from 11.1% at 10-14 years to 16.8% at 15 - 19 years, but its values are lower compared to the prevalence of migraine in urban adolescents.

Similar to migraine, the prevalence of tension-type headache increases among adolescents in both urban and rural areas. In the urban area it increases from 8.2% during early adolescence to 12.6% in late adolescence. In rural areas

Table 2

The difference between the prevalence values of primary headaches by age group, gender and diagnosis (p <0.001)

	10–14 years		Total	15–19 years		Total	Total		Total
	Males	Females		Males	Females		Males	Females	
W/A headache	846	616	1462	371	233	604	1217	849	2066
	76.4%	59.8%	68.4%	64.2%	35.5%	48.9%	72.2%	50.4%	61.3%
PM	88	138	226	48	102	150	136	240	376
	7.9%	13.4%	10.6%	8.3%	15.5%	12.2%	8.1%	14.2%	11.2%
IEM	67	78	145	54	96	150	121	174	295
	6.0%	7.6%	6.8%	9.3%	14.6%	12.2%	7.2%	10.3%	8.7%
FEM	34	102	136	30	102	132	64	204	268
	3.1%	9.9%	6.4%	5.2%	15.5%	10.7%	3.8%	12.1%	7.9%
CM	7	28	35	10	55	65	17	83	100
	0.6%	2.7%	1.6%	1.7%	8.4%	5.3%	1.0%	4.9%	3.0%
ITTH	48	53	101	54	47	101	102	100	202
	4.3%	5.1%	4.7%	9.3%	7.2%	8.2%	6.0%	5.9%	6.0%
FTTH	18	13	31	9	18	27	27	31	58
	1.6%	1.3%	1.4%	1.6%	2.7%	2.2%	1.6%	1.8%	1.7%
CTTH	0	2	2	2	3	5	2	5	7
	0.0%	0.2%	0.1%	0.3%	0.5%	0.4%	0.1%	0.3%	0.2%
TOTAL	1108	1030	2138	578	656	1234	1686	1686	3389
	100%	100%	100%	100%	100%	100%	100%	100%	100%

Table 3

Difference between primary headache prevalence values by age groups, home environment, and type (p <0.001)

	10-14 years			15-19 years			Total		
Headache type	Rural	Urban	Total	Rural	Urban	Total	Rural	Urban	Total
No headache	923	539	1462	305	299	604	1228	838	2066
	73.5%	61.2%	68.4%	61.7%	40.4%	48.9%	70.2%	51.7%	61.3%
PM	130	95	225	66	84	150	196	179	375
	10.4%	10.8%	10.5%	13.4%	11.3%	12.1%	11.2%	11.0%	11.1%
IEM	71	74	145	38	112	150	109	186	295
	5.7%	8.4%	6.8%	7.7%	15.1%	12.1%	6.2%	11.5%	8.8%
FEM	56	80	136	30	102	132	86	182	268
	4.5%	9.1%	6.4%	6.1%	13.8%	10.7%	4.9%	11.2%	8.0%
CM	14	20	34	15	51	66	29	71	100
	1.1%	2.3%	1.6%	3.0%	6.9%	5.3%	1.7%	4.4%	3.0%
ITTH	49	52	101	33	68	101	82	120	202
	3.9%	5.9%	4.7%	6.7%	9.2%	8.2%	4.7%	7.4%	6.0%
FTTH	12	19	31	6	21	27	18	40	58
	1.0%	2.2%	1.5%	1.2%	2.8%	2.2%	1.0%	2.5%	1.7%
CTTH	1	1	2	1	4	5	2	5	7
	0.1%	0.1%	0.1%	0.2%	0.5%	0.4%	0.1%	0.3%	0.2%
Total	1256	880	2136	494	741	1235	1750	1621	3389
	100%	100%	100%	100%	100%	100%	100%	100%	100%

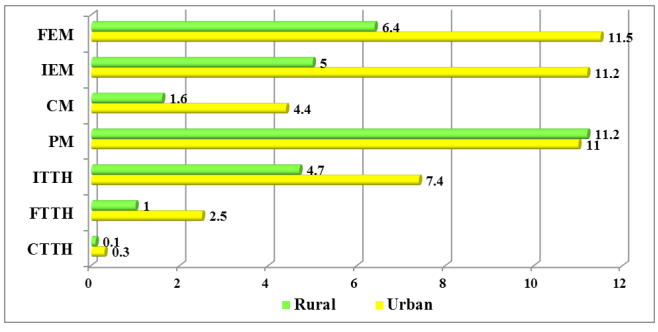

Fig. 7. Specific prevalence of primary headaches by residence environment (%).

the prevalence of tension-type headache at 10-14 years old is 5% and increases to the age of 15-19 years to 8.1%. For the North, Center and South geographic areas, there were not statistically significant differences in prevalence values for both migraine and tensional headaches, and for their forms (tab. 3).

Conclusions

1. The overall prevalence of primary headaches in adolescents in the Republic of Moldova is 38.75%. The prevalence of primary headaches in girls is 1.7 times higher than in boys. Adolescents in rural areas are 1.6 times less likely to have primary headaches than urban adolescents.

2. The prevalence of definite migraine is 19.7% (IEM – 8.8%, FEM – 8.0%, CM – 2.9%, PM-11.1%). The prevalence of migraine in girls is 2.3 times higher than the prevalence of migraine in boys. The prevalence of migraine increases in both sexes from 14.8% in early adolescence to 28.2% in late adolescence. Depending on the residence environment, the prevalence of migraine is 2 times higher in urban adolescents than in rural ones.

3. The prevalence of TTH is 7.9% (ITTH – 6.0%, FTTH – 1.7%, CTTH – 0.2%). The prevalence of tensional headache is almost equal in both sexes (8.0% in girls and 7.7% in boys). The prevalence of TTH is similar in both genders in both age groups, with no statistically significant difference between prevalence values. The prevalence of TTH in urban adolescents is 1.7 times higher than in adolescents in rural areas.

4. The present research is the first Moldovan survey on epidemiology of primary headaches in adolescents and it is very important to continue studying different aspects of epidemiology of adolescents headaches in the Republic of Moldova.

References

1. Abu-Arafeh I, Razak S, Sivaraman B, et al. Prevalence of headache and migraine in children and adolescents: a systematic review of population-based studies. Dev Med Child Neurol. 2010;52(12):1088-1097.
2. Abu-Arafeh I. Childhood headache. 2nd ed. London: Mac Keith Press; 2013. 329 p. ISBN: 978-1-908316-75-2.
3. Ruut Virtanen. Epidemiological studies of childhood and adolescent's headache. Turku: University of Turku; 2008. 84 p.
4. International Headache Society. The International Classification of Headache Disorders, 3rd edition (beta version). Cephalalgia. 2013;33:629-808.
5. Russel MB. Childhood migraine: clinical features. In: Abu-Arafeh I, editor. Childhood headache: clinics in developmental medicine. 2nd ed. London: Wiley; 2013. p. 31-39.
6. Winner P. Childhood migraine: clinical features. In: Abu-Arafeh I, editor. Childhood headache: clinics in developmental medicine. 2nd ed. London: Wiley; 2013. p. 93-107.
7. Stovner LJ, Hagen K, Jensen R, et al. The global burden of headache: a documentation of headache prevalence and disability worldwide. Cephalalgia. 2007;27(3):193-210.
8. World Health Organization, Lifting the burden. Atlas of headache disorders and resources in the world 2011. Geneva: WHO; 2011. 69 p.
9. Kroner-Herwig B, Heinrich M, Morris L. Headache in German children and adolescents: a population-based epidemiological study. Cephalalgia. 2007;27(6):519-27.
10. Ozge A, Şaşmaz T, Bugdaici R, et al. The prevalence of chronic and episodic migraine in children and adolescents. Eur J Neurol. 2013;20(1):95-101.
11. Moldovanu I, Odobescu S, Rotaru L, Vovc V. The prevalence of episodic and chronic migraine in the Republic of Moldova. Socio-demographic and clinical characteristics. Buletinul Academiei de Stiințe a Moldovei. Științe medicale. 2012;(2):35-40.
12. Sedlic M, Mahovic D, Kruzliak P, et al. Epidemiology of primary headaches among 1,876 adolescents: a cross-sectional survey. Pain Med. 2016;17(2):353-359. doi: 10.1093/pm/pnv033.
13. Shivpuri D, Rajesh MS, Jain D. Prevalence and characteristics of migraine among adolescents: A questionnaire based study. Indian Pediatr. 2003;40(7):665-669.

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Atypical pure sensory forms of chronic inflammatory demyelinating polyneuropathies

Eugen Gavriliuc, MD, PhD, **Vitalie Lisnic,** MD, PhD, Professor

Department of Neurology No 1, Nicolae Testemitsanu State University of Medicine and Pharmacy Chisinau, the Republic of Moldova

*Corresponding author: eugen.gavriliuc@medpark.md
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Abstract

Background: There are still not enough data on clinical and laboratory peculiarities of atypical chronic inflammatory demyelinating polyneuropathy (CIDP), ranging from only sensitive symptoms without weakness to asymmetric motor deficit. Recent epidemiological data do not clearly elucidate the percentage of cases with atypical CIDP from total CIDP types. Nerves conduction study, the gold standard in diagnosing demyelinating polyneuropathies has low sensibility for atypical forms of CIDP. The purpose of this study was determining the criteria for clinical and laboratory diagnosis of atypical sensory CIDP.

Material and methods: Two groups of study were identified: 30 patients with typical CIDP and 30 patients with atypical CIDP. All patients underwent nerves conduction studies, blood was drawn for biochemical tests, also electrophoresis and serum protein immunofixation were done. Fibular nerve biopsy was performed in 9 patients. Overall Neuropathy Limitation Scale (ONLS) questionnaire was used for the assessment of functional disability.

Results: Nerves conduction studies in cases with sensory CIDP show normal motor conduction velocity in 10 cases, and diminished only in 4 cases. Total ONLS in patients with sensory CIDP is equal to 1.85 ± 0.21 points compared to total 4.17 ± 0.240 points in patients with typical CIDP ($p < 0.001$).

Conclusions: Nerves conduction study is not a gold standard for diagnosis atypical sensory CIDP. According to functional scores results, sensory CIDP is less disabling compared with typical CIDP.

Key words: sensory CIDP, demyelination, functional tests, polyneuropathy.

Introduction

The classic form of chronic inflammatory demyelinating polyneuropathy (CIDP) is symmetrical damage of motor and sensory nerves; motor involvement is greater than sensory [1]. The mechanism of nerves damage is presumed to be immune mediated [2]. Recent epidemiologic data have shown that up to 35% of CIDP patients may have only sensory symptoms [3, 4].

Several clinical variants of CIDP have been reported widening the spectrum of this neuropathy. According to the European Federation of Neurological Societies (EFNS) and Peripheral Nerve Society (PNS) guideline (EFNS / PNS, revised in 2010) CIDP can be classified into two clinical forms: typical CIDP and atypical CIDP [5].

Atypical forms can be classified according to the clinical manifestations in 4 major groups: pure motor, pure sensory, multifocal and of distal symmetrical impairment [6,7]. The diagnostic criteria for these forms are however not well defined possibly explaining their variable frequency ranging from 1% to 49% in different series and the reported differences in their treatment response [8,9].

A recent study fulfilled in USA showed that the majority of community neurologists had familiarity with the clinical presentations of typical CIDP, but many thought that atypical phenotypes were more various than what have been described in guidelines [10]. The aim of this study was to underline the clinical and paraclinical pecularities of pure sensory CIDP.

Nerve conduction studies (NCS), the gold standard in diagnosing demyelinating polyneuropathies, have low sensitivity for atypical forms of CIDP, that's why it's necessary to identify new ways of diagnosis [11]. Often the clinical picture of a sensory CIDP can simulate idiopathic axonal polyneuropathy, losing opportunity of proper immunomodulation treatment with subsequent resolution of symptoms [12]. Additive tests are required to establish the correct diagnosis of sensory CIDP: lumbar puncture, somatosensory evoked potentials (SSEPs), magnetic resonance imagings of the proximal portions of the cervico-brachial plexus and lumbosacral plexus, if necessary sural nerve or fibular nerve biopsies [13].

Two particular forms of sensory CIDP are described in scientific papers:

a) Clinical picture with distal, symmetrical, sometimes painful paraesthesia with a predominant onset of the soles or hands (feeling of socks tied to the talocrural joints, feeling of sand between the toes and on the soles of the feet, sensation of invisible sandals on the feet) which then progress ascending to the level of the thighs. The neurological objective examination shows a thermo-algic hypoesthesia in socks and gloves, diminished or preserved deep tendon reflexes. Muscle strength according to Medical Research

Council (MRC) scale – 5 points in all limbs. Romberg sign is negative in all the patients [14].

b) Clinical picture of chronic, ataxic neuropathy associated with distal paraesthesia. Ataxia manifests predominantly during walking with the presence of the positive Romberg sign and advanced self-perception disorders. A generalized areflexia is observed. NCS show the reduction in nerve conduction velocity across multiple trunks, increased distal motor latency and increased proximal F wave latency). Conductions blocks are uncommon, which explains the presence of normal muscle strength – 5 points in all muscle groups according to the MRC scale [15].

Material and methods

Two study groups were identified: 30 patients with typical CIDP and 30 patients with atypical CIDP according to the EFNS/PNS guideline (revised 2010).

Clinical examination included the following scales: Overall Neuropathy Limitation Scale – (ONLS), INCAT sensory score, 9-hole peg test, 10 meters test, MRC scale [16,17]. NCS were performed in all the patients. A full routine biochemistry, electrophoresis and immunofixation of serum proteins, all spectrums of anti-myeline and anti-ganglioside antibodies were performed. The proximal segments of the sensory peripheral nervous system can only be assessed by SSEPs [18]. SSEPs were considered to be suggestive of proximal demyelination when they revealed: (i) a significant increase in radicular conduction time with normal distal conduction time in at least 1 nerve and/or (ii) absence of N9/N18 potential or N13/N22 potential and/or delayed proximal volleys (N9 or N18) with normal distal conduction time in at least 2 nerves [19].

Cerebral spinal fluid (CSF) macroscopic/microscopic examination was performed in all the patients. Fibular nerve biopsies were obtained under local anesthesia from the lateral and inferior part of the shank. 5 patients with typical CIDP and 4 patients with atypical CIDP underwent superficial peroneal nerve biopsies. The 5 centimeters long superficial peroneal nerve specimen was divided into three pieces: first piece was fixated in paraformaldehyde and stained with haematoxylin-eosin; second piece was fixated in glutaraldehyde and the subsequent generationof semi-thin sections were stained with toluidine blue; third piece was frozen in liquid nitrogen and stored at -80 degrees Celsius - for immunohistological research [20].

Semi-thin (0.5 μm) sections allow much greater resolution than that provided by specimens embedded in paraffin and allow accurate quantification of demyelination markers: the presence of onion bulbs, decreased number and density of large and small myelinated fibers, decreased thickness of the myelin sheath [21,22]. Statistical analysis was performed using statistical methods Mann-Whitney and Fisher (SPSS statistics 20). Cases with $p \leq 0.05$ were considered statistically significant.

Results and discussion

The percentage of patients with atypical CIDP was the following: 10 patients with Lewis-Sumner syndrome represent 33% of patients with atypical CIDP, 6 patients with distal acquired demyelinating symmetric (DADS) polyneuropathy – 20% of patients and 14 patients with sensory CIDP – 47% of patients with atypical CIDP. Our results suggest that sensory CIDP represents the most frequent form of atypical CIDP.

From the group of 14 patients with sensory CIDP – 4 patients fulfilled the EFNS/PNS quideline 2010 criteria for NCS demyelination, 10 patients didn't fulfill these criteria but instead were selected according to the criteria of the French Group of CIDP Experts [2].

The mean age of onset of the disease in the group of patients with sensory CIDP is 57.71 years. The clinical course of the disease is less disabling in sensory CIDP than in cases with typical CIDP. 14 patients were diagnosed with sensory CIDP, 10 patients had a monophasic disease course, 2 patients had evolution in relapses and remissions, and only 2 patients presented progressive disease course. As compared to patients with typical CIDP forms: 6 cases with monophasic evolution, 6 patients with relapsing and remitting disease courses and 18 patients had progressive evolution.

Ataxia and numbness are the main symptoms of patients with sensory CIDP: all patients had numbness in the lower limbs and 7 patients had postural instability. No muscle weakness according to MRC scale was identified in sensory CIDP patients. In the group of patients with typical CIDP all patients had a predominant muscle weakness in the proximal regions of upper and lower limbs, postural instability had 24 from 30 patients with typical CIDP. Regarding positive sensory symptoms, feet constriction sensation predominates in 5 out of 14 patients with sensory CIDP versus 4 out of 30 patients with typical CIDP ($p < 0.05$). In 12 cases, pain was also described in addition to numbness. Deep tendon reflexes were diminished in 6 cases, in 3 cases only ankle jerk reflex was abolished, diffuse areflexia was observed in 5 cases.

Total ONLS in patients with sensory CIDP is equal to 1.85 ± 0.21 points compared to total ONLS 4.17 ± 0.240 points in patients with typical CIDP ($p < 0.001$). Patients with Lewis-Sumner forms of atypical CIDP and patients with typical CIDP have the longest time of fulfilling the 9-hole peg test (fig. 1). This means that functional ability of

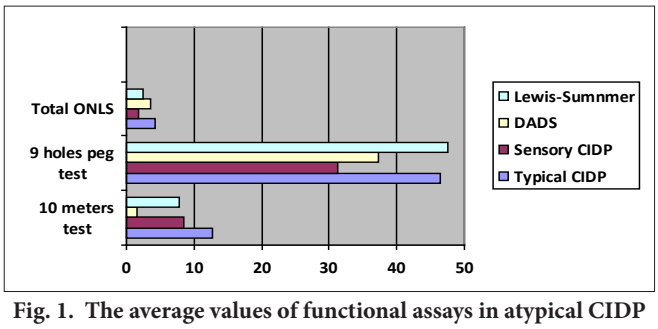

Fig. 1. The average values of functional assays in atypical CIDP subtypes compared to average values in typical CIDP.

their upper limbs is more affected than the functional ability of lower limbs. Also patients with DADS forms of atypical CIDP and patients with typical CIDP have the longest time of fulfilling the 10 meters test (fig. 1). It means that walking is most affected in this group of patients. Patients with sensory CIDP are less affected and have better prognosis of preserving their functional abilities.

According to NCS results presented in fig. 2, 3 – distal motor latencies, motor conduction velocities, proximal motor amplitudes, F waves latencies of median, ulnar, peroneal and tibial nerves are more preserved in atypical CIDP than in typical CIDP ($p < 0.001$). These data suggest a less demyelinating and degenerative process in atypical CIDP patients compared with typical cases of CIDP.

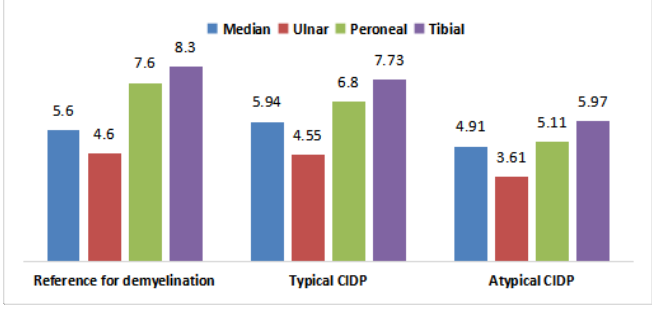

Fig. 2. Median values of distal motor latency (DML) in typical and atypical CIDP.

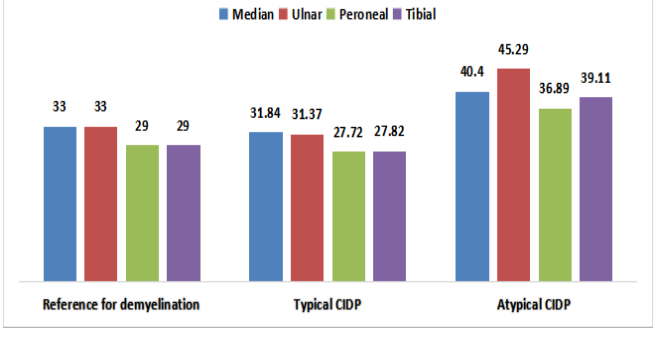

Fig. 3. Median values of motor conduction velocities (MCV) in typical and atypical CIDP.

NCS in 14 cases with sensory CIDP show normal motor conduction velocity in 10 cases, and diminished only in 4 cases. Also distal motor latency was diminished in 4 cases. Conduction block was present only in one case of sensory CIDP. Sensory conduction velocities in median and sural nerves were diminished in 6 cases. The amplitudes of the sensory nerve actions potentials (SNAP) in sural nerves were absolutely normal in 7 patients out of 14 with sensory CIDP (50% of patients with sensitive PDIC). From these 7 patients 6 out of them (43%) have so-called inverse ratio – amplitude of the sural nerve SNAP is greater than the amplitude of median nerve SNAP, which is an important supportive criteria for diagnosis of CIDP (fig. 4).

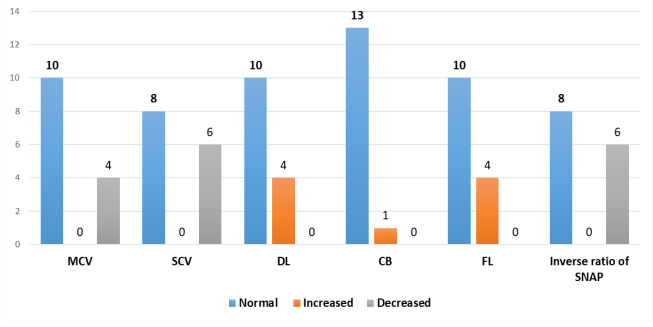

Fig. 4. MCV – motor conduction velocity (m/s), SCV – sensory conduction velocity, DL – motor distal latency (ms), CB – conduction block, FL – F-wave latency (ms), SNAP – sensory nerve action potential.

NCS show no evidence of demyelinating criteria for 10 patients with sensory CIDP, but these patients show clinical examination abnormalities that are not typical for chronic axonal polyneuropathies like: ataxia, generalized areflexia, distal hypoesthesia progressing toward the proximal portions of the limbs. Therefore, the SSEPs investigation was performed to demonstrate proximal demyelination, at pre- or post-ganglion levels, levels that are not accessible for the conventional NCS [23]. SSEP examinations were done in 10 patients diagnosed with sensory CIDP but with no signs of demyelination on NCS and compared with SSEP results of 10 patients with typical CIDP. 6 patients with sensory CIDP had prolonged radicular conduction time in at least 1 limb compared to 7 patients in typical CIDP ($p > 0.05$), and 7 had abnormal/delayed N9/N18 potentials and/or absent spinal potential in at least 1 limb compared to 8 patients with typical CIDP ($p > 0.05$). In summary, all patients with sensory CIDP had evidence of proximal demyelination on SSEPs with no statistical difference from the patients with typical CIDP.

CSF protein was elevated in 10 patients, ranging from 0.5-1.9 g/l, and normal in 4 cases. Data from our study are similar to the results of the French study [24]. CSF protein level was increased in 16 out of 22 patients with sensory CIDP (73% of patients studied) compared to 71% in our study.

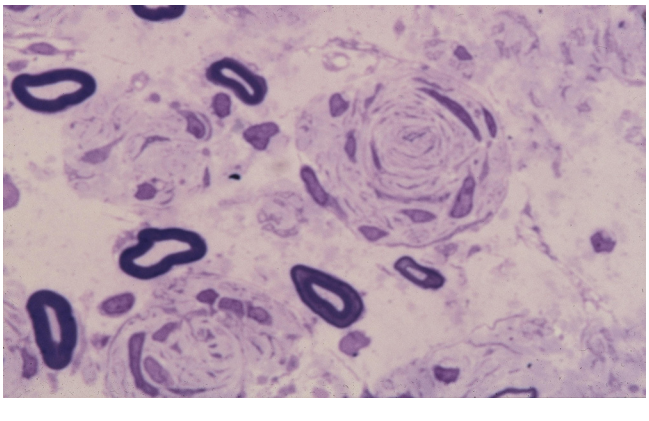

Fig. 5. Semi-thin transversal section of peroneal superficial nerve showing onion bulb formation in a patient with sensory CIDP.

Nerve biopsy findings were the following: reduction in myelinated fiber density was most frequent (100%), followed by demyelination (90%), inflammation (44%), and onion bulb formation (55%). Endoneurial inflammation was more frequent in the relapsing-remitting form (fig. 5).

Conclusions

1. NCS is the most important test used to diagnose demyelinating polyneuropathies. However, NCS are normal when demyelinating lesions are distributed proximally. This may lead to misdiagnosis or mismanagement.

2. SSEPs should be carried out in all cases of atypical sensory polyneuropathy (accompanied by ataxia, areflexia) to demonstrate the proximal demyelination (at pre- or post-ganglionic levels) not accessible for conventional NCS.

3. ONLS and 9 whole peg tests are efficient to evaluate the level of disability in patients with CIDP. According to ONLS scale, patients with typical CIDP are more impaired than sensory atypical CIDP patients.

4. Fibular nerve biopsy is performed only if the NCS don't bring any demyelinating findings, but the clinical evolution of the disease is progressive and disabling.

5. There is significant phenotypic variability in the clinical spectrum of CIDP suggesting that there are different immunopathological mechanisms at play. Future research is needed to identify disease markers.

6. NCS is not a sensitive test to diagnose sensory CIDP, in 70% of cases motor conduction velocities were not affected.

References

1. Eftimov F, van Schaik I. Chronic inflammatory demyelinating polyradiculoneuropathy: update on clinical features, phenotypes and treatment options. Curr Opin Neurol. 2013;26(5):496-502.
2. French CIDP Study Group. Recommendations on diagnostic strategies for chronic inflammatory demyelinating polyradiculoneuropathy. J Neurol Neurosurg Psychiatry. 2008;79:115-118.
3. Ayrignac X, Viala K, Morizot Koutlidis R, Taieb G, Stojkovic T, Musset L, et al. Sensory chronic inflammatory demyelinating polyneuropathy: an under-recognized entity? Muscle Nerve. 2013;48(5):727-732.
4. Lefter S, Hardiman O, Ryan AM. A population-based epidemiologic study of adult neuromuscular disease in the Republic of Ireland. Neurology. 2017;88(3):304-13.
5. Joint Task Force of the EFNS and the PNS. European Federation of Neurological Societies/Peripheral Nerve Society Guideline on management of chronic inflammatory demyelinating polyradiculoneuropathy: report of a joint task force of the European Federation of Neurological Societies and the Peripheral Nerve Society - First Revision. J Peripher Nerv Syst. 2010;15(1):1-9.
6. Doneddu PE, et al. Atypical CIDP: diagnostic criteria, progression and treatment response. Data from the Italian CIDP Database. J Neurol Neurosurg Psychiatry. 2019;90(2):125-132.
7. Mathey EK, Park SB, Hughes RA, et al. Chronic inflammatory demyelinating polyradiculoneuropathy: from pathology to phenotype. J Neurol Neurosurg Psychiatry. 2015;86(9):973-85.
8. Kuwabara S, Isose S, Mori M, et al. Different electrophysiological profiles and treatment response in 'typical' and 'atypical' chronic inflammatory demyelinating polyneuropathy. J Neurol Neurosurg Psychiatry. 2015;86(10):1054-9.
9. Camdessanche JP, Jousserand G, Ferraud K, Vial C, et al. The pattern and diagnostic criteria of sensory neuronopathy: a case-control study. Brain. 2009;132(Pt 7):1723-1733.
10. Gelinas D, Katz J, Nisbet P, England JD. Current practice patterns in CIDP: a cross-sectional survey of neurologists in the United States. J Neurol Sci. 2019;397:84-91.
11. Nobile-Orazio E. Chronic inflammatory demyelinating polyradiculoneuropathy and variants: where we are and where we should go. J Peripher Nerv Syst. 2014 Mar;19(1):2-13.
12. Sinnreich M, Klein CJ, Daube JR, et al. Chronic immune sensory polyradiculopathy: a possibly treatable sensory ataxia. Neurology. 2004;63(9):1662-1669.
13. De Sousa EA, Chin RL, Sander HW, Latov N, Brannagan T. Demyelinating findings in typical and atypical CIDP: sensitivity and specificity. J Clin Neuromusc Dis. 2009;10(4):163-169.
14. Léger JM, Bombelli F, Tran-Thanh H, Chassande B, Maisonobe T, Viala K. Chronic inflammatory demyelinating polyradiculoneuropathy: clinical heterogeneity and therapeutic perspectives. Bull Acad Natl Med. 2010;194(4-5):764-765.
15. Van den Bergh PY, Rajabally YA. Chronic inflammatory demyelinating polyradiculoneuropathy. Presse Med. 2013;42(6 Pt 2):203-215.
16. Graham RC, Hughes RAC. A modified peripheral neuropathy scale: the Overall Neuropathy Limitations Scale. J Neurol Neurosurg Psychiatry. 2006;77(8):973-976.
17. Merkies I, Schmitz P, van Der Mechè F, van Doorn P. Inflammatory Neuropathy Cause and Treatment (INCAT) Group. Psychometric evaluation of a new sensory scale in immune-mediated polyneuropathies. Neurology. 2000;54:943-949.
18. Yiannikas C, Vucic S. Utility of somatosensory evoked potentials in chronic acquired demyelinating neuropathy. Muscle Nerve. 2008;38(5):1447-1454.
19. Kuwabara S, Misawa S. Chronic inflammatory demyelinating polyneuropathy: clinical subtypes and their correlation with electrophysiology. Clin Exp Neuroimmunol. 2011;2(2):41-48.
20. Bosboom WM, van den Berg LH, Franssen H, Giesbergen PC, Flach HZ, van Putten AM, Veldman H, Wokke JH. Diagnostic value of sural nerve demyelination in chronic inflammatory demyelinating polyneuropathy. Brain. 2001;124(Pt 12):2427-2438.
21. Kulkarni GB, Mahadevan A, Taly AB, Nalini A, Shankar SK. Sural nerve biopsy in chronic inflammatory demyelinating polyneuropathy: are supportive pathologic criteria useful in diagnosis? Neurol India. 2010;58(4):542-548.
22. Vallat JM, Tabaraud F, Magy L, Couratier P. Importance of the nerve biopsy for the diagnosis of atypical forms of chronic inflammatory demyelinating polyradiculoneuritis: 8 cases. Bull Acad Natl Med. 2003;187(2):387-399.
23. Bril M, Banach M, Dalakas MC, Deng C. Electrophysiologic correlations with clinical outcomes in CIDP. Muscle Nerve. 2010;42(4):492-7.
24. Viala K, Maisonobe T, Stojkovic T, et al. A current view of the diagnosis, clinical variants, response to treatment and prognosis in chronic inflammatory demyelinating polyradiculoneuropathy. J Peripher Nerv Syst. 2010;15(1):50-56.

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Sympathetic-parasympathetic cardiac autonomic tonus during induction of anesthesia with propofol and fentanyl

*[1,2]**Iuliana Feghiu,** MD, Assistant Professor; [1]**Sergiu Cobiletchi,** MD, Assistant Professor;
[1]**Sergiu Sandru,** MD, PhD, Professor

[1]Valeriu Ghereg Department of Anesthesiology and Intensive Care No 1
[2]Department of Pathophysiology and Clinical Pathophysiology
Nicolae Testemitsanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova

*Corresponding author: i.dimitriu@yahoo.com
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Abstract

Background: Administration of propofol and fentanyl for induction of general anesthesia is often associated with cardiovascular instability. This effect can be caused by changes in the cardiac autonomic tonus induced by the drugs. In the literature there is no consensus regarding the effect of propofol and fentanyl on sympathetic or parasympathetic balance of the heart.
Material and methods: There was performed a randomized prospective study which was approved by the Ethic Committee. Written informed consent was signed by all patients. The study group involved 47 patients scheduled for surgical intervention, anesthetic risk ASA I-II. The analysis of heart rate variability and the changes in cardiac autonomic tonus was performed with Holter ECG at rest, after premedication with fentanyl solution and after induction of general anesthesia with propofol and fentanyl.
Results: After administration of fentanyl in doses of 1.0 mkg/kg for premedication there were not significant changes of heart rate variability and autonomic heart tonus. Administration of propofol 2.5 mg/kg combined with fentanyl 1.0 mkg/kg for induction of general anesthesia leads to significant changes in heart rate variability. There was a considerable reduction of heart rate variability. The LFun (marker of sympathetic heart tonus) has enhanced by 6.8% compared with previous stage (67.1 (95% CI 63.1-71.1) vs 72.0 (95% CI 67.9-76.1) (p=0.004). The HFun (marker of parasympathetic cardiac tonus) has reduced by 19.8% (32.9 (95% CI 28.9-36.8) vs 26.4 (95% CI 20.4-34.3) (p=0.007). After administration of propofol and fentanyl for induction of general anesthesia the LFun/HFun ratio has enhanced by 30.8% (2.7 (95%CI 2.1-3.4) vs 3.9 (95%CI9 2.9-4.8) (p=0.003), signaling an enhanced sympathetic heart tonus.
Conclusions: Administration of fentanyl solution in doses 1.0 mkg/kg for premedication is not associated with significant changes of autonomic tonus of the heart. Administration of propofol 2.5 mg/kg in combination with fentanyl 1.0 mkg/kg for induction of general anesthesia leads to significant enhanced sympathetic cardiac tonus.

Key words: heart rate variability, sympathetic cardiac tonus, parasympathetic cardiac tonus.

Introduction

Heart rate variability (HRV) is a noninvasive electrocardiographic marker which reflects the sympathetic and parasympathetic influences on sinus node of the heart, and in this way can show the ability of the heart to adapt to different physiological situations. HRV expresses the variations of heart rate and duration of RR intervals (intervals between QRS complexes on ECG) when depolarization in the heart is controlled by normal pacemaker. In other words, HRV analysis shows the baseline autonomic function of the heart [1,2,3]. In a healthy heart with normal sympathetic- parasympathetic influences on sinus pacemaker, there will be continuous changes of the sinus cycles. Normal HRV reflects a balanced sympathovagal state of the heart. Gender, age, circadian rhythm, respiratory rate and body position are physiological factors which may influence HRV [4,5,6]. Measurements of HRV are noninvasive, and highly reproducible. They may be performed on the basis of 24 hour Holter recordings or on shorter periods ranging from 0.5 to 5 minutes particularly in the field of dynamic electrocardiography. Most Holter devices manufactured nowadays have HRV analysis programs which are incorporated into their instrument systems [7,8]. Most studies in anesthesia and intensive care which used the HRV for analysis of changes in sympathetic-parasympathetic balance of the heart performed the 5 minutes analysis of HRV [9-15].

In 1996 a Task Force of the European Society of Cardiology (ESC) and the North American Society of Pacing and Electrophysiology (NASPE) defined and established standards of measurement, physiological interpretation and clinical use of HRV. Time domain indices, geometric measures and frequency domain indices constitute nowadays the standard clinically used parameters [16].

Heart rate variability has been used in different clinical settings, including diabetes, arterial hypertension, coronary artery disease, sudden cardiac death, and for the screening of patients with obstructive sleep apnea. Furthermore, the effects of a variety of pharmacological and non-pharmacological interventions on HRV have been studied, such as antiarrhythmic drugs, physical effort and after radiofrequency ablation procedures [2.9]. In the field of Anesthesiology and Intensive Care, HRV analysis was used for assessment of se-

dation and analgesia, risk for development of hypotension after spinal or epidural anesthesia, for assessment of vegetative effects of different hypnotic drugs used in general anesthesia [2,14,15,17,18].

General anesthesia is usually associated with changes in sympathetic activity that may be due to mechanical ventilation, specific anesthetic drugs effects, the direct circulatory effects they induce, and/or their effects on central or peripheral nervous system. Most anesthetics used nowadays interfere with sympathetic neural outflow and cardiovascular regulation [19-22]. Propofol is a frequently used hypnotic for induction of general anesthesia but it can induce hypotension, particularly when injected rapidly. Many mechanisms have been involved for explanation of propofol induced arterial hypotension, mainly direct depression of myocardium, reduced peripheral vascular resistance caused by direct vasodilatory effect of the drug, reduction of preload and afterload. The studies anyway, showed controversial results, and any of these factors could be imputed for hemodynamic instability after administration of propofol for sedation or for induction of general anesthesia. The observed decrease of peripheral vascular resistance in patients with artificial hearts points to a direct vasodilatating effect of propofol or a decrease in sympathetic vasoconstrictor activity. On the other hand, when propofol was infused in the brachial artery vasodilatation did not occur. Accordingly, other mechanisms must be responsible for the observed vasodilatation during propofol anesthesia [20,23,24].

Most published studies regarding the effects of propofol or fentanyl on heart autonomic tonus were performed with sedative doses of drugs. Administration of propofol for moderate or deep sedation is frequently associated with a significant decrease in mean blood pressure. This hypotensive effect of the drug can be caused by reduction of sympathetic cardiac tonus or disturbances in baroreceptor-mediated cardiac activity [20,25,26,27].

The purpose of this clinical research was to find changes in sympathetic and parasympathetic heart tonus by analyzing HRV after administration of propofol in combination with fentanyl for induction of general anesthesia.

Material and methods

We performed a prospective randomized study to evaluate the changes of vegetative heart tonus after induction of general anesthesia with fentanyl and propofol. The protocol of the study was approved by the Ethic Committee of Nicolae Testemitsanu State University of Medicine and Pharmacy, No 20 of 02.02.2016.

Between March 2017 and September 2017, ASA physical status I-II patients scheduled for elective surgical procedures aged under 60 years (to exclude age-related changes of HRV), and with normal sinus rhythm on ECG were enrolled in the study. We obtained an informed consent from all participants in the study. Patients with diseases that could interfere with vegetative heart tonus (endocrine, neurological, cardiovascular diseases) were excluded from the study. Another exclusion criterion was the presence of more than 20% of artifacts on ECG trace.

In the operating room, the patients were monitored with electrocardiogram (ECG), non-invasive blood pressure, pulse oximetry and capnography. Baseline heart rate, blood pressure and respiratory rate were recorded. During induction of general anesthesia, oxygen was delivered to ensure a SpO_2 above 95%. The patients received 10 ml/kg crystalloid intravenously before induction of anaesthesia.

We attached 10 electrodes on the chest and abdomen of the patients and connected them to Holter monitor (Holter TLC 5000, USA) within 25-30 minutes after admission to surgical room. HRV parameters were analyzed at rest (baseline), after premedication with fentanyl 1.0 mkg/kg and after induction of general anesthesia with propofol 2.5 mg/kg and fentanyl 1.0 mkg/kg (fig. 1). The dose of propofol and fentanyl was given over 30 s, until a loss of consciousness, while the patient was breathing 100% oxygen. The loss of consciousness was defined as a loss of the eyelash reflex and no reaction to subsequent positive-pressure mask ventilation. After administration of propofol and fentanyl and development of bradypnea or apnea, the mask ventilation was initiated in order to ensure a frequency of ventilation of 14-16/min and a tidal volume 7-8 ml/kg, an important requirement for correct registration and analysis of HRV and interpretation of sympathetic-parasympathetic heart tonus.

HRV parameters and changes in sympathetic and parasympathetic vegetative heart tonus were analyzed by Holter computerized system. Parameters of HRV and their significance are presented in table 1 and were interpreted according to the recommendations of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [16].

Statistical analysis of the results was done in accordance with the statistical program GraphPad Prism 6 (GraphPad Software, San Diego, California, USA). Values of parametric distribution were analyzed by t-pair and repeated measures of ANOVA tests. Values of non-parametric distribution were analyzed by Wilcoxon and Friedman tests. Results are presented in the form of average and 95% confidence interval (for parametric data) and median with interquartile range (IQR – for non-parametric data). Value of $p<0.05$ was

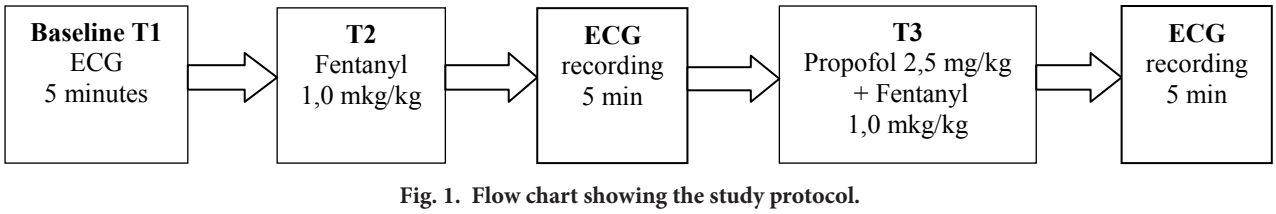

Fig. 1. Flow chart showing the study protocol.

considered statistically significant. The number of patients involved in the study group was determined in order to ensure a study power of 80%, α-error of 5% at a detectable difference of heart tonus between stages of at least 0.5. As well, there was considered a proportion of 10% of patients that couldn't be involved in final analysis for different reasons. Such study group involved 47 patients.

Table 1

Parameters of HRV analyzed by ECG Holte

Parameters of HRV	Significance	Reference values
TP – Total spectral power of HRV (ms^2)	All vegetative influences on the heart (sympathetic, parasympathetic, influences from peripheral and central chemoreceptors, baroreceptors)	3466.0±1018.0
Normalized spectral power of low frequency (**LFun** – *Low Frequency*)	Sympathetic and baroreceptor influences on the heart	54.0 ±4.0
Normalized spectral power of high frequency (**HFun** – *High Frequency)*	Parasympathetic influences on the heart	29.0 ±3.0
LFun/HFun ratio	Sympathetic-parasympathetic heart balance	1.5-2.0

Interpretation and normal ranges are presented according to the recommendations of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [16].

Results

The group of patients who benefited from the induction of general anesthesia with propofol and fentanyl comprised 47 patients (26 females and 21 males) at the age of 37.5±11.9 years. Body mass index varied from 16.1 kg/m^2 to 30.0 kg/m^2 with the average 24.6±3.4 kg/m^2. Most patients in the group benefited from the induction of general anesthesia with fentanyl and propofol for laparoscopic cholecystectomy (24 cases – 51.1%), followed by 7 patients with mandible osteosynthesis (14.9%), 4 cases for discectomy (8.5%), 2 cases for syaloadenectomy (4.2%), 2 patients for excision of maxilar cyst (4.2%), 2 cases for scar excision and lip remodeling (4.2%). The other six cases (12.8%) were for different surgical procedures (maxillary osteoplastia, plastia of frontal bone, removal of metallic blade from the arm, resection of cervical cyst, remodeling of ears, and reposition of nasal bones). All patients involved in the study were with minimal anesthetic risk (ASA I-II).

After administration of fentanyl solution for premedication purposes there were no attested significant changes of HRV parameters when compared with baseline values (tab. 2). Total spectral power of HRV has reduced by 12.6% compared with baseline (1400.0 ms^2 (CI 95%1069.0-1731.0) vs 1223.0 ms^2 (CI 95% 949.4-1496.0), (p=0.2). The LFun – marker of sympathetic cardiac tonus – has increased by 0.4% (66.8 (CI 62.6-70.9) vs 67.1 (CI 95% 63.1-71.1), (p=0.8). On the other hand, HFun – marker of parasympathetic cardiac tonus – has reduced, although this reduction is statistically insignificant compared with baseline value. The spectral power of HFun has reduced by 0.9% (33.2 (95% CI 29.0-37.4) vs 32.9 (95% CI 28.9-36.8), (p=0.8), (fig. 2). The LFun/HFun ratio didn't change significantly and was 2.7±0.3 both, in baseline and after administration of fentanyl for premedication (fig. 3). So, after administration of fentanyl 1.0 mkg/kg for premedication there were not attested significant changes in autonomic heart tonus, and the HRV parameters show the presence of enhanced sympathetic tonus of the heart in patients involved in the study.

Significant changes of HRV were attested after administration of propofol 2.5 mg/kg and fentanyl 1.0 mkg/kg for the induction of general anesthesia (tab. 3). The total spectral power of HRV has reduced by 70.4% (1223.0 ms^2 (95% CI 949.4-1496.0) vs 362.1ms^2 (95% CI 257.3-466.9), (p=0.0001). There was noted a significant reduction of spectral power of HFun – marker of heart parasympathetic vegetative tonus. The spectral power of HFun has reduced by 19.8% (32.9 (95% CI 28.9-36.8) vs 26.4 (95% CI 20.4-34.3), (p=0.007). The reduction of the power of HFun is a proof of the cardiac vagolitic effect of propofol given in doses for the induction of general anesthesia. On the other hand, there was registered enhanced spectral power of LFun, such marking an enhanced sympathetic heart tonus and baroreceptor influences on the sinus node of the heart. The spectral power of LFun has enhanced by 6.8% compared with previous stage (T2) (67.1 (95% CI 63.1-71.1) vs 72.0 (95% CI 67.9-76.1), (p=0.004), (fig. 2). The ratio LFun/HFun has enhanced by 30.8% (2.7 (95%CI 2.1-3.4) vs 3.9 (95%CI9 2.9-4.8), (p=0.003) after administration of fentanyl and propofol (fig. 3). Both, significantly enhanced LFun and LFun/HFun ratio in patient who received propofol 2.5 mg/kg and fentanyl 1.0 mkg/kg for the induction of general anesthesia proved the presence of enhanced sympathetic heart tonus and the cardiac sympathomimetic effects of propofol.

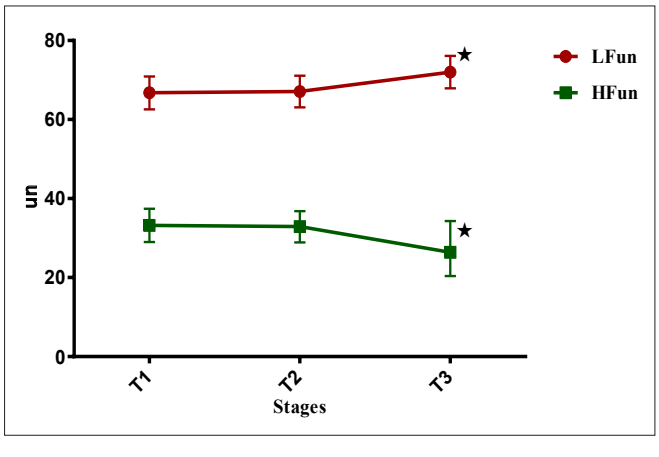

Fig. 2. Changes of LFun and HFun during anesthesia induction with propofol and fentanyl (*p<0.05). Values are presented as mean with 95% confidence intervals (error bars).

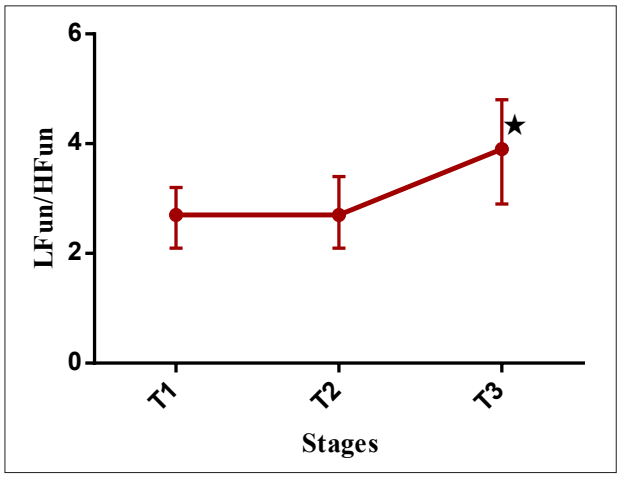

Fig. 3. Changes of sympathetic-parasympathetic heart tonus during general anesthesia induction with propofol and fentanyl (*p<0.05). Values are presented as mean with 95% confidence intervals (error bars).

Table 2

Changes of HRV parameters at rest, after premedication and induction of general anesthesia with propofol and fentanyl (Repeated measures ANOVA and Friedman's test*)

HRV parameters	T1	T2	T3	p
TP (ms²) *	1400.0 (1069.0-1731.0)	1223.0 (949.4-1496.0)	362.1 (257.3-466.9)	0.0001
LFun	66.8 (62.6-70.9)	67.1 (63.1-71.1)	72.0 (67.9-76.1)	0.09
HFun	33.2 (29.0-37.4)	32.9 (28.9-36.8)	26.4 (20.4-34.3)	0.04
LFun/ HFun	2.7 (2.1-3.2)	2.7 (2.1-3.4)	3.9 (2.9-4.8)	0.02

*Values are presented as mean and 95% confidence interval for parameters with normal distribution and median with intercvartilic range for values with non-parametric distribution.

If in baseline, 38.3% of patients presented enhanced sympathetic cardiac tonus, 38.3% – enhanced parasympathetic cardiac tonus and 23.4% – cardiac eutonia, after administration of fentanyl 1.0 mkg/kg for premedication there was detected an enhanced proportion of patients with increased sympathetic tonus of the heart (42.5%). The rate of patients with enhanced parasympathetic heart tonus didn't change and was attested in 18 patients (38.3%), exactly as in baseline. Meantime, the rate of patients with cardiac eutonia has decreased to 19.1%. After administration of propofol 2.5 mg/kg and fentanyl 1.0 mkg/kg for the induction of general anesthesia there was attested a significant increase in the proportion of patients with enhanced sympathetic tonus of the heart (51.1%) and reduction in the proportion of patients with enhanced parasympathetic tonus of the heart (27.7%), (fig. 4).

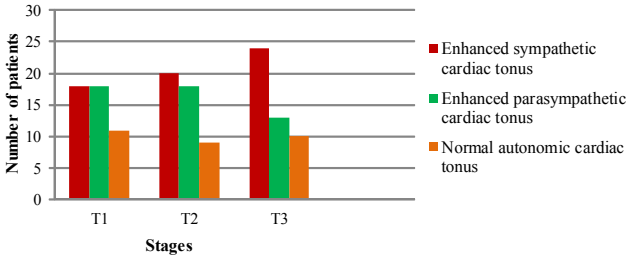

Fig. 4. Structure of the group in function of autonomic heart tonus at rest, after premedication and induction of general anesthesia with propofol and fentanyl.

Discussion

Many factors control perfusion in the peripheral tissues. From these should be mentioned cardiac output, fraction of ejection, stroke volume, microcirculation and vascular tone. Most of these factors are modulated by the autonomic nervous system. Disturbances in cardiac autonomic tonus can lead to adverse cardiovascular events. For anesthesiologists these aspects can be important during perioperative period, especially during the induction of anaesthesia, patient's positioning, episodes of blood loss and surgical stimulation, when cardiovascular instability can be life threatening [9,11,12].

HRV has gained importance in recent years as a technique employed to explore the autonomic nervous system. This method is widely used for studying physiology of arrhythmogenesis [1,6]. Frequency domain (power spectral density) analysis describes the periodic oscillations of the heart rate signal decomposed at different frequencies and amplitudes, and provides information on the amount of their relative intensity (termed variance or power) in the heart's sinus rhythm [2,16]. The HRV analysis may quantitatively and rapidly appreciate the balance of cardiac sympathetic and parasympathetic activities, as well as their effects on cardiovascular system. This method is also useful for evaluation of changes in autonomic cardiac tonus after administration of anesthetic drugs in anesthesia [2,9].

The interpretation of HRV (LF, HF, LF/HF ratio) is to some degree still controversial. Nevertheless, interpretation of HF is more certain than that of LF. Based on many studies it is considered that LF reflects (at least in part) the sympathetic activity of the autonomic nervous system. Another parameter of HRV is HF which reflects the cardiac parasympathetic activity, and the LFun/HFun ratio which reflects the sympathetic/parasympathetic influences on the heart [16,28,29].

Propofol is a hypnotic agent widely used in anesthesia because of its favorable recovery profile and low incidence of side effects. However, induction of general anesthesia with propofol is often associated with a significant decrease in arterial blood pressure and heart rate. The hypotensive effect of propofol has been attributed to many factors. Mostly, reduction in arterial pressure after administration of propofol is caused by low systemic vascular resistance or cardiac

output, impaired baroreflex mechanisms, and depression of myocardial contractility. Inhibition of the sympathetic nervous system and reduced sympathetic cardiac tonus may explain all propofol-induced hemodynamic changes during anesthesia and was proposed as a mechanism of hypotensive effect of the drug, but the precise mechanism by which this may occur is still unknown [20,23,26]. Although there is general agreement that induction of anesthesia with propofol is associated with a reduction in HRV, there are some conflicting data regarding the effects of propofol on cardiac sympathetic or parasympathetic tone [25-33].

The present study aimed to investigate the changes in the HRV in patients with minimal anesthetic risk undergoing the induction of general anesthesia with a combination of propofol and fentanyl.

In the literature there are studies which analyzed the changes in cardiac autonomic tonus during general anesthesia with propofol but it is difficult to compare the results between them due to different anesthesia management, doses and combination of drugs, respiratory pattern, and method for HRV assessment. More than that, it was confirmed by some studies that the changes in sympathetic-parasympathetic cardiac balance are different when there is administration of drug in sedative doses or doses for the induction of general anesthesia [27-31].

In our study administration of fentanyl 1.0 mkg/kg for premedication didn't change significantly the HRV parameters when compared to baseline values. In both time points (T1 and T2) the LFun/HFun ratio showed an enhanced sympathetic tonus of the heart. The values of LFun and HFun after fentanyl administration didn't change significantly in our study. A representative study that examined the cardiac vegetative effects of fentanyl by analysis of HRV is the study conducted and published by Vettorello M. et al. [22]. HRV as a measure of sympathovagal balance was prospectively analyzed in 11 subjects during spontaneous and paced breathing at 20 breaths/min both before and after fentanyl 1.0 mkg/kg administration. Conclusion of this study was that low-dose fentanyl administration in healthy volunteers decreases sympathetic cardiac tonus with a trend toward vagal activation of the heart. Anyway, the number of subjects involved is too small for a relevant conclusion. In another study, fentanyl was administered intravenously for premedication in doses of 3.0 mkg/kg. The analysis of HRV showed that HRV and LFun decreased, but not HFun, indicating a greater reduction of cardiac sympathetic activity [30]. To compare these results with our study is difficult as the doses of fentanyl given for premedication in our research are twice smaller. But, the general conclusion is that fentanyl tends to enhance parasympathetic cardiac tonus and decrease the sympathetic one.

Most studies which analyzed the cardiac vegetative changes after administration of propofol for sedation purposes show also conflicting results. Tarvainen M. et al. analyzed changes of autonomic cardiac tonus in 9 healthy males, at the age of 18-29 years. In this study, propofol was given intravenously using target control infusion aiming at pseudo steady-state plasma concentrations at 10 min intervals starting from 1.0 μg/ml and followed by 0.25-0.5 μg/ml increases until loss of consciousness was reached. The results showed that there is an overall increase in HRV and especially in HF component [24]. So, propofol in small doses has a vagotonic effect on the heart. In another research authors studied the difference in the effects of midazolam and propofol on the cardiac nervous system during combined spinal and epidural anesthesia. The study showed that propofol given in sedative doses during combined spinal epidural anesthesia produced little changes in LF and HF, and such doses do not influence the cardiac vegetative balance [27]. In a study conducted by Tsugayasu R. et al. propofol was infused using a target controlled infusion pump at an initial target effect-site concentration of 0.7g/mL [25]. The final result and conclusion in this study are similar to ours as in the same way after administration of propofol LFun/HFun ratio enhanced thus showing an increased sympathetic cardiac tonus. Exactly as in our study, the LFun increased and HFun decreased proving the vagolitic effect of propofol. As only 7 subjects were enrolled in the study, the validity of the results obtained could be limited. Win N. et al. studied the effect of propofol sedation on vegetative cardiac balance by analysis of HRV in 30 dental implantation patients (ASA I physical status, age 30-62). Propofol was infused at an initial target effect-site concentration of 1 g/ml. The results of this study demonstrated that intravenous conscious sedations with propofol induced significantly decreases in TP, LF, HF and LFun/HFun ratio, indicating predominance of parasympathetic activity during sedation [26]. So, most studies which used propofol in sedative doses showed the predominant vagotonic effect of it on the heart. These results are different from our results, but in our study the dose of propofol administered for the induction of general anesthesia was higher and it was combined with fentanyl.

One representative study which examined the changes in cardiac balance by analysis of HRV after administration of propofol for the induction of general anesthesia was published in 2017 [23]. In this prospective observational study, consecutive adult patients undergoing surgeries for supratentorial tumour (study group) and brachial plexus injury (control group) were recruited. Electrocardiogram was recorded for 5 min at three time points – before propofol induction, at propofol concentration of 2.0μg/ml and at propofol concentration of 4.0 g/ml. We will compare the results obtained in the control group. In brachial plexus group the sympathovagal balance, assessed by LFun/HFun ratio significantly increased at propofol concentration of 4μg/ml and was due to low HF power. Total power of HRV decreased at 4μg/ml. These results are similar to our findings as in our study administration of propofol reduces total power of HRV more than by 70% and the HFun reduced by 19.8%, thus proving the vagolitic effect of propofol. Riznyk L. et al. in a research on one hundred patients proved the fact that fentanyl-based induction of general anesthesia with propofol increases the ratio of LFun/HFun [30]. Their results suggest that induction of anesthesia with propofol reduces the cardiac parasympathetic tone more than sympathetic tone. This result is similar to that obtained in our research, even if

the doses of fentanyl were higher. Three other studies proved these results [31-33]. Kanaya N. et al. [31] and Hamada Y. et al. [32] using a maximum-entropy method for HRV assessment, confirmed that anesthesia with propofol caused reduction in HFun power but not in LFun power, indicating that induction of anesthesia with propofol might reduce a cardiac parasympathetic tone more than sympathetic tone. In another study forty patients were randomly allocated to the propofol group and the midazolam-propofol group co-induction. Propofol was administered at 2.5 mg/kg in the propofol group. The result revealed a greater decrease of the HFun as compared with that of the LFun in both groups, resulting in an increase of the LFun/HFun ratio.

Our report showed that HRV analysis is a noninvasive method that is applicable to the assessment of changes in sympathovagal regulation that are associated with hemodynamic changes during the induction of general anesthesia. Our findings imply that administration of propofol and fentanyl for the induction of general anesthesia enhances the dominance of sympathetic nervous system on the heart. This finding should be considered during general anesthesia, especially in patients at risk of cardiovascular complications.

Conclusions

Administration of fentanyl 1.0 mkg/kg for premedication during general anesthesia is not associated with significant changes in the autonomic cardiac tonus.

Administration of propofol 2.5 mg/kg and fentanyl 1.0 mkg/kg for the induction of general anesthesia is associated with a significant enhancement of sympathetic cardiac tonus and reduction of vagal influences on the heart.

References

1. Pichot V, Roche F, Celle S, Barthélémy J, Chouchou F. HRV analysis: a free software for analyzing cardiac autonomic activity. Front Physiol. 2016 Nov 22;7:557.
2. Anderson T. Heart rate variability: implications for perioperative anesthesia care. Curr Opin Anaesthesiol. 2017;30(6):691-697.
3. Rodriguez-Linares L, Mendez AJ, Lado MJ, Olivieri DN, Vila XA, Gomez-Conde. An open source tool for heart rate variability spectral analysis. Comput Methods Programs Biomed. 2011;103(1):39-50.
4. Tarvainen MP, Niskanen JP, Lipponen JA, Ranta-Aho PO, Karjalainen PA. Kubios HRV – heart rate variability analysis software. Comput Methods Programs Biomed. 2014;113(1):210-220.
5. Billman GE. Heart rate variability - a historical perspective. Front Physiol. 2011;2:86.
6. Lombardi F, Stein PK. Origin of heart rate variability and turbulence: an appraisal of autonomic modulation of cardiovascular function. Front Physiol. 2013;2:95.
7. Nicolini P, Ciulla MM, De Asmundis C, et al. The prognostic value of heart rate variability in the elderly, changing the perspective: from sympathovagal balance to chaos theory. Pacing Clin Electrophysiol. 2012;35:622-638.
8. Piskorski J, Guzik P. Compensatory properties of heart rate asymmetry. J Electrocardiol. 2012;45(3):220-224.
9. Mazzeo AT, La Monaca E, Di Leo R, Vita G, Santamaria LB. Heart rate variability: a diagnostic and prognostic tool in anesthesia and intensive care. Acta Anaesthesiol Scand. 2011;55(7):797-811.
10. Buchman TG, Stein PK, Goldstein B. Heart rate variability in critical illness and critical care. Curr Opin Crit Care. 2002;8(4):311-315.
11. Stein PK. Challenges of heart rate variability research in the ICU. Critical Care Medicine 2013;41(2):666-667.
12. Reimer P, Máca J, Szturz P, Jor O, Kula R, Ševčík P, Burda M, Adamus M. Role of heart-rate variability in preoperative assessment of physiological reserves in patients undergoing major abdominal surgery. Ther Clin Risk Manag. 2017;13:1223-1231.
13. Bradley BD, Green G, Ramsay T, et al. Impact of sedation and organ failure on continuous heart and respiratory rate variability monitoring in critically ill patients: a pilot study. Crit Care Med. 2013;41(2):433-444.
14. Sakata K, Yoshimura N, Tanabe K, Kito K, Nagase K, Iida H. Prediction of hypotension during spinal anesthesia for elective cesarean section by altered heart rate variability induced by postural change. Int J Obstet Anesth. 2017;29:34-38.
15. Jess G, Pogatzki-Zahn EM, Zahn PK, Meyer-Frießem CH. Monitoring heart rate variability to assess experimentally induced pain using the analgesia nociception index. Eur J Anaesthesiol. 2016;33(2):118-125.
16. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: Standards of measurement, physiological interpretation and clinical use. Circulation. 1996;93(5):1043-1065.
17. Padley J, Ben-Menachem E. Low pre-operative heart rate variability and complexity are associated with hypotension after anesthesia induction in major abdominal surgery. J Clin Monit Comput. 2018;32:245-252.
18. Hanss R, Bein B, Weseloh H, Bauer M, Cavus E, Steinfath M, et al. Heart rate variability predicts severe hypotension after spinal anesthesia. Anesthesiology. 2006;104:537-45.
19. Omerbegović M. Short-term parameters of heart rate variability during balanced anaesthesia with administration of two different inhalation anesthetics. Med Arch. 2014;68:268-71.
20. Rawal P, Bajracharya U. Hemodynamic response to sevoflurane and propofol induction: a comparative study. J Soc Anaesthesiol Nepal. 2015;2(1):2-7.
21. Yeganeh N, Roshani B, Almasi A, Jamshidi N. Correlation between bispectoral index and predicted effect-site concentration of propofol in different levels of target-controlled, propofol induced sedation in healthy volunteers. Arch Iran Med. 2010;13:126-34.
22. Vettorello M, Colombo R, De Grandis CE, Costantini E, Raimondi F. Effect of fentanyl on heart rate variability during spontaneous and paced breathing in healthy volunteers. Acta Anaesthesiol Scand. 2008;52:1064-1070.
23. Mohit M, Radhakrishnan M, Umamaheswara R, Kavyashree K, Vishnuprasad K. Assessment of heart rate variability during different propofol effect site concentrations in patients with supratentorial tumours: a pilot study. J Neuroanaesthesiol Crit Care. 2017;4:108-113.
24. Tarvainen MP, Georgiadis S, Lipponen JA, Laitio T, Karjalainen PA, Scheinin H, Kaskinoro K. Analysis of heart rate variability dynamics during propofol and dexmedetomidine anesthesia. In: 32nd Annual International Conference of the IEEE EMBS; 2010 Aug 31- Sept 4; Buenos Aires, Argentina; 2010. p. 1634-7.
25. Tsugayasu R, Handa T, Kaneko Y, Ichinohe T. Midazolam more effectively suppresses sympathetic activations and reduces stress feelings during mental arithmetic task than propofol. J Oral Maxillofac Surg. 2010;68:590-6.
26. Win NN, Fukayama H, Kohase H, Umino M. The different effects of intravenous propofol and midazolam sedation on hemodynamic and heart rate variability. Anesth Analg. 2005;101:97-102.
27. Hidaka S, Kawamoto M, Kurita S, Yuge O. Comparison of the effects of propofol and midazolam on the cardiovascular autonomic nervous system during combined spinal and epidural anesthesia. J Clin Anesth. 2005;17:36-43.
28. Billman, GE. The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. Front Physiol. 2013;4:26.
29. Smith AL, Owen H, Reynolds K. Heart rate variability indices for very short-term (30 beat) analysis. Part 1: survey and toolbox. J Clin Monit Comput. 2013;27:569-576.
30. Riznyk L, Fijałkowska M, Przesmycki K. Effects of thiopental and propofol on heart rate variability during fentanyl-based induction of general anesthesia. Pharmacol Rep. 2005 Jan-Feb;57:128-34.
31. Kanaya N, Hirata N, Kurosawa S, Nakayama M, Namiki A. Differential effects of propofol and sevoflurane on heart rate variability. Anesthesiology. 2003;98:34-40.
32. Hamada Y, Kamevama T, Iizuka T, Nishiyama T, Ishizaki T, Isshiki A. Effects of propofol and fentanyl anesthesia on heart rate variability using two analytical methods. Eur J Anesth. 2004;21 Suppl 32:A-98.
33. Win N, Kohase H, Yoshikawa F, Wakita R, Takahashi M, Kondo N, Ushito D, Umino M. Haemodynamic changes and heart rate variability during midazolam-propofol co-induction. Anaesthesia. 2007;62:561-8.

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Topographico-anatomic peculiarities of the external carotid artery in the perinatal period

*[1]**Oleksandr Slobodian,** MD, PhD, Professor; [2]**Lalita Gerasym,** MD, Assistant Professor

[1]Department of Anatomy, Topographic Anatomy and Operative Surgery
[2]Department of Surgical Dentistry and Maxillofacial Surgery
Bukovinian State Medical University, Chernivtsi, Ukraine

*Corresponding author: slobodjanaleksandr@ukr.net
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Abstract

Background: the importance of the given issue is in clarification of ontogenetic transformations of the external carotid artery during the perinatal and early neonatal periods, which is essential from the view of surgical treatment of congenital pathology of the cervical vessels in fetuses, neonates and infants.
Material and methods: the study was performed on 50 specimens of dead fetuses (from 4 to 10 months) and 9 neonates (5 isolated complexes of organs in particular) without external signs of anatomical defects or deviations, and without visible macroscopic anomalies from the normal structure of the cardio-vascular system. Adequate anatomical methods of investigation were applied during examination: macropreparation, injection of the blood vessels, making topographic-anatomical sections, morphometry, and statistical analysis.
Results: the cervical part of the external carotid artery during the perinatal period is a distance from its origin to the point of crossing with the stylohyoid muscle. The major branches of the cervical part of the external carotid artery are: the superior thyroid one, hyoid, facial, occipital and posterior auricular arteries. The two types are peculiar for the branching of the external carotid artery: main (77%) and scattered (23%). Variability of emerging branches from the external carotid artery is found in 3.5% of cases.
Conclusions: determination of typical and variant topography of the external carotid artery and its branches will promote implementation of new methods to perform radical and reconstructive-restorative surgery on the cervical vessels.

Key words: external carotid artery, topography, fetus, neonate, human subject.

Introduction

Numerous defects found in clinical practice in most cases can be explained on the basis of finding the origin and relations of the organs and structures which in the course of time acquire the shapes peculiar for them, investigating their unusual topography and understanding appropriate embryonic phenomena comprehensively [1, 2, 3]. Development of new directions in operative surgery, elaboration of new surgical methods, always require their anatomical substantiation [4, 5, 6, 7]. Congenital vascular defects are third among other diseases including hypoxic lesions and birth injuries. In 83% of cases ischemic strokes in children occur due to vascular defects. Approximately 70% of infants develop convolution of the carotid arteries associated with coarctation of the aorta (CoA), developmental variants of Willis' circle, aortic aneurism, underdevelopment of the anterior cerebral artery, high degree of bifurcation of the common carotid artery [8, 9, 10, 11]. A general frequency of defects of the carotid arteries depending on the results of angiographic and pathologic anatomical examinations ranges within 10 to 40% [12, 13, 14]. Nowadays pathologic convolution of the carotid arteries is considered to be congenital pathology occurring due to embryogenesis disorders of the carotid arteries. According to the data of autopathies this pathology is found in 14% of cases, and according to the findings of Doppler scanning C-like convolution of the carotid arteries is found in 33.6%, and S-like – in 66.4% of cases [15, 16, 17].

Priority of the study consists of finding ontogenetic transformations of the external carotid artery during the perinatal period of human ontogenesis which is an important issue from the view of surgical treatment of congenital pathology of the cervical vessels in fetuses, neonates and infants.

The objective is to determine topographic-anatomical peculiarities of the external carotid artery and its branches during the fetal and early neonatal periods of human ontogenesis.

Material and methods

The study was performed on 50 specimens of dead fetuses (from 4 to 10 months) and 9 neonates (5 isolated complexes of organs in particular) without external signs of anatomical defects or deviations, and without visible macroscopic anomalies from the normal structure of the cardio-vascular system. Adequate anatomical methods of investigation were applied during examination: macropreparation, injection of the blood vessels, making topographic-anatomical sections, morphometry, and statistical analysis. Injection of the blood vessels, and the cervical arteries in particular, was performed after catheterization of the descending part of the aorta. The catheter was directed to the cranium, the mixture of red-lead paint for injection was introduced. After fixation of the specimens of dead fetuses and neonates macropreparation of the external carotid artery and its branches was performed by means of forceps and scissors.

The study was conducted according to the major requirements of the Declaration of Helsinki on ethical principles to provide scientific-medical research involving human subjects elaborated by the World Medical Association (1964-2000) and the Order of the Ministry of Health of Ukraine № 690 dated 23.09.2009. It is a fragment of a complex planned initiative scientific-research work

of M. G. Turkevych Department of Human Anatomy and Department of Anatomy, Topographic Anatomy and Operative Surgery of Bukovinian State Medical University: "Peculiarities of Morphogenesis and Topography of the Organs and Systems During Prenatal and Postnatal Periods of Ontogenesis " (State Registration № 0115U002769).

Results and discussion

Within the borders of the carotid triangle the common carotid artery is found to be dichotomically divided into the internal and external carotid arteries. The external carotid artery extends in the cranial direction joining the muscles of the supra- and subhyoid groups of the neck. In our opinion, the cervical part of the external carotid artery is determined from the point of its origin (division of the common carotid artery into the final branches) to the point of the artery joining the stylohyoid muscle. During the perinatal period the major branches emerge from the external carotid artery upwards in the cranial direction: the superior thyroid, hyoid, facial, occipital and posterior auricular ones.

Usually the superior thyroid artery branches from the median surface of the external carotid artery 0.3-0.5 cm higher from the point of bifurcation of the common carotid artery. The superior thyroid artery passes upwards close to the lateral border of the omohyoid muscle, forms an arch, and then changes its direction downwards (fig. 1). It joins the posterior surface of the omohyoid muscle. The superior thyroid artery branches the superior laryngeal artery; it divides dichotomically into the final branches near the superior border of the thyroid gland. These branches participate in blood supply of an appropriate lobe of the thyroid gland.

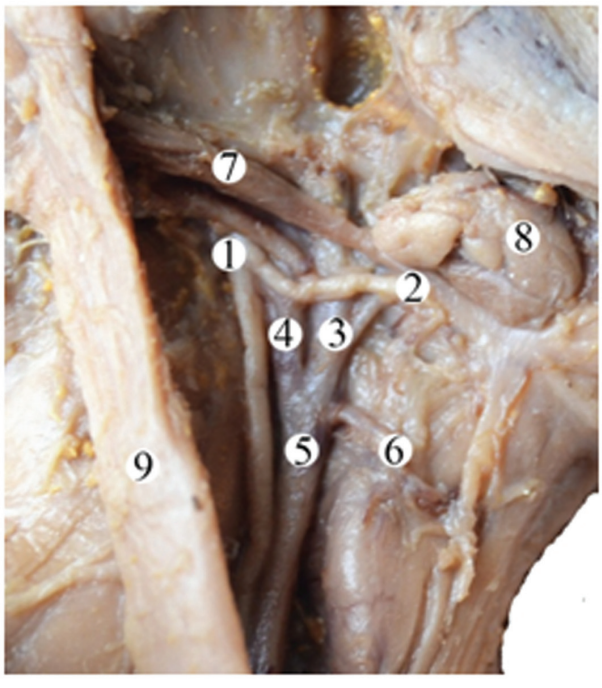

Fig. 2. The anterior part of the neck (from the right) of the fetus, 380.0 mm of the parietal-calcaneal length. Macrospecimen, magnification 1.6ˣ: 1 – vagus. 2 – superior laryngeal nerve, 3 – external carotid artery, 4 – internal carotid artery, 5 – common carotid artery, 6 – superior thyroid artery, 7 – digastric muscle, 8 – submandibular gland, 9 – sternocleidomastoid muscle.

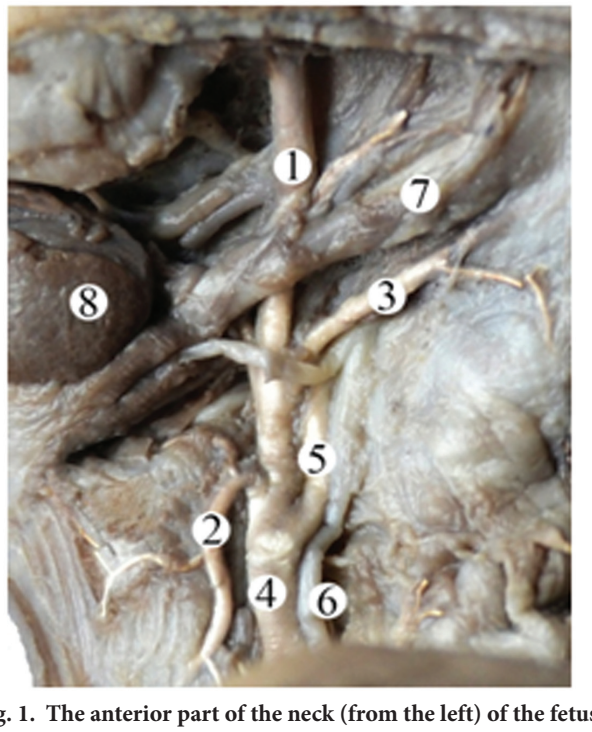

Fig. 1. The anterior part of the neck (from the left) of the fetus, 300.0 mm of the parietal-calcaneal length (the superficial layer of cervical muscles is removed). Macrospecimen, magnification 2.1ˣ: 1 – external carotid artery, 2 – superior thyroid artery, 3 – occipital artery, 4 – common carotid artery, 5 – internal carotid artery, 6 – internal jugular vein, 7 – digastric muscle, 8 – submandibular gland.

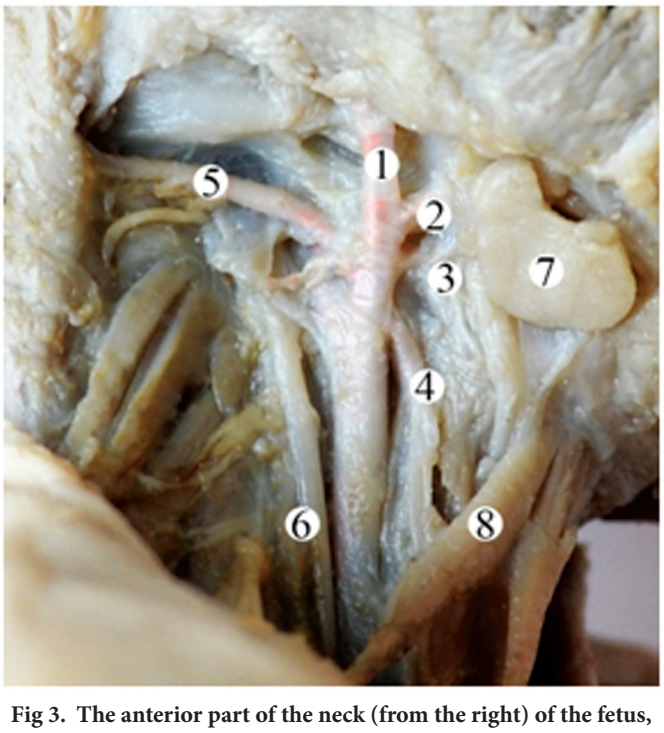

Fig 3. The anterior part of the neck (from the right) of the fetus, 210.0 mm of the parietal-calcaneal length (subcutaneous and sternocleidomastoid muscles are removed). Macrospecimen, magnification 1.3ˣ: 1 – external carotid artery, 2 – facial artery, 3 – hyoid artery, 4 – superior thyroid artery, 5 – occipital artery, 6 – internal jugular vein, 7 – submandibular gland, 8 – omohyoid muscle.

Only in two cases (3.5%) during the perinatal period the superior thyroid artery emerges from the median surface of the common carotid artery lower from the point of its bifurcation (fig. 2). In these cases the superior thyroid artery extends downwards forming branches in the same direction and dichotomically divides into the final branches.

The hyoid artery emerges from the median surface of the external carotid artery. Usually in 75% of cases its direction is upward, in the rest of observations (25%) it extends horizontally. The hyoid artery is located posteriorly from the posterior ventricle of the digastric muscle approaching the inferior border of the submandibular gland. Further topography of the hyoid artery depends on the syntopic effect of the submandibular gland, and its sizes in the perinatal period in particular.

Higher from the hyoid artery the occipital artery emerges from the lateral surface of the external carotid artery. The direction of the occipital artery is dorsoascending. It extends along the inferior border of the posterior ventricle of the digastric muscle (fig. 1).

The facial artery emerges from the external carotid artery practically on the same level of the occipital artery, but from the opposite surface, that is from the median one. The facial artery usually extends in the ventrodorsal direction, and close to the superior border of the posterior ventricle of the digastric muscle it joins the superior border of the submandibular gland. The facial artery crosses the inferior border of the mandible near the anterior masticatory muscle and passes to the mandibular-facial area.

Cranially from the point where the occipital artery emerges from the external carotid artery the posterior auricular artery originates. It has dorsocranial direction and extends between the posterior ventricle of the digastric muscle and stylohyiod muscle practically parallel to the occipital artery.

Beginning from the third trimester of the perinatal development the median group of branches of the external carotid artery is found in 12% of cases, namely the branches of the ascending pharyngeal artery penetrating the muscles and participating in blood supply of the pharynx.

The presented topography of the branching of the external carotid artery is found in 77% of cases. This type of branching of the external carotid artery is the main one. Only in 23% of observations a scattered type of branching of the external carotid artery is found. The hyoid, facial, occipital and posterior auricular arteries emerge from the external carotid artery practically on the same level, that is in a fan-shaped manner (fig. 3). At the same time, the superior thyroid artery possesses a typical feature of emerging from the external carotid artery. A scattered type of branching of the external carotid artery in the majority of observations (70%) is peculiar for the early fetuses (4-5-months). It is usually found in the right side.

Conclusions

1. The cervical part of the external carotid artery during the perinatal period is a distance from its origin to the point of crossing with the stylohyoid muscle.

2. The major branches of the cervical part of the external carotid artery are: the superior thyroid one, hyoid, facial, occipital and posterior auricular arteries.

3. The two types are peculiar for the branching of the external carotid artery: main (77%) and scattered (23%).

4. Variability of emerging branches from the external carotid artery is found in 3.5% of cases.

References

1. Akhtemiichuk IuT, Slobodian OM, Hmara TV, et al. Narisi perinatalnoi anatomii [Essays on perinatal anatomy]. Chernivtsi; 2011. 300 p. Ukrainian.
2. Akhtemiichuk IuT. Aktual´nist´ naukovikh doslidzhen´ u galuzi perinatal´noi anatomii [Actuality of scientific research in the field of perinatal anatomy]. Neonatologiia, Khirurgia ta Perinatal´na Meditsina [Neonatol Surg Perinatal Med]. 2012;2(1):15-21. Ukrainian.
3. Menshawi K, Mohr JP, Gutierrez J. A functional perspective on the embryology and anatomy of the cerebral blood supply. J Stroke. 2015;17(2):144-58. doi: 10.5853/jos.2015.17.2.144.
4. Benouaich V, Porterie J, Bouali O, et al. Anatomical basis of the risk of injury to the right laryngeal recurrent nerve during thoracic surgery. Surg Radiol Anat. 2012;34(6):509-12.
5. Fluss J, Garcia-Tarodo S, Granier M, Villega F, Ferey S, Husson B, et al. Perinatal arterial ischemic stroke related to carotid artery occlusion. Eur J Paediatr Neurol. 2016;20(4):639-48. doi: 10.1016/j.ejpn.2016.03.003.
6. Popović R, Radovinović-Tasić S, Rusović S, Lepić T, Ilić R, Raičević R, et al. Urgent carotid stenting before cardiac surgery in a young male patient with acute ischemic stroke caused by aortic and carotid dissection. Vojnosanit Pregl. 2016;73(7):674-8.
7. Katory Y, Kawase T, Ho Cho K, et al. Suprahyoid neck fascial configuration, especially in the posterior compartment of the parapharyngeal space: a histological study using late-stage human fetuses. Clin Anat. 2013;26(2):204-12.
8. Agrawal R, Agrawal SK. Dangerous anatomic variation of internal carotid artery – a rare case report. Int J Anat Var (IJAV). 2011;4:174-6.
9. Beigelman R, Izaguirre A, Robles M, Grana D, Ambrosio G, Milei J. Kinking of carotid arteries is not a mechanism of cerebral ischemia: a functional evaluation by Doppler echography. Int Angiol. 2011;30:342-8.
10. Dadashov SA, Lavrent'ev AV, Frolov KB, Vinogradov OA, Dziundzia AN, Ul'ianov ND. Khirurgicheskoe lechenie patologicheskoi izvitosti vnutrennei sonnoi arterii [Surgical treatment of the pathological tortuosity of internal carotid artery]. Angiologiia i Sosudistaia Khirurgiia [Angiol Vasc Surg]. 2012;18(3):116-21. Russian.
11. Pfeiffer J, Ridder GJ. A clinical classification system for aberrant internal carotid arteries. Laryngoscope. 2008;118(11):1931-36.
12. Avazashvili ID, Skorokhoda II, Tysh II. Otsenka effektivnosti karotidnogo stentirovaniia pri stenoziruiushchikh porazheniiakh sonnykh arterii [Assessment of the efficiency of carotide stenting during penyring damages of the carotid arteries]. Zhurnal Natsional´noi Akademii Medichnikh Nauk Ukraini [J Natl Acad Med Sci Ukr]. 2013;19(Suppl):15. Russian.
13. Gavrilenko AV, Abramian AV, Kuklin AV, Ofosu D. Patologicheskaia izvitost' vnutrennei sonnoi arterii: klinika, diagnostika i khirurgicheskoe lechenie [Internal carotid artery kinking: the clinic, diagnosis and surgical treatment]. Kardiologiia i Serdechno-sosudistaia khirurgiia [Cardiol Cardiovasc Surg]. 2016;9(1):29-33. doi: 10.17116/kardio20169129-33. Russian.
14. Gavrilenko AV, Abramyan AV, Kuklin AV. Sravnitel'nyi analiz rezul'tatov khirurgicheskogo i konservativnogo lecheniia bol'nykh s patologicheskoi izvitost'iu sonnykh arterii [Comparative analysis of the results of the surgical and conservative treatment of patients with pathological tortuosity of carotid arteries]. Angiologiia i Sosudistaia Khirurgiia [Angiol Vasc Surg]. 2012;18(4):93-9. Russian.
15. Abramova MF, Shurupuva NS. Ul'trazvukovoe dupleksnoe skanirovanie i klinicheskie osobenosti ekstrakranial'nykh anomalii vnutrennykh sonnykh arterii u detei [Ultrasonic duplex scanning and clinical peculiarities of the extracranial abnormalities of the internal carotid arteries in children]. Pediatr Farmakol. 2009;6(3):80-3. Russian.
16. Kaplan ML, Bontsevich DN. Techenie sosudistoi mozgovoi nedostatochnosti pri patologicheskoi izvitosti sonnykh arterii, ee rol' pri opredelenii pokazanii k operativnomu lecheniiu [The course of cerebral vascular insufficiency in pathological tortuosity of carotid arteries, its role in indications for surgical treatment]. Problemy zdorov'ia i ekologii [Probl Health Ecol] (Gomel). 2014;3:95-100. Russian.
17. Kathuria S, Gregg L, Chen J, Gandhi D. Normal cerebral arterial development and variations. Semin Ultrasound CT MR. 2011;32:242-51. doi: 10.1053/j.sult.2011.02.002.

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Treatment of inflamed skin wounds with biodegradable polymeric film "Biodep nano"

*[1]**Oleg Popadyuk,** MD, PhD, Associate Professor; [2]**Roman Kutsyk;** MD, PhD, Professor;
[1]**Maryana Voloshyn,** MD, PhD, Associate Professor

[1]Department of General Surgery, [2]Department of Microbiology
Ivano-Frankivsk National Medical University, Ivano-Frankivsk, Ukraine

*Corresponding author: popadyukoleg@ukr.net
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Abstract

Background: Experimental and clinical studies show that wound healing cannot be evaluated as optimally effective today. For the treatment of wounds today, various methods and means are used. Wound dressing was developed from both natural and synthetic materials. However, polymeric biodegradable materials, saturated with various active drugs, which are effective, easy to use, have insignificant disadvantages and need further study. The aim was to study the effectiveness of the use of biodegradable polymeric film "biodén nano" in the treatment of suppurable postoperative wounds of the skin in the experiment.

Material and methods: The research was carried out on 45 guinea-pigs of the species "Murchaky" in the clinical and biological base (Vivarium) of the Ivano-Frankivsk National Medical University, in accordance with the requirements for the maintenance and handling of laboratory animals. The pelvic wounds were simulated after *staphylococcus aureus* $2.0*10^8$ KU/ml infection.

Results: The change in the wound area of the group IV and group V decreased slightly up to the third day, and after 7 days the area of the wound surface was reduced by 7% and 13.6% respectively. During the observation period, 14 days, the wound area in relation to the initial area was 35.5% in group IV and 43% in group V.

Conclusions: The biodegradable polymeric film "biodep nano" demonstrated high antimicrobial and wound healing properties in an experimentally simulated peptic cutaneous wound of the skin.

Key words: purulent wound, polymer films, treatment.

Introduction

Experimental and clinical studies show that the results of wound treatment cannot be estimated today as optimally effective, and success depends on their local treatment. It is important to search for new methods and means of local treatment of multi-directional actions that provide antimicrobial, anti-inflammatory and reparative effects [1].

The process of wound healing includes processes for restoring the barrier function of the skin, preventing dehydration and reducing the risk of bacterial infection [2].

The problem of effective healing of purulent wounds is also associated with high polyresistance of pathogenic microorganisms to modern antibacterial drugs [3].

Bacteria die under the action of antibiotics and antiseptics, which are used for wound dressing. In general, antibiotics for local wound therapy are not recommended because of minimal efficacy and also do not reach the bactericidal concentrations in situ, thus they can form resistant strains and further sensitization. However, in some indications, local antibiotic use still plays an important role in the clinical management of specific infections (e. g., keratitis, conjunctivitis, and otitis media). In the case of such wound infections, as a rule, systemic use of antibiotics is recommended [4].

For the treatment of wounds today, different methods and means are used. Wound dressing was developed from both natural and synthetic materials. The ideal material should be elastic, maintain the moisture and pH in the wound environment, prevent bacterial contamination and promote painless and rapid healing of the wounds [5].

For example, the latest silk biomaterial bandages consisting of nano-sized silk fibers showed good result *in vivo* studies in treating skin wounds in mice [6].

However, polymeric biodegrading materials, saturated with various active drugs, which are effective, easy to use, have pitfalls and require further study [7, 8].

For the treatment of purulent wounds of different genesis, we have developed a biodegradable polymer film that contains nanosized zinc oxide and nanosized hydrated fullerene C_{60}.

Our comparative experimental studies *in vitro* have shown that zinc nanoxide is a highly effective antimicrobial agent in both gram positive and gram negative pathogen flora [9].

The first fullerenes of the C_{60} and C_{70} were discovered in 1985, and almost immediately fullerenes attracted the attention of many researchers, including from the point of view of the possibility of their use in biology and medicine [10, 11].

The biological activity of fullerene is due, of course, to its physical and chemical properties, and therefore it is capable

of illuminating the properties of an oxidant, while in the dark it acts as a highly active antioxidant due to its ability to "capture" free radicals [11].

Goal: To study the effectiveness of using a biodegenerative polymeric film "Biodep nano" with nanosized hydrated fullerene C_{60} in the treatment of inflamed wounds of the skin in the experiment.

Material and methods

The study was conducted on 45 guinea-pigs of the species "Murchaky" weighing 323 (303-346) grams, which were on a balanced diet of clinical and biological base (Vivarium), Ivano-Frankivsk National Medical University, according to sanitary and hygienic standards (Scientific and Practical Guidelines for the Maintenance and Operation of Laboratory Animals, 2002), in accordance with the requirements of the General Ethical Principles of Animal Experiments, approved by the National Congress on Bioethics (September 20, 2004, Kyiv, Ukraine), agreed upon with "The rules of the slaughter of animals using experimental animals", approved by the order of the Ministry of Health of Ukraine and the Law of Ukraine "On the Protection of Animals from Cruel Treatment" (No 1759-VI of 15.12.2009) and the rules of the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes [12].

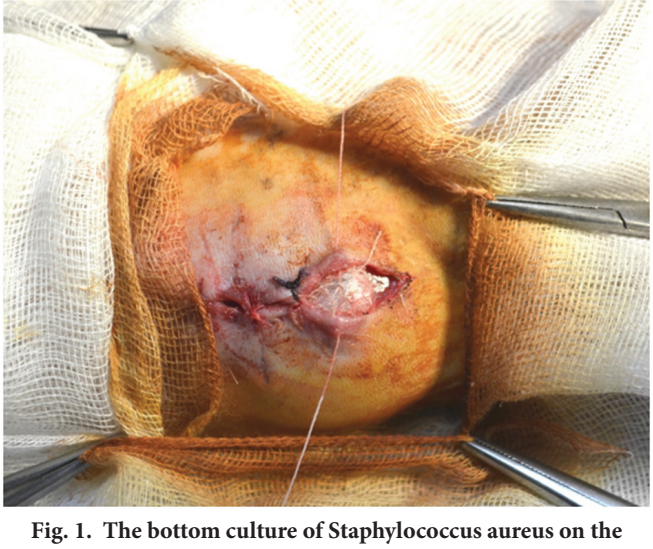

Fig. 1. The bottom culture of Staphylococcus aureus on the wound.

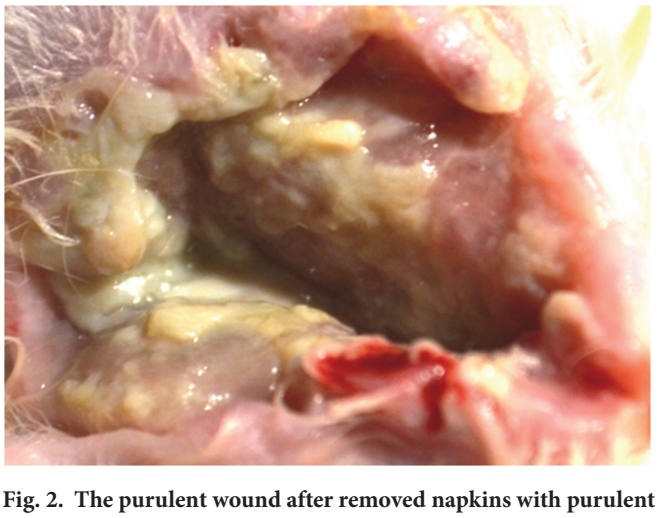

Fig. 2. The purulent wound after removed napkins with purulent contents and purified contents by physically sterile tupfer.

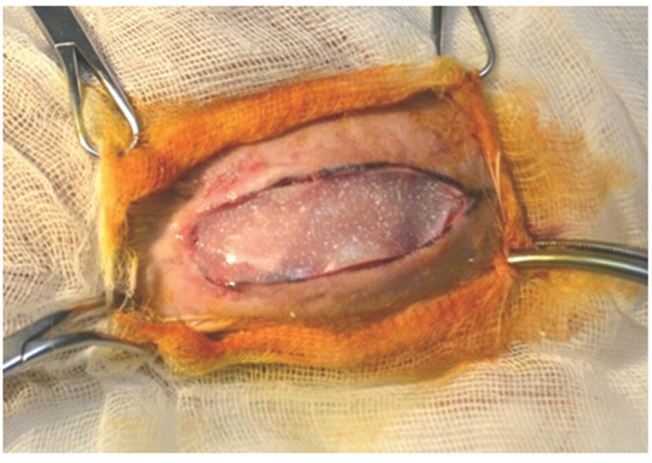

Fig. 3. Attachment of a polymer film to the wound.

For reproduction of puffy skin injury to animals under general anesthesia of oxybutyrate by the calculation of 0.15 ml per 100 g of animal weight in the interlopatal region, a cutaneous wound of skin covering 50 mm long was modeled. At the bottom of the wound, a cloth dampened with a culture of *Staphylococcus aureus* (concentration $2.0*10^8$ KU/ml) was placed on the wound and 10 mm apart of the nodule seams were applied, the wound was treated with a betadine solution from the outside (fig. 1). 3 days later we removed seams, napkins with purulent contents and purified the wound from purulent contents by physically sterile tupfer, washing with 0.9% physiological saline solution of sodium chloride (fig. 2). A sterile napkin was applied and fixed with a fixing tape on paper basis in two places.

Animals were divided into four groups: group I intact (5 animals); group II (10 animals) were not infected; group III (10 animals) wounds were infected and managed without additional treatment with the replacement of sterile gauze napkins every other day; group IV (10 animals) used curasorb ZN wounds; group V (10 animals) applied a biodegradable polymeric film "Biodep nano", which was additionally saturated with a solution of hydrated fuller C_{60}.

The area of the wound was measured at the beginning of the study and according to the specified observation dates at 3,7,14 and 21 days.

The curasorb ZN wound coat used in the control group is made in the form of a plate, consisting of natural alginate fibers and contains zinc [13].

Results

After infecting and waiting for the time required to form purulent wound contents, it was found that the *Staphylococcus aureus* content in the wound exudate was $4*10^8$ KU/ml.

After removal from the wound of wipes with the causative agent and purification of purulent contents, films were applied and observed (fig. 3).

By the 3rd day in the group III of infected animals, the wounds had purulent contents, swollen edges, and a significant excretion of the exudate and an increase of the wound area.

The decrease in the number of bacteria by the 3rd day was recorded in group IV and V, in contrast to group III, in two orders of magnitude, where the level of bacteria remained high, indicating an active increase in their number in the wound without a specific local antiseptic effect.

Wound coatings used in group IV and group V showed high sorption ability and gradually degraded the active substance. Visually, in animals of these groups, the wounds were well cleaned, and their bottom had virtually no purulent content. More pronounced sorption and regenerative effects were observed in group V up to the 7th day, as evidenced by the results of microbiological studies and measurement of the wound area. At day 7, the level of bacteria in the wound of group IV decreased sharply by 3 orders of magnitude and in the group V by 4 orders of magnitude comparatively. This testified to the high efficiency of absorbent roofing materials saturated with antiseptic agents. On the 14th day the pathogen was sown only in the group without treatment (tab. 1). One animal in this group died on the 7th day, one on the 14th day.

The change in the wound area of the group IV and group V decreased slightly until the third day, and by the 7th day the area of the wound surface was reduced by 7% and 13.6%, respectively. During the observation period, 14 days, the wound area in relation to the initial area was 35.5% in group IV and 43% in group V.

The results obtained by us testify to the effective influence of the biodegradable coating on the healing of the cutaneous wounds of the skin of infected *Staphylococcus aureus*. The closure of the wound defect occurred most rapidly by the application of our innovative roofing material for wounds in the form of a biodegradable nanofill polymer film saturated with zinc oxide and hydrated fullerene C_{60}. By the 21st day, the free area of the wounds of the animals of the group where the film was applied was 9.6%, which is critically low and indicates the high level of film exposure to wound healing (tab. 2).

Discussion

Local antiseptic is an anti-infectious treatment of choice. In the case of wound infections, it is advisable to use a local antiseptic to immediately stop the microbial replication, as well as the appropriate spread of the infection in the wound environment. This is of particular importance in the treatment of infections by multi-resistant strains such as *Staphylococcus aureus* resistant to methicillin, resistant to enterocomycin enterococci, and organisms that produce the extended spectrum of betalactamase [4].

The synthesis of the polymer proposed by us became possible due to microwave irradiation in accordance with the timelines, which facilitated the polymerization of the applied components. Thanks to the peculiarities of the composition and manufacturing technology, it was possible to achieve elasticity, gradual degradation, the required steam and moisture permeability. At the final stage of manufacture, zinc nanoparticles were added to the film, which provided excellent antimicrobial properties. Thus, on the third day, the concentration of *Staphylococcus aureus* was such that the pathogen was not at risk, which compared with uninfected

Table 1

The level of bacterial contamination of wounds in different terms of observation

Group		Terms of observation, the day				
	Infection	0	3	7	14	21
		Level of microbial contamination, CFU / ml				
II	-	-	-	-	-	-
III	$2,0*10^8$	$(4.1±0.5)*10^8$	$(5.1±3.6)*10^7$	$(6.6±2.4)*10^6$	$(6.1±6.2)*10^5$	-
IV		$(4.7±0.7)*10^8$	$(3.6±0.4)*10^5$	$(2.9±0.5)*10^3$	-	-
V		$(6.7±2.8)*10^8$	$(3.1±0.3)*10^5$	$(2.1±0.7)*10^2$	-	-

Table 2

Level of wound area at different observation periods

Group	Terms of observation, the day				
	0	3	7	14	21
	area S, mm^2				
II	500(488-514)	453(444-463)	357(344-365)	149(144-154)	
III	521.5(509-534)	555.5(528-580)	510(487-530)	443.5(401-459.5)	265(255-281)
IV	534.8(593-578)	528(514-575)	497(482-505)	345(336-374)	84(78-96)
V	540.3(528-575)	508(480-517)	466(450-474)	307(280-336)	21(14-26)

wounds showed an effective stable effect of the polymer. The results of the application of polymer materials in the world literature confirm the results of the data, namely, the use of nanosized metal oxides such as MgO, TiO_2, Al_2O_2, CuO, SeO_2 and ZnO is highly effective against pathogenic strain-forming strains of *Staphylococcus aureus* [14].

The results of the study of the properties of polymeric materials in the treatment of wounds presented in literature confirmed the high efficiency of polymers due to high sorption and moisture-retaining properties, but at the same time revealed shortcomings and the need for their further improvement [5].

The results obtained by us showed and confirmed the effectiveness and feasibility of the use of fullerene in the treatment of wounds. The assertion that fullerene C_{60} is an antioxidant is found in scientific literature. Thus, studies have demonstrated that the surface modified by fullerene has a pronounced antioxidant effect [15].

Prospects: The polymer material we have developed in the form of a film can be manufactured in different sizes and thicknesses, has good elasticity and takes the form of a wound or other surfaces, releases the active substance gradually over several days, thus reducing the bonding tarmines and their quantity, possesses moisture-preserving and sorption properties. That is why this polymer has the prospect of further research, study and application in surgery.

Conclusions

1. Biodegradable nanocontaining polymeric film "Biodep-nano", which is additionally saturated with hydrated fullerene C_{60}, showed high antimicrobial properties in relation to pathogenic strains of *Staphylococcus aureus* in a comparative study with known roofing materials in esperimentally simulated purulent wounds of the skin.

2. The proposed new biodegradable polymeric material is a promising means of local wound healing, requiring further study and implementation in practical surgery.

References

1. Mnikhovich MV, Eremin NV. Eksperimental'no-morfologicheskii analiz gistogeneza kozhnoi rany pod vliianiem nizkointensivnogo lazernogo izlucheniia [Experimental and morphological analysis of histogenesis of skin wounds under the influence of low-intensity laser radiation]. Vestnik Novykh Meditsinskikh Tehnologii [J New Med Technol] (Tula, Russia). 2013;20(2):113-120. Russian.
2. Rowan MP, Cancio LC, Elster EA, Burmeister DM, et al. Burn wound healing and treatment: review and advancements. Crit Care. 2015;(19):243. doi: 10.1186/s13054-015-0961-2.
3. Daunton C, Kothari S, Smith L, et al. A history of materials and practices for wound management. Wound Pract Res. 2012;20(4):174-186.
4. Daeschlein G. Antimicrobial and antiseptic strategies in wound management. Int Wound J. 2013;10 Suppl 1:9-14.
5. Wiegand C, Hipler UC. Polymer-based biomaterials as dressings for chronic stagnating wounds. Macromol Symp. 2010;294:1-13.
6. Gil ES, Panilaitis B, Bellas E, Kaplan DL. Functionalized silk biomaterials for wound healing. Adv Healthc Mater. 2013;2(1):206-217.
7. Hakkarainen T, Koivuniemi R, Kosonen M, et al. Nanofibrillar cellulose wound dressing in skin graft donor site treatment. J Control Release. 2016;244(Pt B):292-301.
8. El-Feky GS, Sharaf SS, El Shafei A, et al. Using chitosan nanoparticles as drug carriers for the development of a silver sulfadiazine wound dressing. Carbohydr Polym. 2017;158:11-19. doi: 10.1016/j.carbpol.2016.11.054.
9. Popadyuk OY. Antimicrobial effect of biodegradable wound healing nano-containing polymer materials. Mold Med J. 2017;60(1):35-38.
10. Kroto HW, Heath S, O'Brien SC, Curl RF, Smalley RE. C60: Buckminsterfullerene. Nature. 1985;318:162-163.
11. Piotrovskii LB, Kiselev OI. Fullereny v biologii [Fullerenes in biology]. SPb: Rostok; 2006. 336 p. Russian.
12. Popadyuk OY. Patomorfologichni osoblivosti vidnovlennia poshkodzhenikh miakikh tkanin iz zastosuvanniam biorozchinnoi polimernoi plivki v eksperymenti [Pathomorphological features of restoration of damaged soft tissues using a biodegradable polymer film in an experiment]. Ukrayins'kii Zhurnal Khirurgii [Ukr J Surg]. 2013;(4/23):67-72. Ukrainian.
13. Thomas S. Surgical dressings and wound management. Cardiff: Medetec; 2010. 708 p.
14. Brayner R, Ferarri-Iliou R, Brivois N, Djediat S, Benedetti MF, Fiévet F. Toxicological impact studies based on Escherichia coli bacteria in ultrafine ZnO nanoparticles colloidal medium. Nano Lett. 2006;6:866-870.
15. Piotrovskii LB, Eropkin MIu, Eropkina EM, Dumpis MA, et al. Mekhanizmy biologicheskogo deistviia fullerenov - zavisimost' ot agregatnogo sostoianiia [Mechanisms of biological action of fullerenes – dependence on the aggregate state. Psikhofarmakologiia i Biologicheskaia Narkologiia [Psychopharmacol Biol Narcology] (St. Petersburg, Russia). 2007;7(2):1548-1554. Russian.

REVIEW ARTICLES

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Disturbance of bioelectric transmission in carcinogenesis

*[1]**Ilarion Draguta,** MD, PhD; [1]**Anatolie Mustea,** MD, PhD; [3]**Constantin Popescu,** MD, PhD, Associate Professor; [1]**Cornel Iurcu,** MD, PhD; [2]**Valeriu Palade,** MD, PhD

[1]Division of Urology, [2]Division of Head and Neck Surgery, Institute of Oncology
[3]Department of Oncology, Nicolae Testemitsanu State University of Medicine and Pharmacy
Chisinau, the Republic of Moldova

*Corresponding author: ilariondraguta@yahoo.com
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Abstract

Background: Despite significant financial resources invested in the field of cancer research, there has been a steady increase in the registration of new cases of malignant neoplasms. Modern technical capabilities for analyzing the molecular substrate of tumor genesis have revealed a large number of such factors. The latest studies point out the paramount importance of the integral bioelectric field as contrasted with molecular mechanisms in oncopathology. Clear evidence has emerged that the "decision" of a certain part of the body to develop a tumor depends on the bioelectric state of remote regions. In the light of these findings, it becomes obvious that the difficulties in solving the cancer problem are associated with a simplified approach focused only on molecular components.

Conclusions: It can be assumed that the difficulties in solving the cancer problem are associated with a simplified approach, focused only on molecular components. It is difficult to identify clear differences between the blastomic and healthy cells, as they work according to the same biological principles, although differently expressed. Despite functioning with almost identical molecular components, tumor and healthy tissues differ significantly in the dynamics of growth and pattern formation. The above data indicates that the "decision" of a certain part of the body to develop a tumor depends on the bioelectric state of remote regions. In this context, the prognosis and treatment of malignant neoplasms can most likely be achieved not by local, gene-targeting technology, but by methods for the detection of tumor signatures in the morphogenetic field of the organism.

Key words: carcinogenesis, bioelectric patterns, non-coding RNAs.

Introduction

Cancer incidence is one of the most pressing problems of modern medicine. According to the International Agency for Research on Cancer GLOBOCAN, there will be an estimated 18.1 million new cancer cases (excluding 17.0 million nonmelanoma skin cancers) in 2018. Cancer is the second leading cause of death globally. The 2018 statistics show that cancer was responsible for an estimated 9.6 million deaths, and about 1 in 6 deaths was due to cancer [1]. In 2016, in the Russian Federation, the number of new registered cases of malignant neoplasms increased by 1.7% compared to 2015, and was by 20.6% higher than in 2006 [2]. Economic losses associated with this pathology are significant and are increasing each year. In 2010, the total annual economic cost of cancer diagnosis and treatment was estimated at U.S. $ 1.16 trillion [3].

Despite huge financial resources invested in the field of cancer research, the leading U.S. centers have noted a steady decline in the incidence of low-risk cancers and an absolute increase in intermediate and high-risk cancers. For instance, the proportion of low-grade prostate cancer with Gleason score 3+3 cancers decreased by 2012 from 30.2% to 17.1% in subsequent years while high-grade Gleason score 8+ cancers increased by 2012 from 6.2% to 17.5% today. At the same time, the authors note an increase by 24% in absolute numbers of GS8+ group [4].

The launch of the Human Protein Atlas ushered in a new era in the fight against cancer [5]. Decoding the human genome has become possible due to the next-generation sequencing (NGS) methods. The technology allows describing the primary structure of DNA and RNA. The main difference from earlier sequencing techniques is the possibility to "read" several sections of the genome simultaneously. A single instrument run of the NGS generates up to hundreds of megabases and gigabases of nucleotide sequence [6]. The description of the human genome has made it possible to detect abnormal behaviour of proteins in different cancers [7].

Using these technologies, Japanese researchers have identified independent oncogene panels and have compiled the first guidance for the diagnosis, treatment and prognostic evaluation of oncological diseases. The guidance describes how to use the outcomes of gene panels testing according to the type of blastomic process: childhood cancer, rare cancer, cancer of unknown primary, and cancer of unknown etiology [8].

Another facet of genetics research concerns changes of microsatellites – repetitive DNA segments (ranging in length from 1-6 or more base pairs) [9, 10]. These repeats are

found in numerous places in the genome, and have a higher mutation rate than other regions of DNA [11].

According to Wadhwa N et al. [12], a set of microsatellite markers (D9S63, D9S156, and D9S283) can be used to detect bladder cancer in high-risk population.

The most important methods for early cancer detection and prediction include identification of specific compounds circulating in the body – products of the cancerous process.

Recent studies reveal a number of RNA molecules that do not encode proteins as tumor markers. Such RNAs can be structural components of organelle (ribosomal RNA), participate in protein synthesis, (transfer RNA), have enzymatic activity, or perform regulatory functions by influencing on chromatin structure. The non-coding RNAs include: transfer RNAs (tRNA), ribosomal RNAs (rRNA), small nuclear RNAs (snRNA), small nucleolar RNA (snoRNA), antisense RNA (aRNA), micro RNA (miRNA), small interfering RNA (siRNA), piwi-interacting RNA (piRNA), long noncoding RNA (lncRNA) – Xist, Evf, Air, CTN, PINK, TUG1 [13].

These compounds can act both as oncogenes and as oncosuppressors [14, 15]. The scientists [16] have studied the effect of long non-coding RNA (lncRNA) H19 on the epithelial-mesenchymal transition (EMT) process in patients with colorectal adenocarcinoma (CRA). Genetic analysis showed that high expression of lncRNA H19 was observed in patients with poorly differentiated tumors and lymph node metastases. In the given group of patients, this indicator was also an independent predictor of adverse outcome of the disease. According to researchers, lncRNA H19 can be used as a potential biomarker for the diagnosis and treatment of colorectal adenocarcinoma.

Similar results are provided by the authors [17]. According to their opinion, overexpression of the microRNA miR-3148 promotes an increased resistance of cancer cells under conditions of hypoxia and starvation.

A comparative study of the exhaled-breath-condensate (EBC) proteome was carried out using the method of ion cyclotron resonance mass spectrometry with electrospray ionization in four donor groups: patients diagnosed with lung cancer, patients with chronic obstructive pulmonary disease, community-acquired pneumonia, and healthy nonsmokers [18]. More than 300 proteins were identified, while 19 of them were found in the EBC samples of the donors who were diagnosed with early stage lung cancer and are potentially significant in the development of a diagnostic lung-cancer biomarker panel. Thus, the EBC analysis could be a promising non-invasive method for early diagnosis of lung cancer, since the EBC protein profiles of different donor groups can be distinguished. There is a possibility of identifying a specific group of proteins inherent in a particular condition in respiratory diseases.

The use of optogenetics in the study of cell biology has increased in recent years. The method is based on the introduction of special channelopsins into the cytomembrane that respond to light excitation; channelrhodopsin was the first opsin used. Genetic engineering is applied for building of these structures [19]. Optogenetics can be used to disclose important information about signal transduction networks within cells under normal and pathological conditions [20]. Using this technology, the authors [21] have noted a change in the duration and frequency of the extracellular signal-regulated kinase (ERK) in tumor cells. In particular, blast cells that harbor particular B-Raf mutations (in the kinase P-loop) exhibit a substantially slowed kinetics of inactivation of the dynamic signal (half-time for signal decay is 10-fold longer). In these cancer cells, the active ERK output signal remains abnormally high for ~20 min compared with 1 to 2 min for normal cells.

Signal transduction is the process by which various types of signals (chemical, physical) are transmitted through a cell as a series of molecular events (most commonly protein phosphorylation), which ultimately results in a cellular response [22]. When signaling pathways interact with each other, they form networks, which allow cellular responses to be coordinated [23]. Gene activation and metabolism are examples of cellular responses to extracellular stimulation that require signal transduction. Thus, the initial impulse can activate the expression of a large number of genes, which leads to various physiological processes [24, 25].

According to the transcription process and biochemical cascades depend on the electrical potential of the cells and cell-cell interactions [26, 27, 28, 29, 30]. The electrical potential of a histiocyte is expressed by membrane voltage (Vm). The latter value is defined as the difference in electrical potential between the cytoplasm and the extracellular space [31].

The hypothesis that biological information can be transmitted by electricity was first proved in the late 1700s, when Luigi Galvani electrically stimulated muscle contraction in an amputated frog's leg [32]. Electrical properties are often associated only with excitable cells such as neurons. However, all cells possess an electrical potential across the membrane, and thus generate and receive bioelectric signals [33].

Evidence that the electric field can serve as a vector and conductor-morphogen for growth and regeneration of the soma was first provided by A.P. Matthews in 1903 when he determined the electrochemical gradient in the regenerating hydra [34]. Modern studies confirm the thesis that these voltage gradients can predict morphology, providing information on the structure, growth and formation of the organism as a whole [35, 36].

The significance of bioelectrical potential of the cell for its further differentiation and the morphogenesis of the organism is revealed by the results of researchers' experiments [37]. Using fluorescent voltage reporters CC2-DMPE and DiBAC4, bioelectric phenomena were investigated during normal development in Xenopus embryos. The images of embryos developing from gastrula to tailbud stages revealed remarkable, never-before-seen patterns of hyper′and depolarized subpopulations of visible ectodermal cells. Three courses of hyperpolarization were distinguished during the entire period of animal development. Course I was a wave that moved across the entire embryo, apparently coincident with the appearance of cilia at the blastula surface and the beginning of neurulation. Course II, being distinguished by a bright signal coming from the median ectoderm, accompanied the closure of the neural tube.

Course III represented a series of hyper-polarizations in multiple smaller areas and coincided with the change of embryonic shape from spherical to elongate. For example, the intense region of hyperpolarization of a certain group of cells marked the future stomodeum. The neighboring cells that did not contribute to this structure remained relatively depolarized.

To sum up, the authors argue that bioelectric patterns delimit the "precursor fields" – that is to say, regions within the embryo consisting of cells whose offspring will produce specific morphological features, and they can be distinguished from the neighboring cells or regions.

The results of the experiments also provide evidence that Vm is a field of morphogen that controls development at both cellular and tissue levels, and is not a simple cellular "switch" (Pai et al., 2015) [38]. The authors observed widespread apoptosis or proliferation in the adult central nervous system by the overexpression of hyperpolarizing channels in the blast cells of the frog embryo.

The electrical potential of Vm represents the long-term, slowly changing bioelectric gradient in non-excitable cells [39], and controls critical cell functions including proliferation, migration, and differentiation [40, 41]. Recent studies have also demonstrated that Vm is able to control wound healing, either directly or indirectly [42].

In the late 1960s, while studying mitotic activities in sarcoma cells, Clarence D. Cone Jr. [43] reported that Vm underwent hyperpolarization before entering M phase, and suggested that the level of Vm is correlated with cell cycle progression. Cone's theory [44] was supported by several previous studies, which demonstrated significant Vm depolarization during malignant transformation of normal cells [45, 46]. Direct *in vitro* and *in vivo* comparisons of Vm levels between normal hepatocytes and hepatocellular carcinoma cells [47], normal and neoplastic adrenocortical tissues) [48], normal embryonic fibroblasts and fibrosarcomas [49] showed that cancer cells tended to be more depolarized than their normal counterparts.

The experimental findings serve as a good example of the significance of Vm in tumor genesis [50]. Scientists induced tumor-like structures (ITLSs) in Xenopus model by overexpression of various oncogenes, such as Xrel3, Gli1, p53 (Trp248) and KrasG12D, associated with the development of melanoma, leukemia, lung cancer and rhabdomyosarcoma. Microinjection of mRNAs encoding these genes into a single blastomere resulted in clearly identifiable ITLS. The authors revealed that induced tumor-like structures (ITLSs) generated by overexpression of Xrel3 are clearly demarcated from surrounding tissue by a depolarized transmembrane potential. The unique depolarization in relation to the surrounding tissue was also observed for Gli1 and KrasG12D ITLSs. Experimental findings suggest that the depolarized transmembrane potential is a marker of ITLSs regardless of its genetic origin.

The importance of the bioelectric field as a formative one is also reported by the authors [51]. Investigating the role of bioelectric signals in embryogenesis and tumor formation by modulating chlorine channels, the Vm of individual neural crest cells were changed. These structures represent a temporary group of cells that arise from the embryonic ectoderm. The latter gives rise to multiple cell types, including melanocytes, craniofacial bones and cartilage, smooth muscle, peripheral and intestinal cells, neurons and glia [52]. During the temporary depolarization of the above embryonic cells *in vivo*, a completely different type of cells (melanocytes) acquired a phenotype similar to metastatic melanoma [51]. Melanocytes acquired dendritic morphology, increased mitotic activity, and penetrated into blood vessels and soft tissues, such as the lumen of the neural tube and brain. In addition to the appearance of this melanocyte clone, disorganization and ectopic blood vessels growth were also observed [51]. It is important to mention that the same effect was obtained using any method of depolarization of Vmem (by modulating chlorine, sodium, potassium, or hydrogen channels). This in turn indicates the primary role of a purely physiological perturbation – disturbance in Vmem in the appearance of a metastatic phenotype, and not in case of any specific gene product or ionic disturbances. Furthermore, the authors suggested that forced hyperpolarization can suppress tumorigenesis. Various hyperpolarizing ion channels and the oncogene Xrel3 were co-injected into a single blastomere of different Xenopus embryos. It has been found that hyperpolarization can prevent the formation of tumor-like structures, despite the high levels of oncogene expression in cells. The use of several different hyperpolarizing channels based on Cl– and K+ demonstrated that the suppression of neoplastic transformation is due to the Vmem hyperpolarization, and does not depend on the specificity of the ion channels.

Furthermore, scientists have complicated the experiment with the aim to identify the systemic effects of a single depolarized cell of the Xenopus embryo. One cell of embryos at the 32-cell stage was microinjected with mRNA encoding the depolarizing channel subunit KCNE1 plus mRNA encoding β-galactosidase as a lineage tracer. These embryos were then treated with the MMP-blocking compound NSC-84093, which prevents melanocytes from migrating. As a result of the experiment, despite blocking cell migration, high-dendritic melanocytes appeared in the head and on the opposite side of the experimental animal. The authors conclude that depolarized cells can exert their inductive effect at a long range, crossing the midline to affect the contralateral side. The same conclusion is confirmed by transplantation experiments: small fragments of cells from a depolarized donor transplanted into an untreated embryo induce host melanocytes to arborize and migrate inappropriately [51].

The authors [53, 54] also point to the importance of the integrity of the bioelectric field. Implanting into connective tissue of the experimental rodents rectangles of inert plastic, metal foil, or glass coverslips induces sarcomas when the material is >1 cm^2. If the material is perforated, the incidence is reduced, and the effect is not recapitulated by powders of the same material (which actually increases surface area, ruling out chemical induction or genetic damage mechanisms).

In the context of the importance of the problem concerning intercellular communication for tumor genesis and

regenerative pattern, we think it necessary to consider the early experiments of Seilern-Aspang [55]. The author described planarian experiments in which a carcinogen led to the formation of many head teratomas with irregular nerves and ectopic eyes, and concluded that "the cell-isolating action of the carcinogen prevents formation of a single morphogenetic field and leads to the establishment of several separated fields of reduced dimensions".

Consequently, it is possible that the tumor has, in some practical sense, its own bioelectric autonomous field. The latter leads to a loss of integration with the host's body layout. This phenomenon is indirectly confirmed by the fact that, in contrast to normal somatic tissues, which are reconstructed during transplantation to foreign places [56], the histopathological structure of metastasis reflects the structure of the tissue of origin rather than their destination [57].

The view that cancer is a consequence of some failure in the geometry of the organism formation is confirmed by the reversibility of the cancer process.

Thus, if intercellular communication failure leads to the formation of the tumor, then the presence of a strong formation field can presumably inhibit this pathology. This hypothesis is proved by embryo experiments, as the morphogenetic field ought to be the most active in this period. According to [58, 59], despite high malignancy and euploidy, tumor cells integrated into wild-type embryonic hosts have become integrated as normal tissue. Equally, the embryonic field present in the blastocyst can normalize several types of blastoma cells, including cells isolated from embryonic carcinoma, leukemia, and neuroblastoma [60].

According to the results of recent studies [61, 62, 63], some ion channels have been suggested as potential tumor markers. However, as previously described in the examples of experiments [51], the same effect was achieved by any method of depolarization of Vmem (by modulating -chloric, -sodium, -calcium or hydrogen channels). The researchers [64] also point out the paramount importance of the integral formation field as contrasted with molecular mechanisms at the cellular level for the integral development of an individual. The scientists' research was focused on independent methods for implementing morphogenesis. For example, renal tubules in a triton, having a constant size, can be constructed from cells of various sizes, depending on ploidy. Reaching the same macroscopic state can be realized by various underlying molecular mechanisms. Thus, the renal tubules can be formed both by bending of the cytoskeleton – twisting one very large cell around it, or by numerous small cells. The above discrepancies may, to some extent, explain the absence of a frequent direct dependence between the outcome of the cancer process and the level of tumor markers.

Despite significant efforts to identify cancer "triggers", molecular cell substrate studies have been significantly more modest. Instead of a small amount of biochemical and genetic indicators of specific blastoma cells, molecular analysis of human cancers revealed a much wider variety of such determinants [65]. As noted above, the latest studies identify a number of RNA molecules that do not encode proteins as tumor markers. According to S.A. Lavrov, et al. [66], investigated aspects of the effect of non-protein-coding RNA on chromatin structure, the actual importance of these processes at this stage turns out to be not evaluable, but, undoubtedly, enormous.

Similar conclusions can be drawn from the works of researchers [67]. The authors studied tissue and plasma samples of cancer patients treated with surgical resection using the next-generation sequencing (NGS) method. When somatic alterations identified by each test were combined, the total proportion of patients with actionable mutations increased to 71.43%. Moreover, variants of unknown significance that were assessed as likely pathogenic had a higher percentage in ctDNA exclusively.

Conclusions

Summarizing the above, it can be assumed that the difficulties in solving the cancer problem are associated with a simplified approach, focused only on molecular components. It is difficult to identify clear differences between the blastomic and healthy cells, as they work according to the same biological principles, although differently expressed. Despite functioning with almost identical molecular components, tumor and healthy tissues differ significantly in the dynamics of growth and pattern formation. The above data indicates that the "decision" of a certain part of the body to develop a tumor depends on the bioelectric state of remote regions. In this context, the prognosis and treatment of malignant neoplasms can most likely be achieved not by local, gene-targeting technology, but by methods for the detection of tumor signatures in the morphogenetic field of the organism.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424.
2. Kaprin AD, et al., editors. Sostoianie onkologicheskoi pomoshchi naseleniiu Rossii…v 2016 godu [The state of oncological assistance to the population of Russia ... in 2016]. Moscow; 2017. 236 p. Russian.
3. Stewart BW, Wild CP, editors. World cancer report 2014. Lyon, France: International Agency for Research on Cancer; 2014. 630 p.
4. Harrison P. Prostate cancer more aggressive in post-USPSTF era [Internet]. Medscape Medical News. 2018 August 1 [cited 2018 October 12]. Available from: https://www.medscape.com/viewarticle/900073#vp_1
5. Uhlén M, Fagerberg L, Hallström BM, et al. Proteomics. Tissue-based map of the human proteome. Science. 2015 Jan 23;347(6220):1260419.
6. Weiss GJ, Liang WS, Demeure MJ, et al. A pilot study using next-generation sequencing in advanced cancers: feasibility and challenges. PLoS One. 2013;8(10):e76438.
7. Nolting B. Noveishie metody issledovaniia biosistem [Methods in modern biophysics]. Moscow: Tekhnosfera; 2005. 254 p. Russian.
8. Sunami K, Takahashi H, Tsuchihara K, et al. Clinical practice guidance for next-generation sequencing in cancer diagnosis and treatment (Edition 1.0). Cancer Sci. 2018;109(9):2980-2985.
9. Gulcher J. Microsatellite markers for linkage and association studies. Cold Spring Harb Protoc. 2012 Apr 1;2012(4):425-432.
10. Richard GF, Kerrest A, Dujon B. Comparative genomics and molecular dynamics of DNA repeats in eukaryotes. Microbiol Mol Biol Rev. 2008;72(4):686-727.
11. Brinkmann B, Klintschar M, Neuhuber F, et al. Mutation rate in human microsatellites: influence of the structure and length of the tandem repeat. Am J of Hum Genet. 1998;62(6):1408-1415.

12. Wadhwa N, Mathew BB, Tandon S, et al. Assessment of microsatellite instability for screening bladder cancer in high-risk population. J Cancer Res Ther. 2018;14(5):916-920.
13. Lee TI, Young RA. Transcriptional regulation and its misregulation in disease. Cell. 2013;152(6):1237-1251.
14. Yoon JH, Abdelmohsen K, Gorospe M. Posttranscriptional gene regulation by long noncoding RNA. J Mol Biol. 2013;425(19):3723-3730.
15. Hung T, Chang HY. Long noncoding RNA in genome regulation: prospects and mechanisms. RNA Biology. 2010;7(5):582-585.
16. Li CF, Li YC, Wang Y, Sun LB. The effect of LncRNA H19/miR-194-5p Axis on the epithelial-mesenchymal transition of colorectal adenocarcinoma. Cell Physiol Biochem. 2018;50(1):196-213.
17. Akamine T, Morodomi Y, Harada Y, et al. miR-3148 is a novel onco-microRNA that potentiates tumor growth in vivo. Anticancer Res. 2018;38(10):5693-5701.
18. Fedorchenko KIu, Ryabokon' AM, Kononikhin AS, et al. Ranniaia diagnostika raka legkogo na osnove analiza proteoma kondensata vydykhaemogo vozdukha [Early diagnosis of lung cancer based on the analysis of exhaled-air condensate proteome].Vestnik Moskovskogo Universiteta. Seriia 2: Khimiia [Moscow University Bulletin. Series 2: Chemistry]. 2016;57(2):112-120. Russian.
19. Boyden ES, Zhang F, Bamberg E, et al. Millisecond-timescale, genetically targeted optical control of neural activity. Nat Neurosci. 2005;8(9):1263-1268.
20. Bugaj LJ, O'Donoghue GP, Lim WA. Interrogating cellular perception and decision making with optogenetic tools. J Cell Biol. 2017;216(1):25-28.
21. Bugaj LJ, Sabnis AJ, Mitchell A, et al. Cancer mutations and targeted drugs can disrupt dynamic signal encoding by the Ras-Erk pathway. Science. 2018;361(6405).
22. Bradshaw Ralph A, Dennis Edward A, editors. Handbook of cell signaling. 2nd ed. Amsterdam: Academic Press; 2010. 3048 p.
23. Papin JA, Hunter T, Palsson BO, Subramaniam S. Reconstruction of cellular signalling networks and analysis of their properties. Nat Rev Mol Cell Biol. 2005;6(2):99-111.
24. Lalli E, Sassone-Corsi P. Signal transduction and gene regulation: the nuclear response to cAMP. Journal Biol Chem. 1994;269(26):17359-17362.
25. Rosen OM. After insulin binds. Science. 1987;237(4821):1452-1458.
26. Levin M. Reprogramming cells and tissue patterning via bioelectrical pathways: molecular mechanisms and biomedical opportunities. Wiley Interdiscip Rev Syst Biol Med. 2013;5(6):657-676.
27. Accardi A. Cell signaling. Lipids link ion channels and cancer. Science. 2015 Aug 21;349(6250):789-790 .
28. Huang X, Jan LY. Targeting potassium channels in cancer. J Cell Biol. 2014;206(2):151-162.
29. Monteith GR, McAndrew D, Faddy HM, Roberts-Thomson SJ. Calcium and cancer: targeting Ca2+ transport. Nat Rev Cancer. 2007;7(7):519-530.
30. Patel F, Brackenbury WJ. Dual roles of voltage-gated sodium channels in development and cancer. Int J Dev Biol. 2015;59(7-9):357-366.
31. Levin M. Large-scale biophysics: ion flows and regeneration. Trends Cell Biol. 2007;17(6):261-270.
32. Verkhratsky A, Krishtal OA, Petersen OH. From Galvani to patch clamp: the development of electrophysiology. Pflugers Arch. 2006;453(3):233-247.
33. Levin M. Bioelectric mechanisms in regeneration: unique aspects and future perspectives. Semin Cell Dev Biol. 2009;20(5):543-556.
34. Mathews AP, Whitcher BR. Electrical polarity in the hydroids. Am J Physiol. 1903;8:294-299.
35. Tseng A, Levin M. Cracking the bioelectric code: probing endogenous ionic controls of pattern formation. Commun Integr Biol. 2013 Jan 1;6(1):e22595.
36. Adams DS, Uzel SG, Akagi J, et al. Bioelectric signalling via potassium channels: a mechanism for craniofacial dysmorphogenesis in KCNJ2-associated Andersen-Tawil Syndrome. J Physiol. 2016;594(12):3245-3270.
37. Vandenberg LN, Morrie RD, Adams DS. V′ATPase′dependent ectodermal voltage and pH regionalization are required for craniofacial morphogenesis. Dev Dyn. 2011;240(8):1889-1904.
38. Pai VP, Lemire JM, Chen Y, et al. Local and long-range endogenous resting potential gradients antagonistically regulate apoptosis and proliferation in the embryonic CNS. Int J Dev Biol. 2015;59(7-9):327-340.
39. Levin M. Molecular bioelectricity in developmental biology: new tools and recent discoveries: control of cell behavior and pattern formation by transmembrane potential gradients. Bioessays. 2012;34(3):205-217.
40. Blackiston DJ, McLaughlin KA, Levin M. Bioelectric controls of cell proliferation: ion channels, membrane voltage and the cell cycle. Cell Cycle. 2009;8(21):3527-3536.
41. Sundelacruz S, Levin M, Kaplan DL. Role of membrane potential in the regulation of cell proliferation and differentiation. Stem Cell Rev. 2009;5(3):231-246.
42. McCaig CD, Song B, Rajnicek AM. Electrical dimensions in cell science. J Cell Sci. 2009;122(Pt 23):4267-4276.
43. Cone CD Jr. Electroosmotic interactions accompanying mitosis initation in sarcoma cells in vitro. Trans N Y Acad Sci. 1969;31(4):404-427.
44. Cone CD Jr. Unified theory on the basic mechanism of normal mitotic control and oncogenesis. J Theor Biol. 1971;30(1):151-181.
45. Tokuoka S, Morioka H. The membrane potential of the human cancer and related cells. Gan. 1957;48(4):353-354.
46. Johnstone BM. Micro-electrode penetration of ascites tumour cells. Nature. 1959;183(4658):411.
47. Stevenson D, Binggeli R, Weinstein RC, et al. Relationship between cell membrane potential and natural killer cell cytolysis in human hepatocellular carcinoma cells. Cancer Res.1989;49(17):4842-4845.
48. Lymangrover J, Pearlmutter AF, Franco-Saenz R, Saffran M. Transmembrane potentials and steroidogenesis in normal and neoplastic human adrenocortical tissue. J Clin Endocrinol Metab. 1975;41(4):697-706.
49. Binggeli R, Weinstein RC. Deficits in elevating membrane potential of rat fibrosarcoma cells after cell contact. Cancer Res. 1985;45(1):235-241.
50. Chernet BT, Levin M. Transmembrane voltage potential is an essential cellular parameter for the detection and control of tumor development in a Xenopus model. Dis Model Mech. 2013;6(3):595-607.
51. Blackiston DJ, Adams DS, Lemire JM, et al. Transmembrane potential of GlyCl-expressing instructor cells induces a neoplastic-like conversion of melanocytes via a serotonergic pathway. Dis Model Mech. 2011;4(1):67-85.
52. Huang X, Saint-Jeannet JP. Induction of the neural crest and the opportunities of life on the edge. Dev Biol. 2004;275(1):1-11.
53. Oppenheimer BS, Oppenheimer ET, Stout AP. Sarcomas induced in rodents by imbedding various plastic films. Proc Soc Exp Biol Med. 1952;79(3):366-369.
54. Bischoff F, Bryson G. Carcinogenesis through solid state surfaces. Prog Exp Tumor Res. 1964;5:85-133.
55. Seilern-Aspang F, Kratochwill L. Relation between regeneration and tumor growth. In: Kiortsis V, Trampusch H, editors. Regeneration in animals and related problems. Amsterdam: North-Holland Publishing Company; 1965. p. 452-73.
56. Farinella-Ferruzza N. The transformation of a tail into a limb after xenoplastic transformation. Experientia. 1956;15:304-305.
57. Tarin D. Cell and tissue interactions in carcinogenesis and metastasis and their clinical significance. Semin Cancer Biol. 2011;21(2):72-82.
58. Mintz B, Illmensee K. Normal genetically mosaic mice produced from malignant teratocarcinoma cells. Proc Natl Acad Sci USA. 1975;72(9):3585-3589.
59. Li L, Connelly MC, Wetmore C, et al. Mouse embryos cloned from brain tumors. Cancer Res. 2003;63(11):2733-2736.
60. Pierce GB, Pantazis CG, Caldwell JE,Wells RS. Specificity of the control of tumor formation by the blastocyst. Cancer Res. 1982;42(3):1082-1087.
61. Voloshyna I, Besana A, Castillo M, et al.TREK-1 is a novel molecular target in prostate cancer. Cancer Res. 2008;68(4):1197-1203.
62. Diss JK, Stewart D, Pani F, et al. A potential novel marker for human prostate cancer: voltage-gated sodium channel expression in vivo. Prostate Cancer Prostatic Dis. 2005;8(3):266-273.
63. Zhiqi S, Soltani MH, Bhat KM, et al. Human melastatin 1 (TRPM1) is regulated by MITF and produces multiple polypeptide isoforms in melanocytes and melanoma. Melanoma Res. 2004;14(6):509-516.
64. Fankhauser G. Maintenance of normal structure in heteroploid salamander larvae, through compensation of changes in cell size by adjustment of cell number and cell shape. J Exp Zool. 1945;100:445-455.
65. Hahn WC, Weinberg RA. Rules for making human tumor cells. N Engl J Med. 2002;347(20):1593-1603.
66. Lavrov SA, Kibanov MV. Nekodiruiushchie RNK i struktura khromatina [Noncoding RNA and chromatin structure]. Uspekhi Biologicheskoy Khimii [Biological Chemistry Reviews] (Moscow). 2007;47:53-88. Russian.
67. Chang YS, Fang HY, Hung YC, et al. Correlation of genomic alterations between tumor tissue and circulating tumor DNA by next-generation sequencing. J Cancer Res Clin Oncol. 2018;144(11):2167-2175.

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Aqueous humor's biochemical composition in ocular pathologies

***Maria Iacubitchii,** MD, PhD Applicant; **Eugeniu Bendelic,** MD, PhD, Professor; **Suleiman Alsaleim,** MD, PhD

Department of Ophthalmology, Nicolae Testemitsanu State University of Medicine and Pharmacy Chisinau, the Republic of Moldova

*Corresponding author: maria_iacubitkii@yahoo.com
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Abstract

Background: Being analogous to a blood surrogate, the aqueous humor has an important role in the regulation of the homeostasis of the ocular tissues. It has many functions: provides nutrition, removes excretory products, transports neurotransmitters, stabilizes the ocular structures, influences the intraocular pressure, and participates in the immune response against invading pathogens and inflammation. Aqueous humor's unique composition (electrolytes, proteins, biologically active substances, and organic solutes) is required to maintain adequate functionality of the ocular system. Its secretion is a complex biochemical reaction that gives specific properties and makes the difference from other human fluids. Different factors (traumatic, physical, chemical, pharmacological) and eye pathologies influence its composition, modifying its physiological properties, and cause pathological conditions in the anterior segment. In the last decade, it was made massive progress in the characterization of the composition of aqueous humor in different pathologies (glaucoma, myopia, keratoconus, age-related macular degeneration, branch retinal vein occlusion, etc.). It determined the biomarkers for eye's pathologies and identified the progression of the disease.

Conclusions: The detailed knowledge of biochemical and physiological properties of aqueous humor is necessary in understanding the pathophysiology of eye's diseases. The significant variations in the differentially abundant changes in human aqueous humor may be relevant for future diseases treatment in order to get favorable outcomes in patients. Specific markers for pathologies represent nowadays an important field of research. These markers are necessary for early diagnosis and selecting the proper treatment for each individual case by stopping the clinical disease progression.

Keywords: aqueous humor, composition, pathological conditions.

Introduction

Aqueous humor (AH) is the biological fluid produced by the ciliary body in the posterior chamber and fills both chambers (anterior and posterior) [1]. It supplies nutrients and oxygen and removes metabolic waste and toxic substances from posterior cornea, lens and maybe the anterior vitreous [2]. AH provides an optically clear medium for vision, maintains intraocular pressure (IOP) and structural integrity of globe, it has a protective role against ultraviolet [3] and facilitates cellular and humoral responses of the eye to inflammation and infection [4, 5]. Aqueous humor also permits drugs to be distributed to different ocular structures [6].

All AH's properties are due to unique chemical composition. To reach the posterior chamber, the various constituents of aqueous humor must traverse the three tissue components of the ciliary processes – the capillary wall, stroma, and epithelial bilayer [7]. All these structures compose the blood-aqueous barrier which is responsible for the AH's properties [8].

Aqueous humor's dynamics and secretion

The ciliary body represents the main site of aqueous production, secreted into the posterior chamber, AH passes through the pupil in the anterior chamber where it leaves the eye by passive flow via two pathways – conventional and non-conventional route (both located in the iridocorneal angle of the eye). The trabecular meshwork represents the conventional pathway, it is across the inner wall of Schlemm's canal where the AH is drained into its lumen, and after this into the collector channels, aqueous veins and episcleral veins. The uvealscleral pathway refers to the leaving of AH through intercellular spaces among ciliary muscle by diffusion into the suprachoroid and out through the sclera [5, 7, 9, 10, 11].

AH is secreted by ciliary processes, each of which is composed of a double layer of epithelium over a core of stroma and rich supply of fenestrated capillaries [12]. The two layers of the epithelium (pigmented and nonpigmented cells) are with the apical surfaces in apposition to each other [13, 14]. The nonpigmented epithelium has shown to have a large number of mitochondria, rough endoplasmic reticulum, zona occludens, lateral and surface interdigitations. These cells are considered the actual site of AH production. The pigmented epithelium contains numerous melanin granules. The non-pigmented layer is in contact with the aqueous humor in the posterior chamber, and an external, pigmented layer in contact with the ciliary process stroma [7, 12, 15]. Sympathetic and parasympathetic nerves supply the ciliary body [16].

The secretion involves three main processes: diffusion, ultrafiltration and active secretion [5]. Diffusion and ultrafiltration are passive, do not require cellular participation [17, 18, 19] and are responsible for the accumulation of plasma ultrafiltrate in the stroma. Diffusion involves the

passive movement of ions, based on charge and concentration. Ultrafiltration is a pressure-dependent process-IOP, osmotic pressure of blood and in the ciliary body (the difference between the hydrostatic pressure and IOP favors fluid movement – water and water-soluble substances) [10, 11, 12]. Active secretion needs energy (provided by hydrolysis of ATP-adenosine triphosphate) and is responsible for approximately 80% to 90% of the total aqueous humor formation by the movement of ions and other molecules across a concentration gradient in blood-aqueous barrier [2, 19, 20].

AH formation is a complex process and it began with the pass of an ultrafiltrate through the fenestrated capillaries of the ciliary processes into the stroma. The ultrafiltrate contains a high percentage of proteins, which is important for filtration from the capillaries. A number of solutes are transported from the ultrafiltrate to the posterior chamber across the ciliary epithelium, meaning the extraction of electrolytes and other substances (glucose, amino acids, ascorbate, etc.) against a concentration gradient, by means of diffusion, active or carrier-mediated secretion of solutes [8, 20].

The active process of AH secretion is mediated by two enzymes, which are present in ciliary epithelium – Na^+-K^+- ATPase and carbonic anhydrase [4, 7, 21, 22]. The gap junctions have the role of conducting water in the condition of a high degree of ion coupling [5]. Solutes, primarily Na^+ and Cl^-, and water are transferred from the extracellular stroma of the ciliary processes to the posterior chamber by sequential passage through the pigmented ciliary epithelial cells (PE), gap junctions (direct communication between the two cells, layers of ciliary epithelium at the apical-apical interface), and nonpigmented ciliary epithelial (NPE) cells. Na^+ is ejected through Na^+-K^+- activated ATPase, and Cl^- is released through Cl^- channels at the basolateral surface of the NPE into the aqueous humor [5, 11, 20, 22]. In ocular tissues the enzyme has a special function: control of the corneal hydration and the production of AH [21].

Na^+-K^+- ATPase is the enzyme responsible for Na^+ and K^+ transport and has 4 subunits (2α and 2β-subunits). It generates an electrochemical gradient across the membrane [11, 23]. The enzyme has the function of maintaining the intracellular ionic balance. In the non-pigmented epithelial cells the Na^+-K^+- ATPase excludes the Na^+ at the surface of the cells, causing local accumulation of Na^+ and generates a hyperosmotic environment with the formation of AH by driving water and anions [11].

The carbonic anhydrase has the role of pH regulation, CO_2 and HCO_3^- transport and water and electrolyte balance [11]. Other channels responsible for the transport of fluid are Aquaporins (AQPs). AQPs are transmembrane water channels that contribute to AH secretion, especially AQP1 and AQP4 found on ciliary epithelium, trabecular meshwork and endothelium of the canal of Schlemm, specific for AQP1 [24, 25]. Water transport through them occurs after a local osmotic gradient is established via secretion of ions (Na^+, Cl^-, and HCO_3^-) and small molecules (ascorbic acid) [7, 26, 27]. The role of these specific AQPs in AH production has been identified as potential therapeutic targets for pharmacological inhibition in glaucoma patients [28].

All these biochemical reactions involved in AH secretion prove the liquid's unique composition and functions.

Aqueous humor's biochemical composition

AH is considered to be analogous to interstitial fluid in that no red blood cells are present in it, and it is the source of nourishment for cells of the corneal endothelium, stromal keratocytes and the entire lens [21, 29]. All the physiological properties of AH (refractive index 1.336, pH 7.3 [30, 31]) maintain proper functionality of the ocular system [32]. Human AH is presented as a complex mixture of electrolytes, organic solutes, growth factors, cytokines, proteins that provide the metabolic requirements to the avascular tissues of the anterior segment [33]. These differences make AH's viscosity and density a little higher than that of pure water, while the osmolarity is slightly higher than that of plasma [31]. Due to small eye's chambers (anterior with a volume of 200μl and posterior with a volume of 60 μl [34, 35]), it is hard to do a proper chemical analysis of AH in ocular pathologies, anyway, there were several studies that tried to focus the main differences. We will discuss the most relevant components in AH composition.

The greatest difference between human AH and plasma resides in the very low protein and high ascorbate concentration in the aqueous (tab. 1) [21, 35].

Table 1

Aqueous and Serum protein concentration [21, 35]

Chemical composition	Aqueous	Serum
Total protein	0.013g/100ml	7.5 g/100ml
Globulin	0.003 g/100ml	2.5 g/100ml
Albumin	0.010 g/100ml	5 g/100ml
Ascorbic acid	19 mg/100ml	1.3 mg/100ml
Glucose	47mg/100ml	98mg/100ml

The levels of these constitutes are thought to be involved in the development of several eye diseases [36], and investigating the AH will facilitate generation of new hypotheses regarding the etiology of such pathologies [37].

In the article, there were pointed out the most frequent ocular pathologies that cause blindness: glaucoma, uveitis, and diabetic retinopathy, and their changes in the AH's composition that can be named biomarkers. The variations in AH may be relevant for future diseases treatment. All three pathologies are a significant public health problem, being the leading cause of irreversible visual loss. Glaucoma is a group of optic neuropathies characterized by progressive degeneration of retinal ganglion cells and appears at subjects older than 40 years. It affects more than 70 million

people worldwide with approximately 10% being bilaterally blind [12, 38]. Uveitis is an inflammatory disease affecting the uveal layer of the eye. It accounts for about 10–15% of all cases of total blindness in the USA [39]. Diabetic retinopathy is another leading cause of vision-loss globally, affecting adults aged 20–74 years. Of an estimated 285 million people with diabetes mellitus worldwide, approximately one third have signs of diabetic retinopathy [40].

All these pathologies are influenced by a series of risk factors and are characterized by their own way of pathophysiology in AH secretion/ outflow. In most glaucomas, it was established an increased resistance through the trabecular meshwork that contributes to elevated IOP and influence on AH's composition [26]. Diabetes mellitus is associated with problems of general circulation. Ocular effects are dependent on the duration of diabetes, the age of the patient, and the severity of retinopathy. They include changes in AH dynamics, IOP, aqueous flare, permeability of blood-ocular barrier, and retinal vasculature [22, 26]. Uveitis is characterized by inflammation that can cause iris atrophy and secondary glaucoma in some patients. Several studies pointed out different changes in AH due to the increased permeability of the blood-aqueous barrier [5, 26].

The exact number of human AH constitutes is unknown, and it is possible that tens if not hundreds of components exist in the AH and many of these could fall below current detection limits and difficulty in collecting a big quantity for the biochemical exam due to the small eye's chambers. Specific markers for main pathologies represent nowadays an important field of research. Therefore, it was decided to select the most important constitutes from AH for glaucoma, diabetic retinopathy and uveitis, and to analyze their concentration, properties changes.

So, one of the main constitutes of AH is *ascorbic acid* (*Vitamin C* with a concentration about 10- 15 times greater in the AH than in plasma) has the role of antioxidant, protecting the eye from the deleterious effects of free radicals and toxins [10, 21, 41-43]. The concentration of ascorbate is about 15 times greater in the AH than in plasma, suggesting that vitamin C may protect against harmful factors within the eye [10, 41]. It was detected in cornea, AH, lens, vitreous humor, and retina [41, 44].

However, Vitamin C concentrations in AH are lower in patients with various ophthalmic diseases. At the patients with age-related cataract (from 50 to 70 years old) the concentration of vitamin C in AH decreases suggesting that this phenomenon may play a role in susceptibility to cataract formation in older people [44, 45]. Vitamin C concentrations are lower in patients with exfoliation syndrome and glaucoma [46-49]. The endotoxin-induced ocular inflammation in uveitis caused a decrease in the concentration of ascorbic acid in the AH [50]. Diabetic patients have an imbalance between free radical generation and antioxidant defense (vitamin C, vitamin E) which may play a role in the progression of diabetic retinopathy [51].

The low concentration of *proteins* in AH (0,02g at 100ml comparative to plasma concentration of 7g at 100ml) is essential for maintaining the optical transparency [52], this is due to the blood-aqueous barrier. AH comprises many proteins with various roles and important biological functions. The exact number and concentration of human AH proteins are unknown, as it is supposed that tens if not hundreds of lower abundance proteins exist in the AH and many of these could fall below current detection limits [37]. Most of the proteins identified had catalytic, enzymatic, and structural properties [33]. The most abundant proteins found in normal AH are albumin, immunoglobulin G (IgG), transferrin, haptoglobin and antitrypsin that represent the major ones [32, 33]. In a healthy eye, IgG is present at a concentration of approximately 3mg/100ml, while IgM, IgD, IgA are absent due to their large molecule structure [53].

Amount of proteins and cells in AH was observed after surgery, paracentesis, or uveitis [54]. In Prata T. et al.'s study it was mentioned that the total protein concentration in primary open-angle glaucoma AH was approximately two times higher than that in non-glaucomatous patients, albumin (50% of all the protein content) and transferrin being the most abundant protein [55, 56]. Although, it is considered that the alterations in the protein composition of AH trigger signaling molecules that modify the trabecular meshwork and increasing resistance to outflow and induce glaucoma [57]. Grus E.H. et al. found that transthyretin was one of the proteins that are highly abundant in the aqueous of glaucoma patients. It might play a role in the onset of glaucoma since it has been shown to form amyloid deposits (increasing intraocular pressure by the particles that could cause outflow obstructions) [58].

The pathogenesis of uveitis is associated with abnormal expression of some proteins and aberrant regulation of multiple signaling pathways [59]. The blood-aqueous barrier breaks down [60] and the composition and concentrations of proteins in aqueous are similar to that of plasma [61]. The concentration of IgG increases and IgM and IgA appear [62-64]. When the AH proteins concentration rises significantly above its normal level approximately 20mg/100ml, the resultant light scattering (Tyndall effect) makes visible at slit-lamp [5]. Other sources of proteins are represented by IL-1β, IL-2, IL-6, and IL-10, which are cytokines that actively participate in the pathogenesis of clinical uveitis, and it is higher in the samples of patients with uveitis [65, 66].

In diabetic patients, the proteome composition of AH suffers change too [67, 68]. Chiang S.Y. et al. identified 11 proteins differentially expressed between diabetic retinopathy and control groups. There were detected at lower levels – SERPINF1 (encoded protein is secreted and strongly inhibits angiogenesis) and prostaglandin-H2 D-isomerase (PTGDS – involved in development and maintenance of the blood-retina, blood-aqueous humor barrier) compared to control [68, 69]. These altered proteins are involved in in-

flammation, lipid metabolism and cell proliferation, microstructure reorganization, angiogenesis, anti-oxidation, and neuroprotection [67, 69, 70].

Other important protein found in diabetic AH that has an important role in angiogenesis is vascular endothelial growth factor (VEGF) [71, 72]. Data from several studies support the generally accepted supposition that the VEGF level in the aqueous liquid collected from the anterior chamber adequately reflects the VEGF activity in retinal tissues [72, 73]. The severity of retinopathy and the degree of retinal ischemia is directly proportional to the elevation of VEGF levels (957 pg/ml as detected in Patel J.I.'s study) [72, 74-76].

Glucose levels in AH correlate with blood glucose levels [77]. It is a component of the AH due to the process of diffusion. At young patients, the concentration of the glucose in AH represents 76% from the plasma concentration, but with the age the concentration decrease is 63% [12]. Davies P.D. et al. have observed mean AH glucose concentration in non-diabetic is 3.2mmol/L (57.6mg/dl). There were determinate differences between non-diabetic and diabetic patients. The glucose levels in non-diabetic patients were 5.8 mM in plasma and 3.2 mM in AH, while the values for diabetics were 14.2 and 7.8 mM [78], influencing the metabolism of the lens, the refraction [12]. In addition, the glucose level influences the IOP (intraocular pressure) in patients with uncontrolled diabetes that was significantly higher [79, 80]. The mechanism is still unclear, but *in vitro* studies suggested that high glucose conditions could induce excess extracellular matrix synthesis by trabecular meshwork cells. Accumulation of extracellular matrix in the trabecular meshwork blocks the aqueous outflow [81, 82]. Glucose levels of AH in ocular inflammations as iritis, keratitis and corneal ulcer are elevated, according to Alaerts et al. [83]. There is no evidence about the concentration of glucose in glaucoma.

Anyway, the changes in the most important constitutes of the AH involve modifications in the other components (ions, amino acids etc.), physiological properties and cause pathological conditions in the anterior segment. All the biochemical researches made on specific marker in the AH for eye pathologies are developing.

Conclusions

This study reveals significant variations in the differentially abundant changes in human aqueous humor that may be relevant for future diseases treatment in order to get favorable outcomes in patients. The aqueous humor proper composition is important in the regulation of the homeostasis of the ocular tissues. Every pathology leads to changes to aqueous humor. They influence physiological properties and cause pathological conditions in the eye. The specific identification of these markers will aid in understanding various eye diseases of the anterior segment such as glaucoma, uveitis and diabetic retinopathy. Other areas for future study include determining differences in aqueous humor constitutes levels among patients in different age groups.

References

1. Rosenfeld C, Price M, Lai X, Witzmann F, Price F. Distinctive and pervasive alterations in aqueous humor protein composition following different types of glaucoma surgery. Mol Vis. 2015;21:911-918.
2. Pietrowska K, Dmuchowska D, Samczuk P, et al. LC-MS-based metabolic fingerprinting of aqueous humor. J Anal Methods Chem. 2017:6745932.
3. Ringvold A. The significance of ascorbate in the aqueous humor protection against UV-A and UV-B. Exp Eye Res. 1996 Mar;62(3):261-4.
4. Gold DH, Lewis RA, editors. Clinical eye atlas. 2nd ed. Oxford: Oxford University Press; 2011. p. 314-315.
5. Civan MM, Benos DJ, Simon SA, editors. The eye's aqueous humor. 2nd ed. San Diego: Elsevier; 2008. 483 p.
6. Sires B. Orbital and ocular anatomy. In: Wright K, editor. Textbook of ophthalmology. Baltimore: Williams & Wilkins; 1997.
7. Goel M, Picciani R, Lee R, Bhattacharya S. Aqueous humor dynamics: a review. Open Ophthalmol J. 2010;4:52-59.
8. Shahidullah M, Al-Malki W, Delamere N. Mechanism of aqueous humor secretion, its regulation and relevance to glaucoma. In: Rumelt S, editor. Glaucoma - basic and clinical concepts. Rijeka: Intech; 2011. p. 3-32.
9. Llobet A, Gasull X, Gual A. Understanding trabecular meshwork physiology: a key to the control of intraocular pressure? News Physiol Sci. 2003;18:205-209.
10. Cantor L, Rapuano C, Cioffi G. Fundamentals and Principles of Ophthalmology. San Francisco: American Academy of Ophthalmology; 2016. (Basic and Clinical Science Course; Section 2).
11. To C, Kong C, Chan C, et al. The mechanism of aqueous humor formation. Clin Exp Optom. 2002;85(6):335-349.
12. Cantor L, Cioffi GA, Durcan FJ, Girkin CA. Glaucoma. San Francisco: American Academy of Ophthalmology; 2016. (Basic and Clinical Science Course; Section 10).
13. Smelser GK. Electron microscopy of a typical epithelial cell and of the normal human ciliary process. Trans Am Acad Ophthalmol Otolaryngol. 1966 Sep-Oct;70(5):738-54.
14. Tormey JM. The ciliary epithelium: an attempt to correlate structure and function. Trans Am Acad Ophthalmol Otolaryngol. 1966 Sep-Oct;70(5):755-66.
15. Hara K, Lütjen-Drecoll E, Prestele H, Rohen JW. Structural differences between regions of the ciliary body in primates. Invest Ophthalmol Vis Sci. 1977 Oct;16(10):912-24.
16. McDougal DH, Gamlin PD. Autonomic control of the eye. Compr Physiol. 2015 Jan;5(1):439-473.
17. Netland P, editor. Glaucoma medical therapy: principles and management. 2nd ed. Oxford, New York: Oxford University Press and American Academy of Ophthalmology; 2008. p. 9-10.
18. Gabelt BT, Kaufman PL. Aqueous humor hydrodynamics. In: Hart WM, editor. Adler's physiology of the eye. 9th ed. St. Louis: Mosby; 2003.
19. Mark HH. Aqueous humor dynamics in historical perspective. Surv Ophthalmol. 2010 Jan-Feb;55(1):89-100.
20. Wang Z, Do CW, Valiunas V, et al. Regulation of gap junction coupling in bovine ciliary epithelium. Am J Physiol Cell Physiol. 2010 Apr;298(4):C798-806.
21. Whikehart DR. Biochemistry of the eye. 2nd ed. Philadelphia: Elsevier; 2003. 319 p.
22. Civan MM, Macknight AD. The ins and outs of aqueous humor secretion. Exp Eye Res. 2004 Mar;78(3):625-31.
23. Ueno S, Takeda K, Noguchi S, Kawamura M. Significance of beta-subunit in the biogenesis of Na^+-K^+- ATPase. Biosci Rep. 1997;17(2):173-188.
24. Yamaguchi Y, Watanabe T, Hirakata A, Hida T. Localization and ontogeny of aquaporin-1 and -4 expression in iris and ciliary epithelial cells in rats. Cell Tissue Res. 2006 Jul;325(1):101-9.
25. Schey K, Wang Z, Wenke J, Qi Y. Aquaporins in the eye: expression, function, and roles in ocular disease. Biochim Biophys Acta. 2014 May;1840(5):1513-23.

26. Civan MM. Formation of the aqueous humor: transport components and their integration. In: Civan MM, et al., editors. The eye's aqueous humor. 2nd ed. San Diego: Elsevier; 2008. p. 2-45.
27. Civan MM. Transporters beyond transport. Focus on "Deregulation of apoptotic volume decrease and ionic movements in multidrug-resistant tumor cells: role of chloride channels." Am J Physiol Cell Physiol. 2010;298(1):C11-13.
28. Levin MH, Verkman AS. Aquaporins and CFTR in ocular epithelial fluid transport. J Membr Biol. 2006 Mar;210(2):105-15.
29. Bennett KL, Funk M, Tschernutter M, et al. Proteomic analysis of human cataract aqueous humor: Comparison of one-dimensional gel LCMS with two-dimensional LCMS of unlabeled and iTRAQ®-labelled specimens. J Proteomics. 2011;74(2):151-66. doi:10.1016/j.jprot.2010.10.002.
30. Levin LA, Nilsson SFE, et al. Adler's physiology of the eye. 11th ed. Edingburg: Elsevier; 2011. 795 p.
31. Charman WN, Adnan, Atchison DA. Gradients of refractive index in the crystalline lens and transient changes in refraction among patients with diabetes. Biomed Opt Express. 2012;3(12):3033-42.
32. Perumal N, Manicam C, Steinicke M, Funke S, et al. Characterization of the human aqueous humor proteome: a comparison of the genders. PLoS One. 2017;12(3):e0172481.
33. Chowdhury UR, Madden BJ, Charlesworth MK, Fautsch MP. Proteome analysis of human aqueous humor. Invest Ophthalmol Vis Sci. 2010 Oct;51(10):4921-4931.
34. Hogan MJ, Alvarado JW, Weddell JE. Histology of the human eye: an atlas and textbook. Philadelphia: WB Saunders; 1971. 687 p.
35. Davson H. Physiology of the eye. 5th ed. New York: Pergamon Press; 1990. 830 p.
36. Klenkler B, Sheardown H. Growth factors in the anterior segment: role in tissue maintenance, wound healing and ocular pathology. Exp Eye Res. 2004 Nov;79(5):677-88.
37. Richardson MR, Price MO, Price FW, et al. Proteomic analysis of human aqueous humor using multidimensional protein identification technology. Mol Vis. 2009 Dec 11;15:2740-50.
38. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol. 2006 Mar;90(3):262-7.
39. Acharya NR, Tham VM, Esterberg E, Borkar DS, Parker JV, Vinoya AC, Uchida A. Incidence and prevalence of uveitis: results from the Pacific Ocular Inflammation Study. JAMA Ophthalmol. 2013 Nov;131(11):1405-12.
40. Lee R, Wong TY, Sabanayagam C. Epidemiology of diabetic retinopathy, diabetic macular edema and related vision loss. Eye Vis (Lond). 2015;2:17.
41. Buettner GR, Schafer FQ. Albert Szent-Györgyi: Vitamin C identification. Biochemist. 2006;28:31-33.
42. Hah YS, Chung HJ, Sontakke SB, Chung IY, Ju S, et al. Ascorbic acid concentrations in aqueous humor after systemic vitamin C supplementation in patients with cataract: pilot study. BMC Ophthalmol. 2017;17(1):121.
43. Reiss GR, Werness PG, Zollman PE, Brubaker RF. Ascorbic acid levels in the aqueous humor of nocturnal and diurnal mammals. Arch Ophthalmol. 1986 May;104(5):753-5.
44. Wei L, Liang G, Cai C, Lv J. Association of vitamin C with the risk of age-related cataract: a meta-analysis. Acta Ophthalmol. 2016 May;94(3):e170-6.
45. Canadananović V, Latinović S, Barišić S, Babić N, Jovanović S. Age-related changes of vitamin C levels in aqueous humor. Vojnosanit Pregl. 2015 Sep;72(9):823-6.
46. Ferreira SM, Lerner SF, Brunzini R, Evelson PA, Llesuy SF. Antioxidant status in the aqueous humor of patients with glaucoma associated with exfoliation syndrome. Eye (Lond). 2009;23:1691-1697.
47. Koliakos GG, Kontas AG, Schlotzer-Scherhardt U, Bufidis T, Georgiadis N, Ringvold A. Ascorbic acid concentration is reduced in the aqueous humor of patients with exfoliation syndrome. Am J Ophthalmol. 2002;134:879-883.
48. Leite MT, Prata TS, Kera CZ, Miranda DV, de Moraes Barros SB, Melo LA Jr. Ascorbic acid concentration is reduced in the secondary aqueous humor of glaucomatous patients. Clin Exp Ophthalmol. 2009 May;37(4):402-6.
49. Goyal A, Srivastava A, Sihota R, Kaur J. Evaluation of oxidative stress markers in aqueous humor of primary open angle glaucoma and primary angle closure glaucoma patients. Curr Eye Res. 2014;39(8):823-829.
50. McGahan MC. Ascorbic acid levels in aqueous and vitreous humors of the rabbit: effects of inflammation and ceruloplasmin. Exp Eye Res. 1985;41(3):291-298.
51. Beyazyildiz E, Cankaya AB, Ergan E, et al. Changes of total antioxidant capacity and total oxidant status of aqueous humor in diabetes patients and correlations with diabetic retinopathy. Int J Ophthalmol. 2013;6(4):531- 536.
52. Cole D. Ocular Fluids. In: Davson H, editor. The Eye - Vegetative physiology and biochemistry. Orlando: Academic Press; 1984.
53. Sen DK, Sarin GS, Saha K. Immunoglobulins in human aqueous humor. Br J Ophthalmol. 1977 Mar;61(3):216-217.
54. De Biaggi CP, Barros PS, Silva VV, Brooks DE, Barros SB. Ascorbic acid levels of aqueous humor of dogs after experimental phacoemulsification. Vet Ophthalmol. 2006;9(5):299-302.
55. Prata TS, Navajos EV, Melo LA Jr, et al. Aqueous humor protein concentration in patients with primary open angle glaucoma under clinical treatment. Arq Bras Oftalmol. 2007;70(2):217-20.
56. Zaidi M, Jilani A, Bhattacharya P, Islam N, Alam S. A study of aqueous humor proteins in patients of primary open-angle glaucoma. Adv Biosci Biotechnol; 2010;1:110-114.
57. Anshu A, Price MO, Richardson MR, et al. Alterations in the aqueous humor proteome in patients with a glaucoma shunt device. Mol Vis. 2011;17:1891-900.
58. Grus FH, Joachim SC, Sandmann S, et al. Transthyretin and complex protein pattern in aqueous humor of patients with primary open-angle glaucoma. Mol Vis. 2008;14:1437-45.
59. Guo DD, Hu B, Tang HY, Sun YY, Liu B, Tian QM, Bi HS. Proteomic profiling analysis reveals a link between experimental autoimmune uveitis and complement activation in rats. Scand J Immunol. 2017 May;85(5):331-342.
60. Holland GN. A reconsideration of anterior chamber flare and its clinical relevance for children with chronic anterior uveitis (an American Ophthalmological Society thesis). Trans Am Ophthalmol Soc. 2007;105:344-64.
61. Freddo TF. A contemporary concept of the blood-aqueous barrier. Prog Retin Eye Res. 2013 Jan;32:181-195.
62. Murray PI, Hoekzema R, Luyendijk L, et al. Analysis of aqueous humor immunoglobulin G in uveitis by enzyme-linked immunosorbent assay, isoelectric focusing, and immunoblotting. Invest Ophthalmol Vis Sci. 1990;31(10):2129-35.
63. Norn MS. Immunoglobulins in endogenous uveitis. Br J Ophthalmol. 1976;60(4):299-301.
64. McCoy R, White L, Tait B, Ebringer R. Serum immunoglobulins in acute anterior uveitis. Br J Ophthalmol. 1984 Nov;68(11):807-10.
65. Lacomba SM, Martín MC, Gallardo Galera JM, Estévez CE, Chamond RR, Omar M, Vidal GA. [Aqueous humor and serum interleukin-6 in patients with uveitis]. Arch Soc Esp Oftalmol. 2001 Jun;76(6):345-50. Spanish.
66. Hernandes Garfella ML, Palomares Fort P, Roman Ivorra JA, Cervera Taulet E. Aqueous humor levels of different interleukins 1-β, 2, 6 and 10, tumor necrosis factor-α and vascular endothelial growth factor in uveitis treated with adalimumab. J Ophthalmic Vis Res. 2015;10(1):49-54.
67. Chiang SY, Tsai ML, Wang CY, Chen A, Chou YC, Hsia CW, Wu YF, Chen HM, Huang TH, Chen PH, Liu HT, Shui HA. Proteomic analysis and identification of aqueous humor proteins with a pathophysiological role in diabetic retinopathy. J Proteomics. 2012 Jun 6;75(10):2950-9.

68. Csősz É, Deák E, Kalló G, Csutak A, Tőzsér J. Diabetic retinopathy: proteomic approaches to help the differential diagnosis and to understand the underlying molecular mechanisms. J Proteomics. 2017 Jan 6;150:351-358.
69. Bouhenni R, Deepak E, Sandeep G, Chalam K, Sewell A, Abu-Amero K. The aqueous humor proteome in patients with diabetic retinopathy. Invest Ophthalmol Vis Sci. 2013 June;54:1157.
70. Balaiya S, Zhou Z, Chalam KV. Characterization of vitreous and aqueous proteome in humans with proliferative diabetic retinopathy and its clinical correlation. Proteomics Insights. 2017;8:1178641816686078. doi: 10.1177/1178641816686078.
71. Duffy AM, Bouchier-Hayes DJ, Harmey JH. Vascular Endothelial Growth Factor (VEGF) and its role in non-endothelial cells: autocrinesignalling by VEGF. In: Madame Curie Bioscience Database [Internet]. Austin (TX): Landes Bioscience; 2000-2013. [cited 2018 Dec 19]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK6482/
72. Selim KM, Sahan D, Muhittin T, Osman C, Mustafa O. Increased levels of vascular endothelial growth factor in the aqueous humor of patients with diabetic retinopathy. Indian J Ophthalmol. 2010;58(5):375-9.
73. Qaum T, Xu Q, Joussen AM, Clemens MW, Qin W, Miyamoto K, Hassessian H, Wiegand SJ, Rudge J, Yancopoulos GD, Adamis AP. VEGF-initiated blood-retinal barrier breakdown in early diabetes. Invest Ophthalmol Vis Sci. 2001 Sep;42(10):2408-13.
74. Patel JI, Tombran-Tink J, Hykin PG, Gregor ZJ, Cree IA. Vitreous and aqueous concentrations of proangiogenic, antiangiogenic factors and other cytokines in diabetic retinopathy patients with macular edema: Implications for structural differences in macular profiles. Exp Eye Res. 2006 May;82(5):798-806.
75. Choi GJ, Jung MO, Kim DH. Analysis of VEGF and PEDF concentration in aqueous humor, vitreous humor, and plasma of diabetic retinopathy patients. Invest Ophthalmol Vis Sci. 2012 March;53:2427.
76. Costagliola C, Daniele A, dell'Omo R, Romano MR, Aceto F, Agnifili L, Semeraro F, Porcellini A. Aqueous humor levels of vascular endothelial growth factor and adiponectin in patients with type 2 diabetes before and after intravitreal bevacizumab injection. Exp Eye Res. 2013 May;110:50-4.
77. Lambert JL, Pelletier CC, Borchert M. Glucose determination in human aqueous humor with Raman spectroscopy. J Biomed Opt. 2005 May-Jun;10(3):031110.
78. Davies PD, Duncan G, Pynsent PB, Arber DL, Lucas VA. Aqueous humor glucose concentration in cataract patients and its effect on the lens. Exp Eye Res. 1984 Nov;39(5):605-9.
79. Perez-Rico C, Gutierrez-Ortiz C, Gonzalez-Mesa A, Zandueta AM, Moreno-Salgueiro A, Germain F. Effect of diabetes mellitus on Corvis ST measurement process. Acta Ophthalmol. 2015;93(3):e193-8.
80. Hymowitz MB, Chang D, Feinberg EB, Roy S. Increased intraocular pressure and hyperglycemic level in diabetic patients. PLoS One. 2016;11(3):e0151833.
81. Li A-F, Chen A, Roy S. High glucose-induced fibronectin overexpression inhibits trabecular meshwork cell permeability. Invest Ophthalmol Vis Sci. 2003;44(ARVO).
82. Sato T, Roy S. Effect of high glucose on fibronectin expression and cell proliferation in trabecular meshwork cells. Invest Ophthalmol Vis Sci. 2002;43(1):170-5.
83. The glucose content of the aqueous humor in anterior uveitis. In: Acta Ophthalmologica. 1966;44(S88):55-60. [cited 2019 Feb 4]. Available from: https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1755-3768.1966.tb06447.x

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Interests in knowledge and assistance of epilepsy

*[1]**Oleg Cobileanschi,** MD, PhD, Professor; [2]**Ludmila Baba,** MD; [1]**Alexandru Dandara,** MD; [1]**Alexandru Bobea,** MD

[1]Department of Psychiatry, Narcology and Medical Psychology, [2]National Epilepsy Center
State University of Medicine and Pharmacy Nicolae Testemitsanu, Chisinau, the Republic of Moldova

*Corresponding author: oleg.cobileanschi@usmf.md
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Abstract

Background: Many problems with differential treatment of epilepsy require further clarification. As far as we are concerned, we have developed therapeutic recommendations which, in our opinion, demonstrated to be effective in certain cases, supporting the results of the treatment of epilepsy at its various stages known in the literature: from premonitory forms to status variants. The main element in the choice of anticonvulsant remedies, besides the clinical markings, was the dynamically derived EEG data as well as the subjective pharmacological response of the patients. The preferential associations of anticonvulsant remedies for various topographies of the epileptic outbreak and oscillation of nicotine paroxysms of sleep-wake cycle were given in the formula and strictly individual dosing. Remarkable advances in the field of perturbation in the last decades of the twentieth century, as well as active research in the uninterrupted process, have made epilepsy now called unequivocally a "hopeful affection".

Conclusions: Normal and abnormal neuronal cells are involved in pathological discharges, the exact genesis of this phenomenon is known to be vague and means the involvement of many factors of cellular, vascular and metabolic disorders. Rehabilitation of patients with epilepsy should be gradual, using compliance between drug treatment, psychosocial rehabilitation depending on the dynamics of the disease and the patients' reaction to their own condition. The uptake of concepts and rehabilitation programs introduced into many countries' systems, the formation of the assisted care system, will increase the effectiveness of ambulatory rehabilitation.

Key words: epilepsy, assistance, rehabilitation.

Introduction

Contemporary society imposes an accelerated pace of everyday activity and a need for rapid adaptation to the environment in which the person satisfies his own needs and desires. For quick adaptation, a person needs to have certain abilities to contact with society and to actually carry out his own work [1].

The priorities of contemporary psychiatry are focused on medical-social rehabilitation and the reintegration into society of patients who lost due to the disease some adaptive abilities, but also the education of society to avoid stigmatizing people with psychiatric diagnostics, among which the diagnosis of epilepsy. Considering that most patients with epilepsy are treated as outpatients and many of them are fit to work, the issue of discrimination and social stigmatization is a current problem. Rehabilitation of the epileptic patient includes certain stages, and the success of adaptation depends on many factors, such as: concrete manifestations of epilepsy, social and psychological adaptation skills to different daily circumstances of the patient, his level of intellectual development, work skills, psychological disturbance. Equally important is the condition of the patient and how he resigns his own diagnosis. Such a problem can arise as negativity, isolation from society, denial or resignation with his own illness, fear that the epileptic crisis, social reaction, stigmatization will emerge in the next moment. These issues limit the social activity of the sick and do not allow them to work professionally [1, 2].

This work is in itself a detailed description of the stages of rehabilitation of patients with epilepsy, proposing certain criteria for the conduct of the medical treatment, as well as its adjustment depending on the type of therapy: stationary or ambulatory, description of the unique features of the diagnosis of epilepsy. It is the study of genesis and neuro-anatomical manifestations of epilepsy, particularities of rehabilitation and reintegration of the epileptic patient and proposals for solving problems that arise during the rehabilitation [3]. Presentation of studies and conclusions based on past experience of these issues as well as the assessment of progress made in recent years are considered in this work [1].

Classifications and conceptions of the epilepsy

Most classifications and assumptions made in an attempt to explain the causes of epilepsy are targeted towards deciphering the circumstances generating seizures as convulsions, and finding propensity seizures was indicative of predisposition to developing epilepsy [4].

Sustained efforts that were made over time by clinicians focused on pragmatic objectives: defining clinical variant, determining the frequency and severity of seizures, which are the criteria for therapeutic interventions for physical and psychological rehabilitation of patients with epilepsy and a number of other social aspects of epilepsy.

Classical conception of the central nervous system is based on the teaching of I. Pavlov, the subsequent elaborations of his many disciples and successors about higher nervous activity which remain valuable perennial including neurophysiology and modern medicine [4, 5].

I. Pavlov interpreted neurodinamic character of epileptic crisis, considering the value of decisive factor discrepancies between excitation and response inhibition. Moreover, says

the scientist, "all variations of the disease are defined by the proportioning". But the essence of the focal character is persistent and morbid hypertonic, which is a pathological inertia. And the nature of illness – chronic, latent or explosive – is totally disproportionate, determined by the intensity of the excitation and inhibition, reveals that there are still many unknowns and uncertainties [6].

Several decades ago by the modern concept of epilepsy J. Jackson hypothesized that this condition is caused by "gray matter downloads, fast and local, intermittent, sudden and excessive" and that when brain tissue participates in the normal focus initiated by abnormal, generalized seizures occur. Over time, Jakson's concepts have remained almost unchanged and have been confirmed in medical practice. The experimental research noted that convulsive seizures can be caused easily by chemical and electrical stimulation of the brain tissue, and therefore, the equipment must be an inhibition of the upright body, to prevent the normal neuronal activity of the brain to unleash bursts [6, 7].

Therefore, convulsive seizures may occur when, for various reasons, the normal balance between excitation and inhibition is disturbed in such a way that the ratio exceeds unity. Furthermore, brain excitability is regulated in large areas of inhibitory pathways operating Scab extracorticale. In this way brain possesses mechanisms of self-regulating excitability.

Numerous researches have been conducted to elucidate the nature of the epileptogenic furnace, which is usually taken as a group of neurons in pathological change, which discharge excessively in normal circumstances of the neuronal request. It is possible that the focus of normal brain cells consists of the excessive discharging due to reduced vascular supply or due to some other abnormality. It is quite possible that the outbreak is an area previously affected, as some explorers announced, in neurons a portion of dendritic spines have been destroyed and it would create a stable region overactive inhibitory absent of its internal mechanisms [8, 9].

Such a stable outbreak suppresses the need to create abnormal neuronal requests or pathological changes of each neuron as a cause of access and is consistent with the clinical experience that many epileptic patients continue to have accesses for years without signs of progressive neurological lesions. But when an epileptogenic outbreak is discharged, the spread of seizure activity as a secondary outcome may comprise normal brain cells. If the irradiation is sufficiently extensive, the brain is fully activated and as a result there is a tonic-clonic access associated with a state of unconsciousness. When access convulsive action is localized seizures produce objective and subjective signs characteristic to anatomical area [10, 11].

Other areas and centers can be drawn indirectly without participating themselves in the production of convulsive discharges access, such regions will not exhibit another depressed state.

Since the brain injuries causing seizures are present constantly, while seizures are intermittent, and because the lesions can exist without causing seizures, were conducted many studies of physiological factors, inhibitors and activators that can influence the activity of an epileptogenic outbreak.

These factors announce blood glucose level, blood gas concentrations, pH of the plasma, the total osmotic pressure of the extracellular fluid and electrolyte composition, endocrine disorders, nutritional deficiencies, etc. These trigger factors intervene, influencing predisposition of brain to damage or inherited defects to exhibit seizure activity, therefore, it should not confuse clinicians when patients with seemingly identical types of convulsive seizures often respond differently to drug treatment [12, 13, 14].

Rehabilitation of patients with epilepsy: principles and controversy

Medical opinion in the contemporary world seems to have crystallized certain idea about community attitudes that should be reconsidered in relation to the status of patients with epilepsy, for which there are sufficient only to cover costs of drug therapy. It requires the development of specific reforms that are oriented for reinstatement of this group of sufferers, although the company does not deprive it of its obligations to these citizens. Civil society and various voluntary associations have to intervene more and more frequently to support as many people as possible with marginalized psychosomatic disabilities, who suffer either through the indifference of the responsible officials or through the imperfection of legislation, but most often through the lack of financial subsidies [1, 15].

The problem of rehabilitation of patients with epilepsy, which is a particular issue, but it is the indispensable part of the issues concerned with the general rehabilitation of patients and disabled, has become particularly acute over the past few decades, when due to various social cataclysms and many other adversities of the human psyche cases of affliction through psychological suffering, are continuously increasing. As most patients with epilepsy are marked by different physical disabilities, their recovery is based on the same spectrum of tasks adopted for social reinsertion of mental patients. Many existing centers in countries with potent economies, and a series of health forums and bodies with powers in this area held meetings devoted to the topic in question, the agenda of them aimed at psychologically specific patients with epilepsy that occurs not only through aggressive disease that affects matter of the brain, but also by the reaction of the patient with epilepsy to unusual and scaring manifestation of his own disease that can create the secondary psychic changes. These psychic changes were classified by specialists as: a) responding to an epileptic disease (character depression, hysteria, etc.); b) responding to crises in social entourage (the patients with epilepsy hide their disease); c) responding to restriction in professional activity (different phobias, including preconceived attitude of society towards sickness) [16, 17].

These circumstances define the extremely complex mental profile and often very distorted in relation to the social activity in which the patients tend to work on full rights , when in fact they have different emotional reactions, dysphoria, conflicts, that are serious impediments to their employment in the sphere of production [18].

All experts agreed on several principles absolutely indis-

pensable for rehabilitation of patients with epilepsy: timely diagnosis and accurate clinical and evolutionary characteristics of the disease; to adopt and implement appropriate antiepileptic therapy and to start immediately after the onset of the disease; the third principle of recovery of patients with epilepsy is psychological diagnosis, to capture early changes of personality, defining their evaluation in terms of quantity and quality, and according to the inferred to design exactly the plan for restoration actions to be taken in each case; the fourth principle is that of joint efforts involving various stakeholders (the activity of the treating physician, the psychiatrist, the patient with epilepsy himself, the relatives, the family and other third parties in the social environment), the fifth principle is to strive for cohesion measures of biological activity and psychosocial considering that we do not approach a patient mentality but epileptic pathology with a mentally specific defect parallel with bouts, therefore, and recuperation program must be adequate to disease diagnosis parameters [17].

Although only 20% of all patients with epilepsy end up experiencing psychiatric disorders and the frequency and type of seizures do not act as maladaptive on the patient with epilepsy and generally do not disfigure the person too manifest, however, all patients who have been diagnosed of epilepsy fall under the scope of the classical socio-professional restrictions addressed to this contingent of persons [19, 20].

At present, there is a growing need to relate the psychosocial adaptation measures to the positive dynamics of the epileptic process under the effects of drug therapy or psychological recovery measures, and these increase the possibilities for socio-professional training of patients with epilepsy [21].

To sum up, we can talk about three distinct stages of palliative recovery of patients with epilepsy – rehabilitation therapy, which is essential for the future and the chances of rehabilitation or overall rehabilitation of the patient with epilepsy. This stage begins in a stationary or epileptic center, which has a stationary service within its structure. Stationary assistance is of prime value, because for patients with epilepsy there is an indispensable rule: each patient with epilepsy at onset of the disease should be examined multilaterally in the somatic plane (tumors and other cerebral organic processes) [22].

The second stage – rehabilitation or adaptation – in the situation of patients with epilepsy is practiced under outpatient conditions under the control of specialists from the offices of the family physicians or the special services; the second stage of the rehabilitation process would be rehabilitation (if speaking about young people who did not have any special anatomy), that develops under the control of physicians at the clinic, neurological clinic or epidemiological center.

The primary objective pursued in the rehabilitation of patients with epilepsy is to maintain the socio-professional status to illness or to adapt it to life and social utility activity in extra-hospital conditions, because the return of the patient with epilepsy to the family very often involves exposing to many exogenous factors that have a negative influence on the evolution of the disease. At this stage is recommended biological therapy, which includes according to various authors any measures that optimize the effect of antiepileptic therapy: psycho-correction, which often supports the reorientation of the patient with epilepsy to another profession, the psychotherapeutic and instructive activity with patients and relatives, which is of great utility if they help patients to reconsider their attitude to illness, work, social entourage and curative process; if they suggest how to solve some cardinal problems such as marriage, conception of a child, which will be decided individually and according to the psychobiological parameters of the person with epilepsy [18, 22].

Rehabilitation of patients with epilepsy in ambulatory conditions

The fascinating advances of medical science in the twentieth century have revolutionized not only the diagnostic and curative approaches to epilepsy patients, but the attitude of modern society to the social status and psycho-biological reductions of people who go through the terrible drama of epileptic seizures, which continuously and ruthlessly demolishes the intellect and the human nature [21, 23].

Both in West-European countries with reputed traditions in plenary recovery treatment of people with diseases that damage the health of the human psyche, such as Austria, Switzerland, Germany, etc., and in the new world represented by the US, governments and civil society activate and excel in the field of human psyche protection. In these states there are systems that have demonstrated the usefulness and effectiveness of supervising appropriate assistance of diseases with destructive potential over supreme brain functions.

In former Soviet countries, the observation of patients with epilepsy at the stage of post-treatment care, as well as the treatment required, was the responsibility of neuropathic physicians at the polyclinics or the psychiatrist from the psycho-neurological dispensary. It cannot be said that there was indeed an articulated and orderly service network concerned with the continuous and staged assisting of psycho-neurologic patients, especially the dramatic situation of persons with psychological effects, which in any case in our country could not hope for rehabilitative treatment of volume and quality sufficient to maintain or even return to pre-mental social status [21, 24].

On the other hand, the rehabilitation of the patient with epilepsy in ambulatory conditions is inconceivable without puncturing and solving the problems of organizing epileptologic assistance, post-pharmacological supervision, supportive therapy and socio-psychological insertion measures of epilepsy, which are performed at the level of services of ambulatory [1].

In Switzerland, the tradition of recovery assistance for patients with epilepsy is of the remarkable history, and specialized centers in this field have been organized in the twentieth century, the services of the country gaining evocative experience. Currently there are 4 large centers with one thousand beds, 200 of them are for children and one of the supportive tasks of these services is the treatment and social adaptation of patients and in other countries have been inaugurated antiepileptic centers. Moreover, in Norway, specialized assistance extends not only to urban centers but also to polar areas with extreme climatic conditions and no railway communi-

cations. In these epileptological centers patients' employment problems are examined and solved, there are special instructors here and two-week courses take place for mothers whose children make epileptic paroxysms [25, 26].

The experience of these centers, which is heading towards gradually and rehabilitation system for patients with epilepsy, have demonstrated the opportunity to take it on a wide scale, namely as a principle of deploying national networks, which are calculated numerically according to the global population, the incidence of the disease is defined by epidemiological research. Some of the first data was presented by I. Reid, who has made some raw calculations stating that the need for such centers in Great Britain would be 5-6 epileptic centers that would provide permanent curative and rehabilitative care [27]. Many scholars, as well as researches on disease and socio-prophylactic issues, have demonstrated that only these centers, though endowed, cannot solve all the social aspects of this major problem. Particularly difficult is the situation of patients with epilepsy who cannot return to society and find no occupation to provide them with means of subsistence [16, 25].

The agenda of rehabilitation in epilepsy is a key issue of the recovery of patients with epilepsy. Scientists and experts representing competent services of these countries outlined concrete steps for action: a) organizing centers to combat epilepsy, including planning and zoning; b) development and improvement of rehabilitation medical premises; c) the settlement agreement of rehabilitation issues, involving not just health workers but social organizations, territorial executives etc.; preparation and presentation the spectrum list of professions and occupations that can be recommended for patients with epilepsy at the stage of social reinsertion, considering the possibility of local employment; involving various civil associations, voluntary social services officials in solving problems confronting the person with epilepsy being remedied or serious sequelae left by it [21, 28, 29].

More difficult is the problem of issuing the verdict of healing session; most doctors are reluctant when it comes time to announce the conclusion of recovery. Their reservations are justified because when there are no known mechanisms of the primary occurrence of epilepsy, they failed to issue a response to the question as to why epileptic seizures occur. All statements about fighting disease are at least empirical and no one can predict the future absolutely clearly of a patient who had once clear signs of epilepsy of the encephalus.

Despite some relevant reluctance to the healing of patients with epilepsy, we have to deal with this act of healing. However, we can talk about a sustainable remedy, based on generally accepted medical principles: symptomatic disappearance of the disease, biological and psychological compensation; winding mechanisms defining pathological disease; recovery of the social status of the person concerned [21, 25, 29, 30].

We can talk about the abolition of antiepileptic therapy of a former patient with epilepsy if the following prerequisites are met:

The first criterion is the absence of any form of seizures over the past 3-5 years. This is required stating that the time slot for crises monotype is 3-4 years without such events, if the patient with epilepsy manifested mixed paroxysms for which improvement were needed high doses of anticonvulsant remedies – the duration of the remission of the seizures should be 4-5 years.

The second essential criterion is the regression of epileptic phenomena characteristic of the EEG (paroxysmal activity, dysrhythmia, etc.). If normalization of EEG (during at least one year) is found, patients who no longer have seizures for the last 3-5 years now are taking low dose anticonvulsant remedies, often a monotherapy supervises that if specific events occur they can also be progressively reduced.

The third criterion of healing evolution is the lack of personality changes and mood variations (dystrophy), except for moderate changes, such as those caused by some deficiencies in the intellectual sphere – the slight decrease of memory, etc.

The patient with epilepsy which has evolved thus will be actively observed another year without medication and if during this period out of therapy suspicious events haven't occurred, one can deduce that the patient with epilepsy is practically cured and only periodically (2-3 years) will have to be examined EEG [13, 26, 31].

Patients with long-term remission should be observed with the utmost caution, especially those who developed epilepsy on the background of substantial cerebro-organic changes or sustained functional relapse (infantile brain paralysis, etc.), because remission can be achieved in these patients, but it is not possible to speak in full terms about complete remission, since changes in the brain were not surgically extracted, the outbreak being a potential generator of future epileptic seizures. For them, only the optimal dose of therapy can be defined to support the curative outcome achieved at a given time [17].

For those patients who have completed their antiepileptic therapy it is recommended to consume episodically specific anticonvulsivant remedies better tolerated if possibly exposed to unfavorable factors in prolonged psychogenic situations of long sleep deprivation for various reasons, with fever prolonged due to other diseases, etc. [17, 28].

Social reforms taking place for over a decade in Eastern Europe have mobilized medical associations in Moldova; many specialists are engaged actively in an inspired movement to safeguard disabled people with psychosomatic problems, involving civil society more strongly. Moldovan National League against Epilepsy is involved in rendering unconditional support services to socially excluded patients because of their different physical and intellectual disadvantages. Moldovan Psycho-Social Philanthropy Center is another NGO service that provides psychotherapy and social rehabilitation of patients with mental disabilities.

Anticonvulsant therapeutics traditions and innovative interventions in epilepsy

At least theoretically, antiepileptic drugs may act to prevent seizures accesses, influencing on:

1. Extra neuronal damage;

2. Pathologically modified neurons, in terms of decreasing or preventing their excessive decay;

3. Normal neurons, as preventing alteration of their tone by excessive downloads coming from elsewhere.

In the first category are substances that can alter blood irrigation of abnormal epileptogenic foci. For example, one can mention that the atropine and antihistamine substances have been tested clinically in epilepsy.

The second category allows that selective anticonvulsants neurons can influence the hyperactive, without changing the function of normal brain cells. This is attractive and there is some evidence to support it, but has not been proven in practice [28, 32].

In the third category, which is most important, there are drugs that prevent the dissemination of access convulsively; and all substances used in clinical antiepileptic property change the brain to respond to various stimuli provoking convulsive seizures. It remains to be established exactly how it is accomplished, although a number of authors have reported a slight increase of the threshold of synaptic long chain system reverberant inhibiting the transformation of the excitation chains and other mechanisms. These points should be considered by the teams concerned about ordering antiepileptic programs.

Choosing the best possible product or optimal combination of drugs is sometimes difficult. Perfect antiepileptic substances must be long acting, non-sedating, well tolerated, and highly active against different types of convulsive seizures and lack adverse effects on organs' vital functions. In addition, it should be used for patients with different types of convulsive seizures, must be active in treating seizures and able to restore electroencephalogram of convulsive seizures in its normal form. Finally, it must have positive psychological effects.

It is still questionable whether such a drug will ever be discovered, and especially one that would cure all types of epilepsy. The extent, to which each of the antiepileptic drugs routinely approaches this perfection, is presented with the description of the respective pharmacological properties [25, 33].

Due to the fact that patients vary considerably with regard to their clinical response to treatment, anticonvulsants known as the opportunities associated with drugs, have been investigated only superficially, the search for new substances and new combinations to be of higher efficiency continues.

Since there is a large number of patients with epilepsy and public costs for assisting them are hardly supported by the state budget, the deficient epilepsy treatment should be the problem with many facets of social and other care. The condition, due partially to ignorance and misunderstanding of its nature, produces a lot of unhappiness, personal family tragedies, psychological and social maladjustment and economic losses. So, it is the duty of the medical practitioner who has not only the obligation to treat each patient with epilepsy right away, but to disseminate and correct information about the disease, attitude towards it, preventing stigmatization when the word «epilepsy» is heard, and once the correct diagnosis has been made, the treatment objectives are: complete healing of the seizures and obtaining for the patients the possibility of living a normal life.

We have to make every possible effort to approach the achievement of these goals as much as possible. Drug treatment occupies the central position among the measures used to prevent seizures accesses. Development concepts, which obviously originated in the process of epilepsy patients medication were dependent on the level of quality of investigation methods and diagnostics, but also reflected the clinical thinking of the doctor who performed the observation of patients along their way to recovery [9, 19].

Often it takes care, patience and verification tests to find the best combination of drugs and exact dosing schedule to avoid the phenomena of maximal effects that have almost all possible anticonvulsive remedies used by epileptologists. Sometimes metabolic measures are necessary adjuncts, such as a ketogenic diet and water reduction.

Development and improvement of treatment methods of epilepsy showed that drug therapy should be started early to yoke or re-channel the process to benign evolution. The elective treatment outcomes depend on adequate preparation in various evolutionary types of epilepsy [34]. However, the drug is chosen to match better access with patient's profile, usually a standard line of treatment is being selected for the anticonvulsant therapy.

The principles of epileptic therapy have been developed in the context of basic research and involve joint efforts of many generations of scientists. Ultimately, it was announced that in most cases, there was required continuous, individual and differentiated therapy for various forms of epilepsy. An obstacle for the implementation of these principles is the identification of adverse effects. The choice of the more appropriate formulation of the case, according to the recent literature, devoted to problems raised by epilepsy therapy, indicates that even when a wide spectrum of anticonvulsants is administered, it is possible to control their plasma concentrations precisely by monitoring physiobiological parameters of the medically treated patients [19, 35].

We highlight the contribution of local scientists, for example academician Stanislav Groppa and the author of the current paper, to the formation of the doctors in the field of neurology and psychiatry, elaboration and selection of the most operative treatment guidelines. Recently, under the auspices of the National League Against Epilepsy – a body that focused on the management of antiepileptic care and the research that has been developed in this regard – a series of conferences took place and several published materials appeared on the topic, some of which are extremely useful and easy to use in choosing the remedy with the dose appropriate to the forms of epilepsy onset, and evolution [7].

The authors of this scientific-practical study recommend for all generalized epilepsy with absences the elective preparation Valproate (VPA) with serum levels between 60 and 100 mg/ml, 20-30 mg / kg body mass. The preparation has the quality of not affecting the patient's cognitive functions, or the digestive signs apparent at the initiation of therapy. The same authors suggest that other anticonvulsant remedies from this recent series, for example Ethosuximide (ESM), an equivalent of Valproate, may also be used; both can be combined [36]. Also in the opinion of some auhtors in a number of cases the therapeutic effect can substantially

improve epilepsy with nocturnal seizures by intensifying the hypothermic inhibition (deepening of sleep) with Amobarbital sodium [36].

All specialists opt for monotherapy, or at least direct doctors to aim at such an objective. The objective of any antiepileptic medication is the complete suppression of seizures, and the progression and reduction in frequency and severity of seizures, which are associated with a relatively calm EEG trace, are considered to be successful [12,21,37]. The complete disappearance of seizures must be confirmed by a clean EEG route. In the last few decades, considerable results have been obtained in the treatment of epilepsy. It started from the fact that more and more specialists have begun to reject phenobarbital as a medical remedy for the treatment of paroxysms. The latter was accused of cumulative properties with following degradation in the cerebrospinal fluid 2-3 hours after administration. It was rejected for its long-term removal from the body, its traces also being detected 7-12 days after administration. So, luminal accumulates, creates habit, and also causes adverse phenomena [17, 35].

It has been found, for example, that luminal, although reducing seizures precipitates the formation of epileptic personality. These conclusions were reconfirmed by other researchers.

The idea that the application of barbiturates in the treatment of epilepsy, especially of its benign forms, is irrational has been mentioned in various studies reported at different times. Some clinicians recommend that Luminal be given in combination with Caffeine, Phenamine, Sidnocarb, Tryptophan, to neutralize the soporific effects and inhibition of the drug remedy [12, 38].

Analysis of literary sources with reference to the treatment of epilepsy has shown that this issue is still questionable. The etiological, pathogenetic and symptomatic principles of the described medication programs were not based on principles of pathogenicity, etiocauzality and conformation to symptomatology [17, 30].

Others argued that an important factor in epilepsy onset is arachnoidite or meninges inflammation and neurosurgical treatment of scarring processes in meninges. It should be noted that the latest study reported that the normalization of meninges is the resorption function, combating hypertension with CSF hypersecretion, all of which were obtained from X-therapy [33, 39, 40].

The physician's conduct in the treatment of epilepsy has been reported in many authoritative studies. Most cited scientists mention that a well-adopted behavior can provide positive results to the majority of patients with appropriate epilepsy. We could not derive from these studies that there would be a systematized attitude based on the efficacy criteria specified in relation to epilepsy therapy.

Another important issue, which remains controversial and with many uncertainties, would be the association of anticonvulsants with neuroleptics so, polytherapy in epileptic medication. This curative way becomes imperative in disinhibition syndrome, in anatomies with frequent affective reactions, in *petit mal* type, in paroxysms that do not respond to therapy.

Some authors studying the vitamin metabolism concluded that epilepsy patients consistently suffer from vitamin deficiency, especially from the B group. These complexes of vitamins are useful in all clinical forms of epilepsy.

Folic acid deficiency often occurs due to long-term treatments with phenobarbital, pyramidine (in 25-92% of patients with epilepsy). Carbamazepin treatment can also cause follicular deficiency. Epilepsy causes not only the paroxysms, but also the psychic changes, the characteristic deviations of epilepsy sufferers. Many scientific sources underline that the vicissitudes of position and social status are of particular importance in triggering epileptiform phenomena and largely decide the chances of plenary curative recovery.

In the past few years, epilepsy treatment has been substantially revised and benefited from a more pragmatic and more concrete approach. Here we quote the works of a number of authors. Thus, besides the fact that many medication programs have been modeled and imagined in relation to the most diverse types of epilepsy, it has been promulgated that in a rather small number of patients with epilepsy surgery may prove useful and can lead from a considerable decrease in convulsions to their disappearance. This may be the case with patients with discrete focal lesions, which can be removed by surgical resection and in some cases of psychomotor epilepsy, caused by a temporal lobe dysfunction [41, 42].

Recent literature on epilepsy-related issues shows that using a wide spectrum of anticonvulsant remedies, precise plasma concentrations can be performed, monitoring paraclinically the dynamics of medication patients, but the evolution of the conceptions regarding the medication of patients with epilepsy reflects the level of the methods of investigation and diagnosis, of the clinical thinking of the physician, of observing the patients in the treatment process. The development and improvement of methods to monitor subcurative epilepsy evolution have demonstrated that drug therapy should be started as early as possible not to juggle the process or re-analyze it to benign development, thus the results of the treatment depend on the proper choice of the preparation in various evolutionary types of epilepsy [15, 38].

In cases where the application of an anticonvulsant at the maximum therapeutic dose is impacted by the occurrence of adverse events, it is reasonable to slightly reduce the dose and to associate other anticonvulsants. In such cases, it is basically a summary of the effects of anticonvulsant remedies. Thus their conjugate action became known in various complex mixtures, including the original one. Also, it is known that additive blend is proven to be highly effective, developing a broad spectrum of effects, with minimal toxicity even in long-term application. Combined prescribing of preparations requires a thorough, dynamic control of the functional status of the kidneys, the liver, and the blood vessels [13, 14].

Conclusions

1. Normal and abnormal neuronal cells are involved in pathological discharges; the exact genesis of this phenomenon is known to be vague and means the involvement of many factors of cellular, vascular and metabolic disorders.

2. Rehabilitation of patients with epilepsy should be gradual, using compliance between drug treatment, psychosocial rehabilitation depending on the dynamics of the disease and the patients' reaction to their own condition.

3. The uptake of concepts and rehabilitation programs introduced into many countries' systems, the formation of the assisted care system, will increase the effectiveness of ambulatory rehabilitation.

References

1. Goldberg EM, Coulter DA. Mechanisms of epileptogenesis: a convergence on neural circuit dysfunction. Nat Rev Neurosci. 2013;14(5):337-349. doi:10.1038/nrn3482
2. Beghi E. Efficacy and tolerability of the new antiepileptic drugs: comparison of two recent guidelines. Lancet Neurol. 2004;3(10):618-621.
3. Bishop M, Allen CA. The impact of epilepsy on quality of life: a qualitative analysis. Epilepsy Behav. 2003 Jun;4(3):226-233.
4. Boro A, Haut S. Medical comorbidities in the treatment of epilepsy. Epilepsy Behav. 2003 Oct;4 Suppl 2:S2-12.
5. Choi-Kwon S, Chung C, Kim H, Lee S, Yoon S, Kho H, Oh J, Lee S. Factors affecting the quality of life in patients with epilepsy in Seoul, South Korea. Acta Neurol Scand. 2003;108(6):428-34.
6. Covanis A. Panayiotopoulos syndrome: a benign childhood autonomic epilepsy frequently imitating encephalitis, syncope, migraine, sleep disorder, or gastroenteritis. Pediatrics. 2006 Oct;18(4):e1237-43. Epub 2006 Sep 1.
7. International League Against Epilepsy (ILAE). A Global Agenda: Annual Report 2016. Hartford; 2017. 170 p.
8. Cramer JA, Blum D, Reed M, Fanning K; Epilepsy Impact Project Group. The influence of comorbid depression on quality of life for people with epilepsy. Epilepsy Behav. 2003 Oct;4(5):515-521.
9. Holloway R, French J, Kanner A. Efficacy and tolerability of the new antiepileptic drugs: treatment of new onset epilepsy. Neurology. 2005;64:172-174.
10. Djibuti M, Shakarishvili R. Influence of clinical, demographic, and socioeconomic variables on quality of life in patients with epilepsy: findings from Georgian study. J Neurol Neurosurg Psychiatry. 2003 May;74(5):570-573.
11. Gilliam F. The impact of epilepsy on subjective health status. Curr Neurol Neurosci Rep. 2003 Jul;3(4):357-362.
12. Frey LC. Epidemiology of posttraumatic epilepsy: a critical review. Epilepsia. 2003;44(Suppl 10):11-77.
13. Helmstaedter C, Kurthen M, Lux S, Reuber M, Elger CE. Chronic epilepsy and cognition: a longitudinal study in temporal lobe epilepsy. Ann Neurol. 2003 Oct;54(4):425-32.
14. Lada C, Skiadas K, Theodorou V, Loli N, Covanis A. A study of 43 patients with Panayiotopoulos syndrome, a common and benign childhood seizure susceptibility. Epilepsia. 2003 Jan;44(1):81-8.
15. Kanner AM, Barry JJ. The impact of mood disorders in neurological diseases: should neurologists be concerned? Epilepsy Behav. 2003 Oct;4 Suppl 3:3-13.
16. LaRoche SM, Helmers SL. Epilepsy in the elderly. Neurologist. 2003 Sep;9(5):241-249.
17. Piccenna L, Shears G, O'Brien TJ. Management of post-traumatic epilepsy: an evidence review over the last 5 years and future directions. Epilepsia Open. 2017;2(2):123-144. Published 2017 Mar 17. doi:10.1002/epi4.12049
18. Meletti S, Benuzzi F, Rubboli G, Cantalupo G, Stanzani Maserati M, Nichelli P, Tassinari CA. Impaired facial emotion recognition in early-onset right mesial temporal lobe epilepsy. Neurology. 2003;60(3):426-431.
19. Specchio L, Beghi E. Should antiepileptic drugs be withdrawn in seizure free patients. CNS Drugs. 2004;18(4):201-212.
20. Yerby M, Kaplan P, Tran T. Risk and management of pregnancy in women with epilepsy. Cleve Clin J Med. 2004;71 Suppl 2:S25-37.
21. Korczyn AD, Schachter SC, Brodie MJ, et al. Epilepsy, cognition, and neuropsychiatry (Epilepsy, Brain, and Mind, part 2). Epilepsy Behav. 2013;28(2):283-302.
22. Gekht AB. [Starting and ending antiepileptic therapy in adults]. In: [Diagnosis, treatment, social aspects of epilepsy: Proceedings of the International conference]. St. Petersburg; 2006. p. 126-134. Russian.
23. Vol'f P. [Epilepsy in the literature]. In: Gusev EI, Gekht AB, editors. [Epileptology in medicine of the XXI century]. Moscow: Svetlitsa; 2009. p. 9-14. Russian.
24. Gekht AB. [Epidemiology and the course of epilepsy]. In: Gusev EI, Gekht AB, editors. [Epileptology in medicine of the XXI century]. Moscow: Svetlitsa; 2009. p. 45-50. Russian.
25. Gromov SA, Neznanov NG. [Biopsychosocial aspects of rehabilitation of patients with epilepsy]. In: Neznanov NG, editor. [Epilepsy]. St. Petersburg; 2010. p. 857-891. Russian.
26. Gusev EI, Gekht AB, Melochanova LE, Chiurilin IuIu. [Epidemiology of epilepsy]. In: Neznanov NG, editor. Epilepsy. St Petersburg; 2010. p. 51-64. Russian.
27. Reid CA, Phillips AM, Petrou S. HCN channelopathies: pathophysiology in genetic epilepsy and therapeutic implications. Br J Pharmacol. 2012 Jan;165(1):49-56.
28. Gromov SA. [Controlled epilepsy. Clinic, diagnosis, treatment]. St Petersburg: IIC Baltika; 2004. 302 p. Russian.
29. Gromov SA, Iakunina ON, Eroshina ES. [Epilepsy, personality changes, treatment]. St. Petersburg: IIC Baltika; 2006. 320 p. Russian.
30. Gromov SA, Lipatova LV, Neznanov NG. [Epilepsy. Rehabilitation of patients, treatment]. St. Petersburg: SIC WMA; 2008. 392 p. Russian.
31. Genton P. [Paradoxical aggravation of seizures in patients with epilepsy]. In: [Epilepsy – diagnosis, treatment, social aspects: Proceedings of the international conference]. Moscow; 2005. p. 139-142. Russian.
32. Panayiotopoulos CP, Michael M, Sanders S, Valeta T, Koutroumanidis M. Benign childhood focal epilepsies: assessment of established and newly recognized syndromes. Brain. 2008 Sep;131(Pt 9):2264-86. Epub 2008 Aug 21.
33. Zenkov LR. [Pharmacotherapy of difficult to cure epilepsy]. In: Gusev EI, Gekht AB, editors. [Epileptology in medicine of the XXI century]. Moscow: Svetlitsa; 2009. p. 521-529. Russian.
34. Paprocka J, Kijonka M, Pęcka M, Sokół M. Melatonin in epilepsy: a new mathematical model of diurnal secretion. Int J Endocrinol. 2016;2016:3861461.
35. Mikhailov VA. [Epilepsy: stigmatization, quality of life and rehabilitation of patients]. [Neurological Herald]. 2007;3:135-136. Russian.
36. National Clinical Guideline Centre (UK). The Epilepsies: the diagnosis and management of the epilepsies in adults and children in primary and secondary care: Pharmacological Update of Clinical Guideline 20. London: Royal College of Physicians (UK); 2012. (NICE Clinical Guidelines, No. 137).
37. Saltik S, Angay A, Ozkara C, Demirbilek V, Dervent A. A retrospective analysis of patients with febrile seizures followed by epilepsy. Seizure. 2003. Jun;12 (4):211-216.
38. Herrmann LK, Welter E, Berg AT, Perzynski AT, Van Doren JR, Sajatovic M. Epilepsy misconceptions and stigma reduction: current status in Western countries. Epilepsy Behav. 2016;60:165-173. doi:10.1016/j.yebeh.2016.04.003
39. Litvinovich EF, Savchenko AIu, Pospolit AV. [Current aspects of the pharmacotherapy of epileptic status]. [Clinical Epileptology]. 2007;(1):28-32. Russian.
40. Mironov MB, Mukhin KIu, Petrukhin AS. [Monitoring the effectiveness of treatment of patients with juvenile forms of idiopathic generalized epilepsy and the state of „pseudo-remission"]. [J Neurol Psychiatry SS Korsakov]. 2005;105(8):24-28. Russian.
41. Schmidt D, Löscher W. Uncontrolled epilepsy following discontinuation of antiepileptic drugs in seizure free patients: a review of current clinical experience. Acta Neurol Scand. 2005;111(5):291-300.
42. Petrukhin AS, Pylaeva OA, Voronkova KV. [Aggravation of epileptic seizures under the influence of anti-epileptic drugs]. [J Neurol Psychiatry SS Korsakov]. 2005;105(9):66-70. Russian.

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Correlation between spinal nerves, anterolateral abdominal wall muscle tone and inguinal hernia

Gheorghe Guzun, MD, PhD, Associate Professor; ***Radu Turchin,** MD, PhD, Associate Professor

Department of Topographic Anatomy and Operative Surgery
Nicolae Testemitsanu State University of Medicine and Pharmacy, Chisinau, the Republic Moldova

*Corresponding author: radu.turchin@usmf.md
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Abstract

Background: The complete diagnosis of pathomorphological disorders in case of diseases of the spine determines the choice of effective treatment, pathogenetically argued in various diseases which, according to modern classifications, do not have a direct link with it. Continuous improvement of diagnostic and treatment methods in some pathologies, both surgical and therapeutical requires a more detailed study of clinical anatomy and spinal biomechanics. Thus, perhaps even revising concepts are well-rooted in the consciousness of scientists and modern practitioners. As we will try to analyze the anatomical-clinical component of the appearance and recovery of antero-lateral abdominal wall hernias, we will limit ourselves to some analysis and discussion of one of the predisposing factors, namely the lack of resistance or insufficient resistance of the abdominal muscles. This is closely related to the condition of the constituent elements of the abdominal wall, and in particular depends on the innervation and vascularization of the musculo-aponeurotic layers, which determine the strength and muscle tone.

Conclusions: One of the causes of inguinal hernias is the decrease in the tone of the abdominal muscles. It depends on several factors: the elasticity of the muscular tissue, state of neuro-muscular transmission, the state of peripheral nerve fibers and motor neurons in the spinal cord, superior motion control centers. Thus, the causes of muscle tone decrease can be both muscular in origin and generated by pathology of the nervous system at different levels. Because the causes are multiple, the treatment is different at certain stages of hernia development. In this way, our treatment – qualitative nutrition, vitamins, special exercises, spinal region work, paravertebral muscles massage fit perfectly into hernia etiology and pathogenesis. This treatment is physiologically accessible and can be performed and supervised by physicians with non-surgical specialties in stationary or outpatient settings, after prior consultation with the surgeon.

Key words: spinal nerves, anterolateral abdominal muscle tone, inguinal hernia.

Introduction

The complete diagnosis of pathomorphological disorders in case of diseases of the spine determines the choice of effective treatment, pathogenetically argued in various diseases which, according to modern classifications, do not have a direct link with it.

Continuous improvement of diagnostic and treatment methods in some pathologies, both surgical and therapeutical requires a more detailed study of clinical anatomy and spinal biomechanics. Thus, perhaps even revising concepts are well-rooted in the consciousness of scientists and modern practitioners.

We all know the notion of hernia of the anterolateral wall of the abdomen: which represents the partial or total exteriorization of one or more internal organs, from the peritoneal cavity to the weak areas of the abdominal wall, pre-existing anatomically, with the parietal sheet of the peritoneum. The causes of any acquired hernia are the result of the interaction of two categories of forces: 1. resistance of the abdominal wall determined both by the mechanism of its constitution and by the state of its structural elements; 2. and the intraabdominal pressure exerted from the inside on it represented by the pressure gradient of the abdominal internal organs, overlapping complementary forces that destabilize the existing balance at some point and become the determinant element in the production of hernias [1, 2].

Because we will try to analyze the anatomical-clinical component of the appearance and recovery of antero-lateral abdominal wall hernias, we will limit ourselves to some analysis and discussion of one of the predisposing factors, namely the lack of resistance or insufficient resistance of the abdominal muscles. This is closely related to the condition of the constituent elements of the abdominal wall, and in particular depends on the innervation and vascularization of the musculo-aponeurotic layer, which determines the strength and muscle tone.

Generally, the vascularization and innervation of a muscle is determined by a major artery that is accompanied by 2 veins and a nerve. The vessels are located along the muscle fibers and gradually divide into smaller arteries. Innervation is performed by a single nerve branch, which penetrates the muscles together with the vessels, forming within it a rich plexus intramuscularly. The nerve of the muscle is mixed, having motor, sensory and vegetative fibers. Vegetative fibers enter the muscles and form perivascular plexuses, being sympathetic fibers with action on their vessels. Special anatomo-experimental investigations and clinical observations have shown that *nn. vasorum* go to vessels in the composition of spinal nerves in which they are also sympathetic fibers. Sympathetic fibers, to the periphery at different levels, come out of their composition and penetrate into the perivascular tissue, then into the vessel wall. The arteries have more *nn. vasorum*, than the veins.

The muscle has several properties that characterize it: contractility, elasticity and tonicity. Thus, muscle tone is a state of mild and permanent contraction of the muscle at rest, and is manifested by a low degree of tension. This is the fundamental property of the muscle, which has preserved innervation. The mechanism of muscle tone production is of a nervous nature [3, 4].

Thus, in order to maintain the normal muscle size, it is necessary to receive permanent contractile impulses. When a muscle loses its innervation, it is subject to atrophy that begins almost immediately [5].

The vessels and nerves play an essential role in maintaining muscle strength and tone in the anterior-lateral abdominal region. It can be said that any disruption in their activity, both internal and external, will have effects on the muscles. However, in order to answer questions about the causes of the installation of muscle hypotonia in the abdomen's antero-lateral region, it is necessary to examine the vascularization and innervation.

Superficial arteries and veins in the anterolateral abdominal wall are found in the subcutaneous layer. The arteries in the lower compartment: superficial epigastric arteries, superficial circumflexes iliac artery (femoral artery), and in the region of superficial inguinal ring – external pudenda artery. In the upper compartment are branches of intercostal and lumbar arteries. The veins are better developed than the arteries and form plexuses. In the umbilical region, the toracoepigastric vein begins to flow into the lateral thoracic vein and anastomoses the superficial epigastric vein that flows into the femoral vein (link between the axillary vein and the femoral vein). Superficial veins anatomies in the umbilical region with deep veins (upper and lower epigastric veins) and paraombilical vein that flow into the portal vein. This makes the connection between the lower inferior venous system and the portal. The deep arteries and veins: the upper epigastric artery – the terminal branch of the internal thoracic artery and the umbilical anastomosis with the inferior epigastric artery branch of the external iliac artery. In the lower regions is the deep iliac circumflex artery, branch of the external iliac artery. Likewise, the anterolateral abdominal wall is vascularized by the last 5 intercostal arteries and the lumbar arteries. The veins accompany the arteries.

Both superficial and deep muscular innervation is performed by the lateral and anterior branches of the intercostal nerves from VII to XII, and in the lower portions – the ileohipogastric and ilioinghinal nerves from the lumbar plexus. Thus, in the epigastral region there are the intercostal nerves VII-IX, the intercostal nerves X-XI branch in the mezogastrium, and in the hypogastrium the subcostal nerve XII, the ileohipogastric and ilioinghinal nerves [1, 6-11].

Because the previously named nerves play a primary role in maintaining the muscular tonus of the anterior abdominal wall, we will describe their topography and some causes of disorder of the transmission of nerve impulses to the muscles and blood vessels. As mentioned above, the spinal nerves VII-XII, the ileohypogasic and ilioinghinal (lumbar plexus) nerves, which are emerging from the lower and lumbar thoracic column, are responsible for the anterolateral abdominal wall's innervation.

Generally, the spine is the axis of support of the whole skeleton of the body. It has the form of a resistant and flexible bone column, it consists of bone pieces, called vertebrae, among which there are fibrocartilage formations called intervertebral discs. The vertebrae give the spine the resistance to support the weight of the body, and the intervertebral discs give it the flexibility that ensures its movements. The vertebral body and arch are defining the vertebral opening. The vertebral arch is linked to the vertebral body by two thin blades – the vertebral pedicles. The upper and lower margins of the vertebral pedicle present the vertebral incisions. The vertebral incisions of two neighboring vertebrae form an orifice (the intervertebral orifice) through which the spinal nerves pass. Vertebral bodies are linked together by joint discs and ligaments. An intervertebral disc has at its periphery a fibrous ring made of fibrocartilage and fibrous tissue, and towards the center – a soft, gelatinous elastic substance (pulp nucleus). All spine biomechanics depend on the state of the intervertebral discs. In adults, the discs make up 20-25% of the entire length of the spine. Disc degeneration leads to loss of substance in the pulp nucleus, decrease in intervertebral orifice size, and compression of spinal nerves.

Spinal nerve originates in the spinal cord and is the way to drive the nervous influx to and from the spinal cord. They are formed by joining the nerve fibers of the posterior and anterior roots. They are mixed nerves, consisting of sensitive fibers and motor fibers (somatic and vegetative). They are short and divide into four branches: posterior, anterior, visceral and meningian. 1. The posterior branch innervates the skin, sweat and sebaceous glands, blood vessels, back muscles and muscles in the occipital and parietal regions of the head. 2. The anterior branch is more bulky and embraces the same organs as the posterior branch, but from the regions: cervical, temporal, auricular, upper, lateral and anterior parts of the thorax and abdomen, as well as upper and lower limbs. 3. The visceral branch or the white communicating branch contains only vegetative fibers, through which the vegetative nervous system connects to the central nervous system. 4. The meningeal branch is a thin branch that returns through the conjugation hole into the spinal canal and innervates the spinal meninges.

There are 31 pairs of spinal nerves, placed symmetrically on both sides of the spinal cord and metamerically distributed as follows: 8 pairs in the cervical region, 12 in the thoracic, 5 in the lumbar, 5 in the sacral, 1 in the region coccygeal. Each pair starts from a medullary nerve center (31 nerve centers) and corresponds to a specific skin area called dermatom, parts of the muscle (myotom), osteoarticular elements (sclerotoma), vascular elements (angiotom) and visceral elements (viscerotom), in which there is a permanent anatomical connection.

Nerve plexuses are the anastomoses of the anterior branches of the spinal nerves, with the exception of the thoracic region. Because the subject of our discussion is innervation and vascularization of the abdominal anterolateral wall, we will focus more on the lower thoracic and lumbar column.

The thoracic segment: The anterior branches of the thoracic spinal nerves pass through the intercostal space and form the intercostal neurovascular bundle. The first six intercostal nerves are branched and reach the sternum. Intercostal nerves from VII to XII, pass to the anterior abdominal wall and provide sensory and motor innervation.

The lumbar nerve plexus consists of anastomoses of the anterior branches of T_{12} and the first four lumbar nerves. This plexus innerves lower abdominal muscles, obturator muscles, thigh muscles (medial groups), skin of lower abdomen, scrotum or greater pudendal lips, skin of the thigh, skin of the calf and foot on the medial face. From the lumbar plexus, two types of branches are formed – collateral and terminal.

Collateral branches of the lumbar nerve plexus:

1. The ileo-hypogastric nerve through the muscular branch innerves the wide abdominal muscles and rectus abdominis muscle. Another branch is the cutaneous branch and innervates the skin of the hip, pubic and scrotum region for male, and for female – the large lips.

2. The ilioinghinal nerve innerves the skin of the scrotum to the males, or the large lips in the woman.

3. The lateral cutaneous femoral nerve innerves the skin of the fessier region and the skin of the thigh's lateral region. 4. The genitofemoral nerve innerves the skin of the medial face of the thigh and scrotum or large lips skin.

Terminal branches of the lumbar nerve plexus:

1. The obturator nerve has an anterior and posterior branch. The anterior branch innerves external obturating muscles, pectinum, long and short adductor, and skin in the knee region and mid-thigh. The posterior branch innerves the large adductor muscle and the coxofemoral joint.

2. The femoral nerve is the most voluminous nerve of the lumbar plexus. In the pelvic cavity it gives branches for the iliopsoas and pectinum muscles, for the femoral artery, and under the inguinal arcade forms numerous branches for the anterior muscles of the thigh [1, 6, 12-20].

We may consider that some causes of the installation of the abdominal hernias are vertebrogenic, i.e. pathologies of the peripheral nervous system, which predominantly affect the spinal nerves in the inferior thoracic and lumbar region in our case. According to the literature, these pathologies are the number one among neurological pathologies. Their etiology may be different: trauma, ischemia, infection, endogenous and exogenous intoxications [21, 22, 23].

However, regardless of the nature of this syndrome, the decrease in the tone of the muscles in the anterolateral abdomen region is associated with spinal nerve compression. Among the pathologies of the peripheral nervous system, an important part is its secondary affection, which develops in patients with traumatic discopathy and osteochondrosis of the spine. In case of osteochondrosis, compression of the spinal nerves develops gradually with the installation of edema and muscle spasm. The inflammatory process occurs as a consequence of degenerative processes in connective tissue. The herniated disc can compress the spinal nerves.

Spinal pathologies with compression of a spinal nerve in most cases are associated with acute pain access. But there may be disturbances in the functioning of the muscles and internal organs. It depends on the part that was involved: motor, sensory or vegetative.

Thus, compression of the sensory nerves is manifested immediately by the occurrence of pain, but the motor and vegetative are for a long time asymptomatic, and the patients address to the physician only after the changes and complications of the internal organs or muscles occur [24, 25].

An important role in the normal functioning of the spinal nerves belongs to the intervertebral disc. Degenerative processes in the intervertebral disc gradually arise and affect the disintegration of the function of the blood vessels and nerve fibers that participate in its nutrition. In addition, intervertebral disc cells are sensitive to decreased oxygen concentration, decreased glucose concentration, and local pH [21, 22, 26].

Considering the above mentioned, it can be stated that at the initial stages of degenerative processes in the spine, effective treatment can be conservative treatment: diet, physical culture, massage and healthy lifestyle.

The diet should contain active products and substances directed to the rehabilitation of vessels and nerves in the intervertebral disc region and to the normalization of oxygen and glucose concentration and local pH. These requirements more closely coincide with a predominantly vegetarian diet rich in vitamins and mineral salts, including proper hydration.

Physical culture and massage enhance tonus of paravertebral muscles and trophic adjacent tissues. These procedures are important because the blood vessels and the nerves only reach the periphery of the intervertebral disc, and its nutrition is made from the surrounding tissues.

Healthy lifestyle involves proper sleep, avoiding smoking, toxins of different genesis and stress.

As a demonstration of the above, we want to describe a clinical case of a 9 year old examined patient G. M., a pupil at a secondary school in Chisinau.

The reason for addressing a physician is the presence of unaccountable pain in the right inguinal region, which accentuates a bit in cough, physical effort and prolonged orthostatism, accompanied by the presence of a pseudotumoral formation corresponding to the right inguinal canal (fig. 1). The child's father states that this formation appeared about 4-5 months ago, noticed it by accident when the boy was bathing. The child at the time of the examination did not show any other illnesses or complaints. He is an active boy who plays football in his free time. From the parents' words, the child was born in term, naturally,

without any complications, 3.6 kg weight. 5-6 months after birth, in the region of the right inguinal canal, during the weeping there was a swelling, the size of a cherry, which in the palpation gave a feeling of crepitation. After referring to the pediatric surgeon it was established the diagnosis of incipient congenital inguinal hernia. Hernia has developed from the peritoneovaginal process, which obliterated after birth. In the case of this child, it persisted and was recommended a surgical intervention in order to terminate the process. Parents refused to have the child operated on motivating that the swelling was diminishing in dimensions, compared to its initial ones. They struggled to compress this formation with a diaper, which was fixed just above the hernia. Half a year later hernia disappeared. The pediatric surgeon who repeatedly consulted the child stated that the peritonoevaginal process had stopped and the child had no hernia. Until the age of 9, the child and parents did not notice any formation in this region.

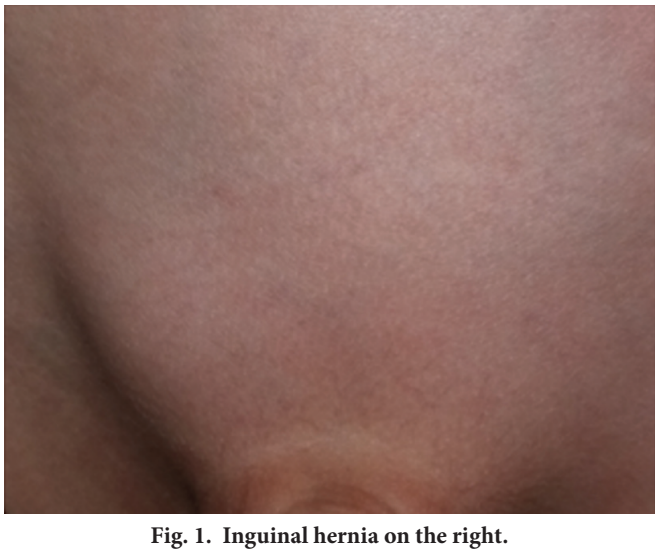

Fig. 1. Inguinal hernia on the right.

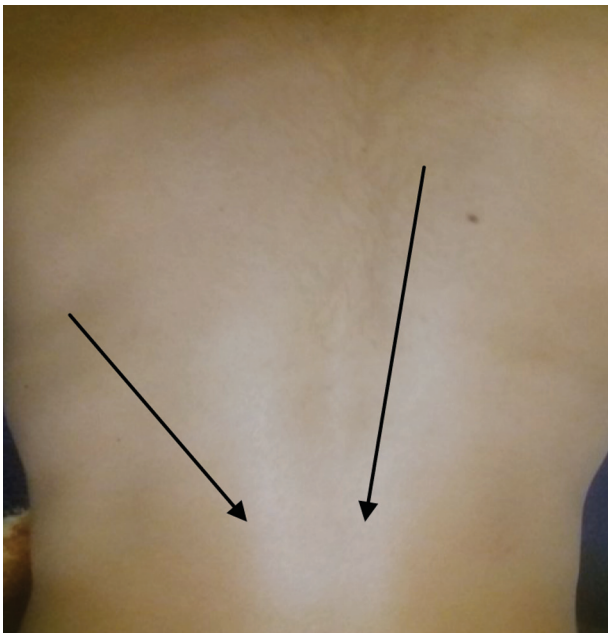

Fig. 2. Appearance of paravertebral muscles before treatment.

Local examination on the day of physician referral, performed at the patient in orthostatism, revealed an elongated, reducible pseudotumoral formation of elastic consistency. At percussion – tympanism and intestinal gargling at auscultation, which corresponds to the inguinal straight canal and extends along its entire tract.

The diagnosis established today is inguinal hernia acquired on the right, which is also shown in the picture below.

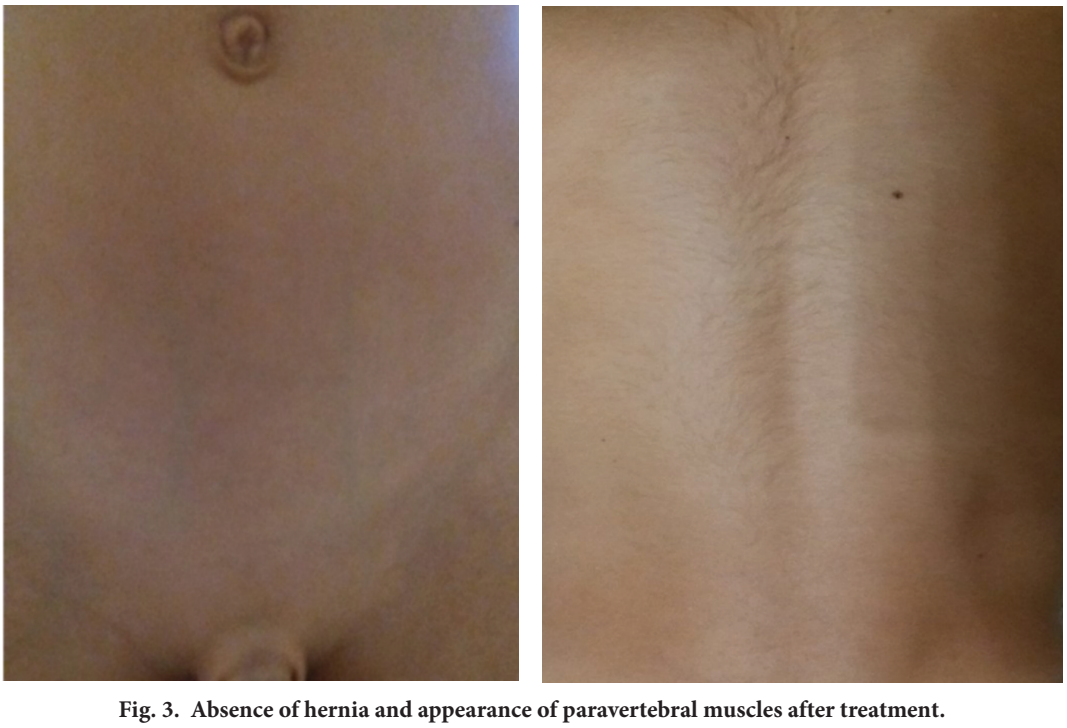

Fig. 3. Absence of hernia and appearance of paravertebral muscles after treatment.

As a result of the back examination, an underdevelopment of the paravertebral muscles was observed in the lower thoracic and upper lumbar segment (fig. 2). In other words, the paravertebral muscles at this level look as if they were interrupted, thus showing the picture of a crater or a muscle defect. That is the muscles above and below this segment are strong, full, elevated, while in this area they seem to be lacking. Moreover, this area is covered with much more developed hair than the rest of the back.

As a result of a treatment of 4-5 months, which consisted of general measures, mentioned above – a predominantly natural and vegetarian diet, excluding products rich in preservatives, dyes and other food additives, gymnastics and local measures – spine work, we obtained the following results.

As shown in the figure 3, the hernia has disappeared and the paravertebral muscles are full and strong.

Conclusions

As a result of studying the literature and our experience we can state that one of the causes of inguinal hernias is the decrease in the tone of the abdominal muscles. It depends on several factors: the elasticity of the muscular tissue; state of neuro-muscular transmission; the state of peripheral nerve fibers and motor neurons in the spinal cord; superior motion control centers. Thus, the causes of muscle tone decrease can be both muscular in origin and generated by pathology of the nervous system at different levels. Because the causes are multiple, the treatment is different at certain stages of hernia development. In this way, our treatment – qualitative nutrition, vitamins, special exercises, spinal region work, paravertebral muscles massage fit perfectly into hernia etiology and pathogenesis. This treatment is physiological, accessible and can be performed and supervised by physicians with non-surgical specialties in stationary or outpatient settings, after prior consultation with the surgeon.

References

1. Kulcițki KI, Bobrik II, Ditkovski AP, et al. Chirurgie operatorie și anatomie topografică [Operative surgery and topographic anatomy]. Chișinău: Știința; 1995. 463 p. Romanian.
2. Suman S, Suman A. Peretele anterolateral al abdomenului [The anterolateral abdominal wall]. Chisinau; 2017. 260 p. ISBN 978-9975-56-415-1. Romanian.
3. Korolev AA. Neirogennye mekhanizmy reguliatsii myshechnogo tonusa [Neurogenic mechanisms of muscle tone regulation]. Uspekhi Sovremennogo Estestvoznaniia. 2013;(5):145-146. Russian.
4. Samsonova AV. Gipertrofiia skeletnykh myshts cheloveka [Human skeletal muscle hypertrophy]. Sankt-Petersburg: Kinetika; 2018. 159 p. Russian.
5. Guyton AC, Hall JE. Tratat de fiziologie a omului [Textbook of human physiology]. 11th ed. București: Callisto; 2007. 1152 p. Romanian.
6. Standring S. Gray's anatomy. 40th ed. Edinburgh: Elsevier; 2008. 1576 p.
7. Kovanov VV. Operativnaia khirurghia i topograficheskaia anatomiia [Operative surgery and topographic anatomy]. Moskow: Meditsina; 1985. 368 p. Russian.
8. Lopukhin IuM. Topograficheskaia anatomiia i operativnaia khirurgiia. T. 1 [Topographic anatomy and operative surgery. Vol. 1]. Moskow: Geotar-Med; 2002. 832 p. Russian.
9. Lopukhin IuM. Topograficheskaia anatomiia i operativnaia khirurgiia. T. 2 [Topographic anatomy and operative surgery. Vol. 2]. Moskow: Geotar-Med; 2002. 592 p. Russian.
10. Lubotskii DN. Osnovy topograficheskoi anatomii [Basics of topographic anatomy]. Moskow: Medgiz; 1953. 648 p. Russian.
11. Sergienko VI, Petrosian EA, Frauchi IV. Topograficheskaia anatomiia i operativnaia khirurgiia. T. 1 [Topographic anatomy and operative surgery. Vol. 1]. Moskow: Geotar-Med; 2012. 832 p. Russian.
12. Cristea I. Anestezia subarahnoidiană și peridurală [Subarachnoid and peridural anesthesia]. București: ALL; 1994. 422 p. Romanian.
13. Ifrim M, Niculescu G, Bareliuc N, Cerbulescu B. Atlas de anatomie umană. Vol. 3 [Atlas of human anatomy. Vol. 3]. București: Editura științifică și enciclopedică; 1985. 280 p. Romanian.
14. Petricu IC, Voiculescu IC. Anatomia și fiziologia omului [Human anatomy and physiology]. București: Editura medicală; 1967. 799 p. Romanian.
15. Turchin R, Guzun G. Aspectele clinice ale anatomiei topografice în specialitatea ATI: îndrumar metodic pentru studenți [Clinical aspects of topographic anatomy in the AIC specialty: methodical guidance for students]. Chișinău: Medicina; 2014. 148 p. Romanian.
16. Gudimov BS, et al. Praktikum po topograficheskoi anatomii [Practical work on topographic anatomy]. Minsk: Visheishaia shkola; 1984. 225 p. Russian.
17. Elizarovskii SI, Kalashnikov RN. Operativnaia khirurgiia i topograficheskaia anatomiia [Operative surgery and topographic anatomy]. Moskow: Meditsina; 1967. 424 p. Russian.
18. Isakov IuF, Lopukhin IuM. Operativnaia khirurghiia s topograficheskoi anatomiei detskogo vozrasta [Operative surgery with topographic anatomy of the child]. Moskow: Meditsina; 1977. 624 p. Russian.
19. Lazort G, Guaze A, Djindjian R. Vaskuliarizatsiia i gemodinamika spinogo mozga [Vascularization and hemodynamics of the spinal cord]. Moskow: Meditsina; 1977. 256 p. Russian.
20. Matiushin IF. Rukovodstvo po operativnoi khirurgii [Operative surgery guide]. Gor'kii (Russia): Volgo-viat; 1982. 320 p. Russian.
21. Barinov AN. Tonnel'nye nevropatii: obosnovanie patogeneticheskogo lecheniia [Entrapment neuropathies: rationale for pathogenetic therapy]. Vrach. 2012;(4):31-37. Russian.
22. Barinov AN. Kompleksnoe lechenie tonnel'nykh nevropatii tazovogo poiasa pri patologii poiasnichnogo otdela pozvonochnika [Complex treatment of tunnel pelvic neuropathies in the pathology of the lumbar spine]. Lechashchii Vrach. 2013:(7):7-11. Russian.
23. Koriachkin VA, Strashnov VI. Epidural'naia i spinomozgovaia anesteziia [Epidural and spinal anesthesia]. Sankt-Petersburg; 1997. 120 p. Russian.
24. Pfirrmann CW, Metzdorf A, Zanetti M, et al. Magnetic resonance classification of lumbar intervertebral disc degeneration. Spine. 2001;26(17):1873-8.
25. Pulatov AM, Nikiforov AS. Nevrologiia [Neurology]. 2nd ed. Dushanbe: Maorif; 1990. 615 p. ISBN 5-670-00243-1. Russian.
26. Urban JP, Roberts S. Degeneration of the intervertebral disc. Arthritis Res Ther. 2003;5(3):120-130. doi: 10.1186/ar629. PMCID: PMC165040; PMID: 12723977.

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Temporomandibular disorders: perspective clinical usage of acupuncture

[1]**Victor Lacusta,** MD, PhD, Professor, Academician;
[*2]**Valeriu Fala,** MD, PhD, Associate Professor; [2]**Gheorghe Bordeniuc,** MD, Assistant Professor

[1]Department of Alternative and Complementary Medicine, [2]Department of Therapeutic Dentistry
Nicolae Testemițsanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova

*Corresponding author: valeriu.fala@usmf.md
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Abstract

Background: One of the oldest medical practices is acupuncture, which was developed for about 3 thousand years. It includes also methods of treatment for various diseases and disorders of the stomatognathic region. Currently, there is an increased interest for the usage of these alternative methods of treatment. Acupuncture methods are widespread worldwide and are endorsed by WHO. The use of alternative medicine methods in dental practice has more positive aspects than negative ones, attracting the attention of patients and doctors and even of countries with different economic levels of development. The most important indications of acupuncture in dentistry are: 1) glossodynia; 2) stomatodynia; 3) primary trigeminal neuralgia; 4) spasm/contracture of the masticatory muscles; 5) myogenous dysfunction of the temporomandibular joint; 6) salivary disorders. For the treatment of various pathologies in dentistry, various stimulation of acupuncture points is being used (needling, electro-puncture, laser-puncture, pressopuncture, thermo-puncture, magneto-puncture, etc.). Currently, there is ongoing research on defining differential indications for these methods, based on the highest efficiency for particular pathologies.

Conclusions: Currently, there is ongoing research on defining differential indications for these methods, based on the highest efficiency for particular pathologies. It is not concluded yet which acupuncture treatment is more efficient: the one based on the reflexogenic theory (local, regional, distal points) or the one based on Traditional Chinese Medicine (meridian theory, etc.). There are being developed complex treatment protocols for dental pathologies, with the inclusion of various acupuncture methods (magneto-puncture, laser-puncture, etc.).

Key words: acupuncture, moxibustion, zhen-jiu, temporomandibular disorders, myogenous disorders.

Introduction

Throughout its history, humanity has shown a special interest in everything that could ensure it with a certain level of health. Much could not stand the test of practice and was lost in its history, and that which had a significant effect in the treatment of certain diseases was preserved in the memory of society and passed down from generation to generation. The latter includes acupuncture, the legendary history of which was founded in China and is perhaps the oldest existing practical knowledge in the field of medicine.

For about 3 thousand years, there already existed methods of treating diseases of the oral cavity and teeth in China – acupuncture, herbal medicine, methods of cleaning and restoration of teeth, etc. [1]. The history of using zhen-jiu therapy (acupuncture, moxibustion) for the treatment of dental diseases goes back many centuries. Ancient Chinese songs and odes, which mention various dental disorders in combination with other disorders and methods for their treatment [2], have survived to the present day. Acupuncture and moxibustion from Eastern countries began to spread more intensively in Europe since the 16th century, through missionaries [3]. In the `70-`90s of the last century, interest in these ancient methods of treating dental diseases, especially traditional in regard to Chinese acupuncture, has increased [4]. In the future, there a deep study of ancient sources of Chinese medicine will be required in order to integrate rich empirical experience into modern academic science.

Acupuncture methods are spread in European countries, despite the lack of fundamental knowledge in this area, attitudes to these methods are changing, innovative research is being conducted using a full scientific potential [5, 6, 7, 8, 9, 10]. Interest in alternative methods of treatment is also associated with the financial capabilities of patients. The use of alternative medicine methods in dental practice has more positive aspects than negative ones, attracting the attention of patients and doctors and even of countries with different economic levels of development [11]. At the same time, the lack of summarizing works based on Chinese fundamental primary sources, as well as the not always objective criticism of the therapeutic importance of acupuncture, due to insufficient knowledge of the conditions, standards and technologies of traditional Chinese medicine create certain distrust on the part of medical workers and patients for this multi-millennial therapy. Despite the fact that up to 80% of the world's population use alternative medicine methods, the attitude towards it by the official health care structures is wary, sometimes punishing [12].

In various countries, the methods of traditional Chinese medicine are used for the treatment and prevention of diseases. In accordance with CAMrella Pan-European Survey (2010-2012), acupuncture was used at least once in the past year by 2.8% of respondents from 29 countries (amounting to 6.2 million Europeans). An analysis of 348 patients with chronic pain syndromes from Finland, who were treated with acupuncture methods for a long period (up to 5 years)

revealed that the minimum course of acupuncture consisted of 5 sessions; 41% of patients received more than one course of treatment; and the intensity of pain on a visual-analogue scale decreased by 40% due to acupuncture [13].

Analysis of PubMed sources for the period 1978-2007 revealed that the methods of acupuncture are most often used for the treatment of musculoskeletal diseases (16.2% of all diseases), lesions of the nervous system (17.13%), psycho-emotional disorders (12.9%). These types of disorders are characteristic of many dental diseases [14]. In the US, the American Dental Association in 1958 included acupuncture in the methods of pain control [15].

When using the methods of acupuncture in dental practice, it is necessary to take into account the recommendations of WHO (2002) regarding four groups of diseases that can be treated with acupuncture and moxibustion:

- Diseases, symptoms and conditions for which control studies proved the effectiveness of acupuncture treatment;
- Diseases, symptoms and conditions for which the therapeutic effect of acupuncture has been demonstrated, but more research is needed;
- Diseases, symptoms and conditions for which the effect of acupuncture and moxibustion is not sufficiently confirmed, but which, nevertheless, can be treated with these methods, since other treatment methods are ineffective;
- Diseases, symptoms and conditions for which acupuncture is possible only in combination with instrumental monitoring and provided that a certified doctor performs acupuncture.

Publications of the late 20th century indicated that the disease's etiology and disorder is an important criterion for the use of acupuncture. The most important indications of acupuncture in dentistry were: 1) glossodynia; 2) stomatodynia; 3) primary trigeminal neuralgia; 4) spasm/contracture of the masticatory muscles; 5) myogenous dysfunction of the temporomandibular joint; 6) salivary disorders.

Clinical evidence of acupuncture in functional disorders in the stomatognathic system

As it is well known, along with various functional disorders of organs and systems, pain syndromes are the main indications for zhen-jiu therapy. In this sense, acupuncture is called pain therapy. When using it, it is necessary to identify the nature and role of the pain syndrome in the pathological process: whether it is purely psychogenic or due to morphological changes, is acute or chronic, etc. Before carrying out acupuncture, in all cases it is necessary to establish the final diagnosis of the underlying disease, find out the pathogenic role of pain impulses. Particularly, caution should be exercised in patients with primary pain syndromes and unspecified diagnoses.

In accordance with the multimodal concept of chronic pain therapy, acupuncture methods are recommended to be incorporated into modern comprehensive pain treatment and control programs [16]. In chronic pain, the effectiveness of acupuncture is higher under the following conditions: 1) the ability to partially or fully control their disease; 2) young age; 3) a shorter period of chronic disease; 4) the absence of surgery for pain; 5) the number of acupuncture sessions held on average is above 8; 6) relatively good overall health [17].

Applying acupuncture to the treatment of bruxism based on the principles of Traditional Chinese Acupuncture has revealed to have positive therapeutic effects with the diminishing of pain and of the tonus of masticatory muscles [18].

In modern Chinese sources, for the treatment of pain in the masticatory muscles and bruxism, there are recommended the following first choice points: GI19, E5, E6, IG16, IG17, TR17, VB3, VB7, VB12, and VG26. The combination of the points of the meridians of the Liver (F1, F3), Gall Bladder (VB43, VB41) and Stomach (E5, E6, and E7) reduces the severity of bruxism [17].

In cases of temporomandibular joint (TMJ) dysfunction with chronic pain syndrome, patients willingly resort to alternative medicine (acupuncture, phytotherapy), as there is a rapid decrease in pain without side effects, as well as improved indices of quality of life [19]. Most patients (62.5%) with TMJ dysfunction desire and request alternative medicine methods, while they prefer manual methods (massage, acupuncture, chiropractic), and the vast majority of them (95.6%) combine these methods with other conventional treatment methods [20].

The greatest number of studies is devoted to TMJ dysfunction, which allowed us to identify the main indications of acupuncture for this disease: 1) early forms of dysfunction; 2) prevalence of functional disorders; 3) the prevalence of reversible disorders; 4) the presence of psycho-emotional disorders; 5) neuromuscular disorders; 6) combination with tension headache [21].

Acupuncture methods as minimally invasive ones (inserting needles into tissues, electro-acupuncture, etc.) and non-invasive ones (electro-puncture, laser-puncture, press-puncture, etc.) are used at various stages in the treatment of disorders of the TMJ area, in accordance with modern recommendations regarding the sequencing of treatment: 1) non-invasive methods; 2) minimally invasive methods: 3) invasive methods [22, 23, 24, 25, 26, 27]. Considering that many symptoms of TMJ dysfunction diminish or go away without any intervention, the treatment of this disease should be started with non-invasive methods. The choice of these methods (physiotherapy, relaxation techniques, behavioral therapy, psychological correction, psychotherapy, hypnosis, acupuncture, etc.) is a difficult task, since many of them do not differ significantly based on the effectiveness of treatment and there is no convincing scientific evidence of their effectiveness.

In the Ancient Chinese poem, *Xi Khun* (960-1279), there was recommended to exert on the *Lieque* point (P7) for unilateral or general headache, and in the *"Song of Acupuncture and moxibustion"* (1329) for unilateral and general headache with dizziness, the *Shenting* point (VG24) is recommended [2]. It has been established that dysfunction of the temporomandibular joint occurs more frequently in

patients with the traditional *Shao Yang* (TR/VB meridians) and *Shao Yin* (C/R meridians) syndromes [28].

In case of TMJ pathology, it is recommended to use the E6, E7, IG18, VG20, VB20, V10, GI4 points with the administering of one session per week (for 30 minutes) for a course of 6 weeks [29]. The authors have noted a decrease in pain and inflammatory processes, as well as an increase in the functional reserves of the muscles and joints. In patients with TMJ dysfunction, acupuncture caused a pronounced decrease in pain, improved temperature in the area of the TMJ and of the microcirculation – the overall effectiveness being 93.1% in a total number of 477 patients [30].

The effectiveness of acupuncture for TMJ disorders is 91% in acute pain syndromes and 70% in chronic pain syndromes [31]. In another study, the use of acupuncture has reduced the chronic pain in 53.3% of cases [32]. In myogenous dysfunctions, the insertion of acupuncture needles at the TR5, TR21, E6 points is effective in 91.7% of cases, and not effective in 4.1% of cases [33].

For the treatment of TMJ dysfunction, the most effective points that reduce pain and harmonize the masticatory muscles are GI4, E6, E7, E44, V2, VB14, VB20, VG18, VG19, F3, E36, VB34, R3. The effectiveness of these points was determined clinically and electromyographically [34]. In patients with myogenic dysfunction of the TMJ and pain syndrome under the influence of acupuncture, a complete cure was achieved in 46% of cases [35].

The use of local acupuncture points has reduced the pain intensity in 34% of patients with TMJ dysfunction; exertion on distal points has reduced pain in 31% of cases, and a combination of local and distal points has shown the same effect in 36% of cases [36]. Using the Pro-TMD Multi-protocol and self-assessment of TMJ dysfunction symptoms, before and after performing various acupuncture options, has revealed that the most effective are the local points (muscle, joint) and distal points, with a pronounced effect on the muscles of the joint [37].

A comparative analysis of the treatment of TMJ disorders using conventional treatment and acupuncture methods has revealed that in the latter case, the effectiveness of treatment was higher, the general condition improved in 85% of patients, the intensity of pain decreased by 75% [21]. Acupuncture in comparison with placebo in patients with TMJ dysfunction has decreased pain [38], without statistically significant changes in the range of motion of the mandible, but with a pronounced tendency of improving protrusion and retrusion. In a number of patients with disorders of the TMJ, acupuncture methods have been found to be more effective when compared with the use of indomethacin and vitamin B1 [38].

In the pathology of the TMJ, acupuncture has a positive, but short-term effect. Analysis of information in various databases (MEDLINE, EMBASE, CINAHL, CISCOM) for the period 1997-2008 has revealed that the vast majority of studies are on the short-term therapeutic effects of acupuncture methods in TMJ dysfunction, and in fact, there are no data on the long-term results of treatment [25], which makes it difficult to assess the real value of the method [39]. A positive therapeutic result in patients with TMJ dysfunction after acupuncture was maintained for up to 12 months [40]. Analysis of the long-term results (after 18–20 years) of treating TMJ dysfunction using acupuncture and occlusal splint therapy revealed that patients respond positively in regard to the quality of treatment and advise it to other patients; headache treatment (at least once a week) affects 73% of women and 77% of men at the beginning of treatment and, respectively, 35% and 54% after 18–20 years; 87% of patients before treatment had pronounced symptoms of joint damage, and after 18-20 years – 38% of patients [41].

Experimentally in rats, a positive effect of acupuncture on the manifestations of TMJ arthritis was observed [42]. Acupuncture techniques are used in patients with arthrosis of the TMJ and with disc damage [43]. The use of laser in patients with TMJ arthrosis has reduced the severity of masticatory muscle spasm, which was most pronounced in cases of reduction of the pain syndrome [44].

The use of acupuncture in myogenous and arthrogenous (TMJ) pain has led to the elimination of pain in 46% of cases [35], in other studies – in 40% of cases [45]. The use of acupuncture in the pathology of the TMJ disc has caused an improvement in the general condition, a decrease in the intensity of pain, an increase in the range of lower jaw movements and an increase in the treatment satisfaction, but the condition and position of the disc did not change according to tomography [43].

Acupuncture techniques have been used successfully in women with TMJ dysfunction, which often is accompanied by migraine and other pain syndromes and tends to have chronic and frequent relapses. Acupuncture, in addition to reducing pain and having a positive effect on the function of the stomatognathic system in patients with TMJ disorders, has influenced the severity of comorbid disorders: has reduced tension headache [21], tinnitus [46], manifestations of depression and anxiety [32]. Acupuncture is used in patients with TMJ dysfunction in cases of marked chewing and swallowing disorders [47]. In the ancient song "*The Magnificent Jasper Dragon*" (1601), it is recommended that if there is difficulty in swallowing food, 7 moxibustion sessions should be conducted at the *Tanzhong* point (VC17) [2].

Among the methods of physio-puncture, electro-acupuncture is the most accessible. Electro-acupuncture at the E6, E7, IG19, VB3, GI4 points causes a decrease in disorders in patients with TMJ dysfunction, including myofascial pain syndromes, especially in cases with spasms of the external pterygoid muscle [48]. The use of high-frequency transcutaneous electrical stimulation (100 Hz) significantly reduced pain in patients with TMJ disorders, in patients with rheumatism, however, lower frequencies (2 Hz) were no different to placebo. Both frequencies (2 Hz and 100 Hz) did not cause a significant improvement in joint function [49].

New opportunities are revealed when using laser-puncture – over the past 50 years, more than 1000 books and monographs were published on the subject of laser therapy. In Russia, tens of thousands of devices are used for laser

treatment [50]. Acupuncture has a more pronounced effect in increasing the pain threshold during pain modeling (thermal stimuli) in comparison with laser puncture, but laser puncture was more effective than placebo [51].

The issues of adequate parameters for laser puncture (dose, wavelength, etc.) have not yet been resolved [52]. New generation lasers significantly increase the efficiency of treatment of dental diseases [52, 53, 54, 55]. The use of laser puncture reduced the severity of pain in 85% of patients with pathology of the temporomandibular joint, with no side effects [56].

Laser puncture significantly improves the condition of patients with TMJ disorders, that are resistant to other methods of treatment [57]. With TMJ dysfunction, the use of a laser over the entire joint surface caused a decrease in pain and has increased the range of lower jaw movements (opening the mouth before treatment – 29 mm, after treatment – 40) [52].

In patients with dysfunction of the TMJ, laser puncture with a combination of exertion on local and distant points caused the appearance of a stable, pain-free period after 5.9 ± 6.08 procedures in acute pain syndromes and after 16.21 ± 17.98 procedures in chronic pain syndromes; accordingly, the intensity of pain on a visual analogue scale decreased to 0.3 ± 0.67 and 0.47 ± 0.84 points [57]. The combination of laser puncture with occlusal splint therapy increases the effectiveness of treatment in chronic TMJ dysfunction. Using a laser for treating TMJ arthrosis breaks the following pathological vicious circle: impaired muscle tone – local ischemia – local metabolic disorders – pain – impaired joint function [52].A promising area for treating TMJ disorders is thermal exposure on acupuncture points (thermos-puncture) – good results have been obtained using hot needling [58], combining moxibustion with *tuina* (Chinese massage and manual therapy) [59].

The generally accepted method of treating TMJ dysfunction is occlusal splint therapy, but this method also has many pros and cons [60]. Examination of the pain threshold at pressure in various areas (pre-auricular zone, masseter, temporal and trapezius muscles), analysis of mandible mobility degree and of mouth opening degree, before the treatment of TMJ dysfunction and after 30 days, has revealed that acupuncture is not inferior to occlusal splint therapy and can be used as an alternative method [61].

In patients with dysfunction of the TMJ, acupuncture caused an increase in the pain threshold and a decrease in subjective symptoms, approximately equal to the effect of the occlusal splint therapy. An analysis of publications for the period 1990-2015 (Cochrane Library, PubMed, Scopus, Web of Science) has revealed that acupuncture in patients with TMJ dysfunction is more effective than placebo and equates to occlusal splint therapy. The combination of auricular acupuncture with occlusal splint therapy for TMJ dysfunction causes the same reduction in disorders as occlusal splint therapy, however, in the first variant, the therapeutic effect occurred already in the first week of treatment [62].

In the process of treating TMJ disorders, acupuncture causes immediate positive subjective changes in comparison with occlusal splint therapy, and after 12 months, positive changes are observed in 57% of patients who received acupuncture and in 68% of patients that have received occlusal splint therapy ($p>0.05$) [63]. Acupuncture has increased the analgesic effect in combination with occlusal splint therapy [38].

A comparative analysis of the effectiveness of acupuncture, occlusal splint therapy, and their combination has revealed that after 3 months there is an improvement in the condition, respectively in 87%, 77.3% and 91.3% of patients [64]. Acupuncture and occlusal splint therapy for TMJ dysfunction after 4 weeks of treatment have reduced the intensity of pain about the same and have increased the range of movement of the mandible (mouth opening), however, acupuncture more pronouncedly has increased the pain threshold on pressure, and occlusal splint therapy has improved the electromyographic parameters of the temporal muscles during rest; after 4 weeks of treatment, pain has decreased in 53.3% of those who received acupuncture vs. 60% of those that received splint therapy, reduction of depression, respectively, in 11.1% vs. 50% of cases; a decrease in signs of somatization (16.7% vs. 44.4%) [32].

The combination of occlusal splint therapy, acupuncture and pharmaco-puncture in patients with temporomandibular joint dysfunction caused a pronounced therapeutic effect in 85% of cases after the sixth treatment session [65].

The research continues for effective options for combining acupuncture with other non-pharmacological methods. With dysfunction of the temporomandibular joint, the use of magneto-puncture seems to be promising [66]. Acupuncture in combination with the usage of vacuum cans in patients with TMJ dysfunction has increased the effectiveness of treatment [67].

The combination of acupuncture at the distal points (GI4, F3) with the use of medicinal cans in the affected area has increased the effectiveness of the TMJ dysfunction treatment: visual analogue scale of pain before treatment 5.39 ± 0.24 vs. 2.13 ± 0.47 after treatment; craniomandibular index, respectively, 0.27 ± 0.02 vs. 0.04 ± 0.01; palpation index 0.19 ± 0.01 vs. 0.05 ± 0.03; the dysfunction index is 0.33 ± 0.04 vs. 0.06 ± 0.02 [68].

The combination of acupuncture with ultrasound physiotherapy has significantly increased the effectiveness of treatment in the TMJ pathology. Acupuncture combined with ultrasound therapy has reduced the spasm of the masticatory muscles and the dysfunction degree of the TMJ, but the use of ultrasound as a monotherapy was not effective. In experiments conducted on animals, it was found that exposing acupuncture points to an electromagnetic field of ultrahigh frequency and a power of 1–20 mWt causes changes in lower jaw movements and of the respiratory rate [69].

Other studies have shown that acupuncture was more effective for treating TMJ dysfunction compared to physiotherapy [38]. The combination of acupuncture with manual therapy has led to a significant reduction in pain and an increase in the range of lower jaw movements [70].

Three-component therapy of TMJ dysfunction using acupuncture (E6, E7, TR17, GI4), moxibustion of wormwood cigarettes at these points through 5-7 layers of gauze and, finally, conducting a finger massage with pressure and with a rotation of the thumb around the joint for 3-5 minutes has led to a pronounced positive result in all 45 patients that were observed [71].

Modern Chinese researchers are recommending a four-stage pressopuncture for TMJ dysfunction: 1) light-moderate pressure with the thumb on the TMJ area for about 10 minutes; 2) pressing for 5-10 min on local points TR21 and IG19 and for 1-3 min on the distal points TR3 and TR6; 3) with one hand of the doctor, maintaining the patient's jaw, while the patient is making rhythmic opening-closing movements of the mouth, and with the other hand pressing on the TMJ area; 4) stroking and rubbing the TMJ area and lower jaw to feel the heat in these areas [17].

In patients with TMJ dysfunction, pharmaco-puncture at point E7 is effective on the affected side – 1 ml of dexamethasone (0.5 mg) and procaine hydrochloride (0.5%) are injected, only 1-2 treatments x 5 procedures, with an interval between injections of 3-5 days [33].

Despite the fact that most literature sources show good efficacy of acupuncture methods for TMJ dysfunction, better studies are now required to determine the place and role of these methods in complex therapy.

Acupuncture is effective for TMJ disorders, having a positive effect on various TMD pathology – pain in the joints, pain and muscle spasm, impaired mastication, headache, and others. Analysis of PubMed literature sources for the period of 1973-2004 revealed that acupuncture in patients with TMJ dysfunction has the most pronounced effect on the myogenous types [37].

The use of transcutaneous electrical stimulation in trigger points by dentists in patients with myogenous dysfunction resulted in a significant improvement in the condition and reduction of pain within 1 hour after the procedure [72].

In Traditional Chinese Acupuncture, there are used points that are painful during palpation or spontaneously painful points (*Ashi* points). The use of trigger points and areas increases the effectiveness of myofascial pain treatment, especially when combined with pharmacotherapy and physiotherapy [60, 73]. The use of trigger points for the treatment of myofacial pain is most effective in acute conditions – needling these points reduces pain and increases the range of motion [56, 74, 75, 76]. Good results were obtained when using hot needling to treat spasms of the facial muscles [77] – hot needles were inserted into the local *Ashi* points on the face and instantly removed; acupuncture in the regional distal points (VB20, VG20, VG24, E36, VB34) was performed for 30 minutes. The method proved to be more effective in comparison with conventional acupuncture.

The needling of trigger points located in the projection of the temporal and masticatory muscles causes a decrease in the severity of nocturnal bruxism starting from the first week of treatment. If the needling of the trigger point cannot be executed, a self- treatment measure that may be applied is the ischemic compression of the trigger point by applying pressure with the thumb until slight pain appears, as the pain decreases, the pressure is to be increased (the compression process lasts for about 1 minute).

In modern neurostomatology, it is being studied the role of trigger points and areas in the diagnosis and treatment of disorders. In affected temporomandibular joints, acupuncture is most effective in cases of needle insertion into painful structures involved in the pathological process (joint capsule, periarticular connective tissue, masticatory muscles, especially the lateral pterygoid muscles).

Epidermal stimulation of trigger zones is also effective in eliminating chronic pain in TMJ dysfunction [78]. Chinese researchers use a treatment technique with effects on the distal points and *Ashi* points: TR3 or TR6 are needled first, then the patient is asked to perform various rhythmic movements with the lower jaw, then *Ashi* points are needled and finally *Ashi* points are burned. 10 sessions are required per treatment [17].

The use of trigger points for acupuncture has caused a pronounced decrease in the intensity of pain in TMJ dysfunction, without significant changes in the functional parameters of TMJ [75]. The introduction of needles into the trigger points of the lateral pterygoid muscle reduced the intensity of pain, the severity of inflammation and has increased the range of movements of the mandible [74]. Acupuncture is more effective compared to placebo for increasing the pain threshold in patients with TMJ dysfunction, especially when there are trigger points and trigger zones, but acupuncture in comparison with placebo, has not significantly changed the pain intensity and the degree of pain-free mandible mobility [79]. The effect on trigger zones in musicians with myogenous TMJ dysfunctions has caused a marked reduction in pain after 8 weeks of treatment [80].

Treatment of pain and spasm of the facial muscles is carried out taking into account their etiopathogenesis and clinical manifestations [81]. For the treatment of chronic pain in the masticatory muscles, it is recommended a combination of the first-choice methods (occlusal splint therapy, physiotherapy, pharmaco-puncture, acupuncture) with patients counseling in regard to how they have to maintain their health [60, 82].

In the Ancient Chinese " *Song to Keep Up Your Sleeve*" (1529), at the bracing of the jaws there is recommended the *Lieque* (P7) point. In the Ancient Chinese sources, there are to be found recommendations for the various types of treatments for muscle spasms.

Acupuncture in spasms of facial muscles (main points – F3, VB20, TR17, RP6 in conjunction with symptomatic points) has caused in 17% of cases recovery (positive effect); in 24.5% – a significant improvement and in 7.5% of cases with no positive change [83]. The usage of pharmaco-puncture (vitamin B12) in the VB34 significantly decreased the facial spasms – overall efficiency of 96.7% vs. 56.7% in the control group [82].

In the case of patients with bruxism, under the influence of acupuncture, it is possible to achieve an improvement of the tonicity of the masticatory muscles and to decrease severity of the concurrent disorders [18, 46, 84, 85, 86, 87]. In the treatment of muscle pain and muscle disorders, it is advocated to use different methods (acupuncture, electro-acupuncture, subcutaneous stimulation, etc.), the choice of which depends not only on the clinical manifestations, but also on the electromyographic indices of the affected muscles [88]. In the case of headaches caused by masticatory muscle dysfunctions, during the first four months of treatment, the treatment has caused a significant improvement in 57.2% of cases, an unstable effect in 9.5% and in 33.3% of the cases there was observed no discernable effect (acupuncture was made in the VB41 point, with needling at a depth of 10-15 mm with continuous manual stimulation for 15-20 minutes). At the same time, there was observed a significant improvement of the electromyographic indices of the masseter and temporal muscles.

One of the most promising methods is auriculo-puncture. There was developed a reflex-bruxism-regulating method, which consists of manual stimulation (massage/stretching/pressure) of the reflexogenic zones of the ears, that correspond to the projection of the stomatognathic system, in combination with the movements of the mandible in a certain rhythm: for three minutes, the jaws are sequentially braced for 5 seconds, with diminishing the intensity for 5 seconds, after which they are to be relaxed for 1-2 min, with a half-open mouth [60].

The use of this method significantly has reduced the number of night episodes of bruxism, their duration and intensity. In addition, the method improved the psycho-emotional state and has increased the resistance to stress. The combination of occlusal splint therapy and reflex-bruxism-regulatory method increased the number of patients with sustained remissions for 6 months. Auricular acupuncture improves the tonus of the masticatory muscles in patients with medulloblastoma, reduces the severity of bruxism in neurodegenerative diseases [84].

Laser-puncture is an effective treatment method for pain and spasm of the masticatory muscles [89]. The use of laser-puncture once a week (10 sessions in total) made it possible to reduce pain and improve the electromyographic indices of the masticatory muscles [90]. The use of a laser (4 J/cm^2) for the purpose of deactivating trigger points and reducing pain in the masticatory muscles was not less effective than the method of administering 2% lidocaine at intervals of 48 and 72 hours [76]. Laser-puncture is successfully used to treat bruxism in children [87] – the method normalizes the activity of the masticatory muscles (based on electromyographic and gnatho-dynamometric data), reduces the reactivity to stress in children (the level of cortisol in the salivary fluid).

A promising method of treatment is electro-acupuncture in connection with a wide range of influencing factors (impulse type, intensity, frequency, duration, etc.). Electro-acupuncture with a frequency of 100 Hz significantly reduced the manifestations of muscle spasticity, and stimulation with a frequency of 2/15 Hz has reduced chronic pain at a higher level [91]. At the early stages of myofascial pain, it is effective to combine electro-acupuncture (points in the facial area in combination with the GI4 point) with massage techniques [48].

Various methods of acupuncture affect the main manifestations and pathogenic mechanisms of myogenous dysfunctions: they reduce muscle spasm [82, 83, 87, 89]; reduce muscle pain [18, 91]; reduce the severity and frequency of bruxism [84, 86, 87]; improve the electromyographic indices of the masseter and temporal muscles [40, 60]; improve the reflex processes of the regulation of masticatory muscles; improve the processes of vegetative regulation in the stomatognathic system [60]; normalize the stress-responsiveness and cortisol levels in the oral fluid [18, 85, 86, 87]; significantly reduce associated headaches in 57.2% of cases; reduce tinnitus in 87% of patients [46]; increase the duration of remission [60].

One of the tasks of modern medicine is the modernization of alternative medicine methods based on the creation of their scientific foundations [92], giving them a full and legal status, acupuncture methods should become part of predictive, preventive, personalized and integrative medicine [93].

Conclusions

1. Acupuncture is having a continuously increasing reach in clinical practice worldwide, and is part of the WHO recommendations regarding treatment for dental diseases.

2. In most studies, it was established that acupuncture is more efficient than placebo, and that it is almost equal to conventional treatment in outcomes.

3. Currently, still it is not concluded which acupuncture treatment is more efficient in dental diseases: the one based on the reflexogenic theory (local, regional, distal points) or the one based on Traditional Chinese Medicine (meridian theory, etc.).

4. Currently, complex treatment protocols for dental pathologies are being developed, with the inclusion of various acupuncture methods (magneto-puncture, laser-puncture, etc.).

References

1. Zhao W, Zhao Q. The overview of the prevention and treatment of dental disease in ancient China. Zhonghua Yi Shi Za Zhi. 2009;39(2):90-92.
2. Tsoi V, Belousov P. [Brief encyclopedia of zhen-jiu therapy]. Alma-Ata; 1995. Russian.
3. Wolfgang M. Japanese acupuncture and moxibustion in 16-18th-century Europe. J Jpn Soc Acupunct Moxibustion. 2011;61(2):150-163.
4. Mattick CR. Stomatology - an intriguing blend of traditional Chinese medicine and Western-style dentistry. Br Dent J. 1995;178(9):350-353.
5. Baatsch B, Zimmer S, Rodrigues Recchia D, et al. Complementary and alternative therapies in dentistry and characteristics of dentists who recommend them. Complement Ther Med. 2017;35:64-69.
6. Gelbier S. The origins and nature of acupuncture in dentistry. Dent Hist. 2016;61(1):5-9.
7. Little JW. Complementary and alternative medicine: impact on

dentistry. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004;98(2):137-145.
8. Mangal B, Sugandhi A, Kumathalli KI, et al. Alternative medicine in periodontal therapy - a review. J Acupunct Meridian Stud. 2012;5(2):51-56.
9. Meng X, Xu S, Lao L. Clinical acupuncture research in the West. Front Med. 2011;5(2):134-40.
10. Thayer ML. The use of acupuncture in dentistry. Dent Update. 2007;34(4):244-246.
11. Goncalo CS, Barros NF. The use of complementary and integrative practices in oral health. Acta Scientiarum. Health Sciences (UEM). 2014;36(2):281-291.
12. Alekseev AA, Larionova IS, Dudina NA. [Mesodermal and alternative medicine]. Moscow; 2001. 408 p. Russian.
13. Junnila SY. Long-term treatment of chronic pain with acupuncture. Part II. Acupunct Electrother Res. 1987;12(2):125-138.
14. Du Y, Xiong J, Huang W, et al. Medical conditions treated by acupuncture: a preliminary review of randomized controlled trials. Med Acupunct. 2009;21(3):207-213.
15. Schoor RS, Sussman HI, Kazandjian GK. Acupuncture: a unique effort to treat periodontal disease. J Am Dent Assoc. 2001;132(12):1705-1706.
16. Grasmuller S, Irnich D. Acupuncture in pain therapy. MMW Fortschr Med. 2007;149(25-26):37-39.
17. Jiang Song-he, Yang Guan-hu. Clinical research and application of acupuncture and tuina. Beijing: People's Medical Publishing House; 2008. 321 p.
18. La Torre Vera RM, Grillo CM, Sousa ML, et al. La acupuntura puede alterar los patrones musculares del bruxismo [Acupuncture could modify muscle activity in bruxism]. Rev Int Acupunt. 2012;6(4):144-150. Spanish.
19. Ritenbaugh C, Hammerschlag R, Dworkin SF, et al. Comparative effectiveness of traditional Chinese medicine and psychosocial care in the treatment of temporomandibular disorders-associated chronic facial pain. J Pain. 2012;13(11):1075-1089.
20. DeBar LL, Vuckovic N, Schneider J, et al. Use of complementary and alternative medicine for temporomandibular disorders. J Orofac Pain. 2003;17(3):224-236.
21. Rosted P, Jorgensen A, Bundgaard M. Temporomandibular dysfunction can contribute to aggravation of tension-type headache: a case report. Acupunct Med. 2010;28(3):154-155.
22. John MT. Complementary and alternative medicine may be effective for reducing TMD pain. J Evid Based Dent Pract. 2009;9(1):18-20.
23. Jung A, Shin BC, Lee MS, et al. Acupuncture for treating temporomandibular joint disorders: a systematic review and meta-analysis of randomized, sham-controlled trials. J Dent. 2011;39(5):341-350.
24. Kuo CE, et al. The application and effectiveness of electroacupuncture in the pain management of temporomandibular disorders. J Tradi Med Clin Natur. 2016;5(2 Suppl):33.
25. La Touche R, Goddard G, De-la-Hoz JL, et al. Acupuncture in the treatment of pain in temporomandibular disorders: a systematic review and meta-analysis of randomized controlled trials. Clin J Pain. 2010;26(6):541-550.
26. Rashid A, Matthews NS, Cowgill H. Physiotherapy in the management of disorders of the temporomandibular joint - perceived effectiveness and access to services: a national United Kingdom survey. Br J Oral Maxillofac Surg. 2013;51(1):52-57.
27. Wu JY, Zhang C, Xu YP, et al. Acupuncture therapy in the management of the clinical outcomes for temporomandibular disorders: a PRISMA-compliant meta-analysis. Medicine. 2017;96(9):e6064.
28. Rasera Zotelli VL, Grillo CM, Bressiani Gil ML, et al. Patterns of energy imbalance of the meridians in patients with temporomandibular dysfunction. J Acupunct Meridian Stud. 2018;11(1):1-6.
29. Chin SJ, Hsu ML, Yip SW. Application of acupuncture in temporomandibular joint disorders. J Prosthod Implantol. 2013;2(2):2-10.
30. Wang C, Long X, Zhu X. A study on the clinical curative effect by acupuncture for myofascial pain dysfunction syndrome. Chin J Stomatol. 1998;33(5):273-275.
31. Noiman M, Garty A, Maimon Y, et al. Acupuncture for treating temporomandibular disorder: retrospective study on safety and efficacy. J Acupunct Meridian Stud. 2010;3(4):260-266.
32. Grillo CM, Canales Gde L, Wada RS, et al. Could acupuncture be useful in the treatment of temporomandibular dysfunction? J Acupunct Meridian Stud. 2015;8(4):192-199.
33. Geng Junying, Huang Wenquan, Sun Yongping. Selecting the right acupoints: a handbook on acupuncture therapy. Beijing: New World Press; 1995. 349 p.
34. Rancan SV, Bataglion C, Bataglion SA, et al. Acupuncture and temporomandibular disorders: a 3-month follow-up EMG study. J Altern Complement Med. 2009;15(12):1307-10.
35. Merchant N. Facial pain: a review of 200 cases treated with acupuncture. Acupunct Med. 1995;13(2):110-1.
36. Kang KW, Kim WY, Kim TH, et al. Adjacent, distal, or combination of point-selective effects of acupuncture on temporomandibular joint disorders: a randomized, single blind, assessor-blind controlled trial. Integr Med Res. 2012;1(1):36-40.
37. Branco CA, Fonseca RB2, Borges RF, et al. Perception of the signs and symptoms of temporomandibular disorder in females by using the ProTMDMulti protocol and the visual analog scale before and after acupuncture treatment. Cranio. 2016;34(2):118-123.
38. Cho SH, Whang WW. Acupuncture for temporomandibular disorders: a systematic review. J Orofac Pain. 2010;24(2):152-62.
39. Fink M, Rosted P, Bernateck M, et al. Acupuncture in the treatment of painful dysfunction of the temporomandibular joint - a review of the literature. Forsch Komplementarmed. 2006;13(2):109-115.
40. de Sousa RA, Semprini M, Vitti M, et al. Electromyographic evaluation of the masseter and temporal muscles activity in volunteers submitted to acupuncture. Electromyogr Clin Neurophysiol. 2007;47(4-5):243-250.
41. Bergstrom I, List T, Magnusson T. A follow-up study of subjective symptoms of temporomandibular disorders in patients who received acupuncture and/or interocclusal appliance therapy 18-20 years earlier. Acta Odontol Scand. 2008;66(2):88-92.
42. Gondim DV, Araujo JC, Cavalcante AL, et al. CB1 and CB2 contribute to antinociceptive and anti-inflammatory effects of electroacupuncture on experimental arthritis of the rat temporomandibular joint. Can J Physiol Pharmacol. 2012;90(11):1479-89.
43. Minakawa Y, et al. Clinical application of acupuncture on temporomandibular disorder in a patient with disc disorders. Zen Nihon Shinkyu Gakkai Zasshi [J Jpn Soc Acupunct Moxibustion]. 2010;60(5):837-845.
44. Hadano T, Koyama S, Sakamoto T, et. al. Clinical application of He-Ne COLD LASER for TMJ arthrosis. J Jpn Soc Temporomandibular Joint. 1990;2(1):133-138.
45. Bressiani Gil ML, Rasera Zotelli VL, Rosário de Sousa ML. Acupuntura como alternativa para el tratamiento de la disfunción temporomandibular [Acupuncture as an alternative in the treatment of temporomandibular dysfunction]. Rev Int Acupunt. 2017;11(1):12-15. Spanish.
46. Ström D, Behrenth E, Ekman K, et al. Management of tinnitus and jaw-muscle tenderness using an intraoral appliance and acupuncture. Swed Dent J. 2013;37(3):105-110.
47. Lu DP, Lu GP, Lu PM. Clinical effectiveness of acupuncture for mandibular subluxation and dislocation. Acupunct Electrother Res. 2010;35(3-4):187-192.
48. Bu LX, Chen T, Chen X, et al. [Clinical observation of acupuncture and massage therapy for temporomandibular joint disorders]. [Shanghai J Stomatol]. 2011;20(3):292-295.
49. Møystad A, Krogstad BS, Larheim TA. Transcutaneous nerve stimulation in a group of patients with rheumatic disease involving the temporomandibular joint. J Prosthet Dent. 1990;64(5):596-600.
50. Moskvin SV. Low-level laser therapy in Russia: history, science and practice. J Lasers Med Sci. 2017;8(2):56-65.
51. Ceccherelli F, Altafini L, Lo Castro G, et al. Diode laser in cervical myofascial pain: a double-blind study versus placebo. Clin J Pain. 1989;5(4):301-304.
52. de Oliveira RF da Silva CV, Cersosimo MC, et al. Laser therapy on points of acupuncture: are there benefits in dentistry? J Photochem Photobiol B. 2015;151:76-82.
53. Kathuria V, Dhillon JK, Kalra G. Low-level laser therapy: a panacea for oral maladies. Laser Ther. 2015;24(3):215- 223.
54. Litscher G. Laser acupuncture - innovative basic research: visual and laser-induced evoked potentials. Laser Ther. 2012;21(4):287-295.
55. Pulido M, Machacon J, Garcia J. Laserpuntura en el tratamiento del

dolor articular temporomandibular [Laserpuncture for temporomandibular joint pain treatment]. Rev CES Odontol. 2009;22(1):39-42. Spanish.
56. Huang Z, Huo J, Zhao J. Efficacy on primary trigeminal neuralgia treated with triple puncture technique and electroacupuncture at trigger points. [Chin Acupunct Moxibustion]. 2017;37(1):31-34.
57. Hu WL, Chang CH, Hung YC, et al. Laser acupuncture therapy in patients with treatment-resistant temporomandibular disorders. PLoS One. 2014;9(10):e110528.
58. Xue W, Ding M, Su XC, et al. Clinical observation on warming needle moxibustion plus exercise for treatment of temporomandibular joint dysfunction syndrome. [Chin Acupunct Moxibustion]. 2007;27(5):322-324.
59. Gong XF. Clinical observation of tuina plus heat-sensitive moxibustion for temporomandibular disorders. J Acupunct Tuina Sci. 2016;14(5):361-365.
60. Romaniuc D. Patternul clinico-neurofiziologic la pacienții cu bruxism și opțiuni de autoajutorare [Clinical-neurophysiological pattern in patients with bruxism and self-help options] [dissertation]. Chișinău; 2019.
61. Vicente-Barrero M, Yu-Lu SL, Zhang B, et al. The efficacy of acupuncture and decompression splints in the treatment of temporomandibular joint pain-dysfunction syndrome. Med Oral Patol Oral Cir Bucal. 2012;17(6):1028-1033.
62. Fernandes AC, Duarte Moura DM, Da Silva LGD, et al. Acupuncture in temporomandibular disorder myofascial pain treatment: a systematic review. J Oral Facial Pain Headache. 2017;31(3):225-232.
63. List T, Helkimo M, Andersson S, et al. Acupuncture and occlusal splint therapy in the treatment of craniomandibular disorders. Part I. A comparative study. Swed Dent J. 1992;16(4):125-141.
64. Elsharkawy TM, Ali NM. Evaluation of acupuncture and occlusal splint therapy in the treatment of temporomandibular joint disorders. Egypt Dent J. 1995;41(3):1227-1232.
65. Wong YK, Cheng J. A case series of temporomandibular disorders treated with acupuncture, occlusal splint and point injection therapy. Acupunct Med. 2003;21(4):138-149.
66. Florian MR, Zotelli VLR, de Sousa MDLR, et al. Use of magnetic neurostimulator appliance in temporomandibular disorder. J Acupunct Meridian Stud. 2017;10(2):104-108.
67. Gellis M. A multifaceted approach to the acupuncture treatment of neuromuscular facial conditions. J Chin Med. 2016;(110):5-12.
68. Han Y, Guo L, Xiao J. Acupuncture combined with medicated cupping for temporomandibular disorders. World J Acupunct Moxibustion. 2015;25(3):31-34.
69. Vagin IuE, Vagina LV, Batsiuro SG. [Somaticoautonomic functions of rabbits exposed to an ultrahigh-frequency electromagnetic field at acupuncture points]. Biol Nauki. 1985;10:50-55. Russian.
70. Shin BC, Ha CH, Song YS, et al. Effectiveness of combining manual therapy and acupuncture on temporomandibular joint dysfunction: a retrospective study. Am J Chin Med. 2007;35(2):203-208.
71. Qing-jie Y. Treatment of temporomandibular joint dysfunction syndrome by acupuncture, Taiyi Moxa-cigar plus Tuina. J Acupunct Tuina Sci. 2006;4(1):56-57.
72. Sun JG, Sun XR. Tongue acupuncture. [Chin Acupunct Moxibustion]. 2010;30(4):347-348.
73. Lacusta VN. Tratat de acupunctura clinică [Manual of clinical acupuncture]. Chișinău: Centrul European de Studii Postuniversitare in Acupunctura; 1999. 777 p. Romanian.
74. Gonzalez-Perez LM, Infante-Cossio P, Granados-Nunez M, et al. Deep dry needling of trigger points located in the lateral pterygoid muscle: efficacy and safety of treatment for management of myofascial pain and temporomandibular dysfunction. Med Oral Patol Oral Cir Bucal. 2015;20(3):326-33.
75. Itoh K, Asai S, Ohyabu H, et al. Effects of trigger point acupuncture treatment on temporomandibular disorders: a preliminary randomized clinical trial. J Acupunct Meridian Stud. 2012;5(2):57-62.
76. Uemoto L, Garcia MA, Gouvea CV, et al. Laser therapy and needling in myofascial trigger point deactivation. J Oral Sci. 2013;55(2):175-181.
77. Qian JW, Xu W. Clinical observation of fire needle for facial spasm. [Chin Acupunct Moxibustion]. 2015;35(12):1221-1224.
78. Berguer A, Kovacs F, Abraira V, et al. Neuro-reflexotherapy for the management of myofascial temporomandibular joint pain: a double blind, placebo-controlled, randomized clinical trial. J Oral Maxillofac Surg. 2008;66(8):1664-1677.
79. Dıraçoğlu D, Vural M, Karan A, et al. Effectiveness of dry needling for the treatment of temporomandibular myofascial pain: a double blind, randomized, placebo controlled study. J Back Musculoskelet Rehabil. 2012;25(4):285-290.
80. Hunter EK. Integration of rehabilitation and acupuncture in the treatment of a professional musician with temporomandibular joint dysfunction. Acupunct Med. 2011;29(4):298-301.
81. Fala V, Lacusta V, Bordeniuc G, et al. Rolul factorilor cotidieni in declansarea/mentinerea manifestarilor clinice ale bruxismului [The role of daily factors in triggering / maintaining clinical manifestations of bruxism]. In: Humboldt Kolleg: Ethical, Ecological and Social Problems of Nanoscience and Nanotechnologies – NANO-2016; 2016 May 11-14; Chisinau : Abstracts. Chisinau; 2016. p. 13. Romanian.
82. Zhou K, Shen Lei. Clinical study of acupuncture of yanglingquan point for the treatment of hemifacial spasm. Biomed Res. 2017; Special Issue:S125-128.
83. Wang L. Treatment of 53 cases of facial spasm with acupuncture. J Acupunct Tuina Sci. 2009;7(4):233-234.
84. Ferreira DC, De Rossi A, Torres CP, et al. Effect of laser acupuncture and auricular acupressure in a child with trismus as a sequela of medulloblastoma. Acupunct Med. 2014;32(2):190-193.
85. Pirnia B, Taheri Nakhost HR, Pirnia K, et al. Ear acupuncture in treating trismus-syndrome: a case report. Ann Med Health Sci Res. 2017;7:97-98.
86. Romoli M. Auricular acupuncture diagnosis. Edinburgh: Churchill Livingstone; 2010. 301 p.
87. Salgueiro MD, Bortoletto CC, Horliana ACR, et al. Evaluation of muscle activity, bite force and salivary cortisol in children with bruxism before and after low-level laser applied to acupoints: study protocol for a randomised controlled trial. BMC Complement Altern Med. 2017;17(1):391.
88. Ozaki A, Wakayama I, Tanaka H, et al. Status of acupuncture and moxibustion: interchange between basic and clinical studies: effects of acupuncture and moxibustion on muscle diseases and muscular functions/metabolism and the status of these techniques. [J Jpn Soc Acupunct Moxibustion]. 2004;54(5):698-716.
89. Law D, McDonough S, Bleakley C, et al. Laser acupuncture for treating musculoskeletal pain: a systematic review with meta-analysis. J Acupunct Meridian Stud. 2015;8(1):2-16.
90. Hotta PT, Hotta TH, Bataglion C, et al. EMG analysis after laser acupuncture in patients with temporomandibular dysfunction (TMD). Implications for practice. Complement Ther Clin Pract. 2010;16(3):158-60.
91. Luo F. A study on the cumulative effect of repeated electroacupuncture on chronic pain. [Prog Physiol]. 1996;27(3):241-244.
92. Yang JW, Li QQ, Li F, et al. The holistic effects of acupuncture treatment. Evid Based Complement Alternat Med. 2014;2014:739708.
93. Bubnov R. Evidence-based pain management: is the concept of integrative medicine applicable? EPMA J. 2012;3(1):13.

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