ISSN 2073-4662

Українська медична стоматологічна академія
Українська Академія наук національного прогресу

# Проблеми екології та медицини

Том 23 № 1-2 2019
Науково-практичний журнал
Заснований у 1997 році



**Журнал внесено до електронних баз даних наукової періодики:**
- "Index Copernicus International",
- "Ulrich's Periodicals Directory",
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**Адреса редакції:** Українська медична стоматологічна академія, вул. Шевченка, 23, м. Полтава, 36011,
Відповідальний секретар Шликова О.А.,
телефон (05322) 2-76-37
e-mail: ecomedpolt@ukr.net

Рекомендовано Вченою радою Української медичної стоматологічної академії МОЗ України

Підписано до друку 12.04.2019 року

Ukrainian Medical Stomatological Academy
Ukrainian Academy of Science for National Progress

# The Medical and Ecological Problems

Vol. 23 No 1-2 2019
Scientific and clinical Journal
Founded in 1997



**Journal is enlisted in the electronic databases of scientific periodicals:**
- "Index Copernicus International",
- "Ulrich's Periodicals Directory",
- electronic library "eLIBRARY.RU",
- Google Scholar

**Editorial office**
Ukrainian Medical Stomatological Academia
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Accepted for publication 12 April 2019

Свідоцтво про реєстрацію серія КВ №2801 від 01.07.1997. Формат 60x84/8. Папір офсетний. Друк офсетний. Ум. друк. арк. 4,19. Тираж 100. Зам. 187.
Редакційно-видавничий відділ Української медичної стоматологічної академії, вул. Шевченка, 23, м. Полтава, 36011
Свідоцтво державного комітету телебачення і радіомовлення України Серія ДК №1691 від 17.02.2004 р.

Українська медична стоматологічна академія
Українська Академія наук національного прогресу

# Проблеми екології та медицини

Том 23 N 1-2 2019

НАУКОВО–ПРАКТИЧНИЙ ЖУРНАЛ *Заснований в 1997 році*

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# КЛІНІЧНА МЕДИЦИНА


UDK 618.19-006.04/.44:611.018

DOI https://doi.org/10.31718/mep.2019.23.1-2.01

## ANALYSIS OF ASSOCIATION BETWEEN THE DENSITY OF INFILTRATION IN PRIMARY CARCINOMA OF THE MAMMARY GLAND BY TUMOR-ASSOCIATED MACROPHAGES AND POSTOPERATIVE PROGNOSIS

***Aikian A.Z., Shynkevych V.I., Kaidashev I.P.***

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*Пухлино-асоційовані макрофаги (ПАМ) М2-типу домінують у пухлинах і продукують сприятливі для їх росту молекули, стимулюючи ріст пухлини. Однак, зміна М2-типу на М1 може уповільнювати або припиняти цей ріст. Для реалізації напрямку модуляції М1/М2 при лікуванні карциноми/раку молочної залози (РМЗ) необхідна обґрунтована діагностика і підтвердження негативного прогнозу ПАМ. Метою роботи було оцінити відношення пухлино-асоційованих макрофагів до післяопераційного прогнозу/виживання пацієнток з карциномою/раком молочної залози (РМЗ). Матеріалом дослідження були інтраопераційні тканини пухлин та іпсилатеральних лімфовузлів при радикальному видалені молочних залоз. Патоморфологічне дослідження лімфовузлів проводилося для уточнення діагностики стосовно N0/1. Щільність інфільтрації ПАМ визначали за допомогою імуногістохімічного забарвлення (ІГХ) CD68 та CD163 на 30 зразках п'яти молекулярно-біологічних типів РМЗ (по три клінічних випадки кожного). ІГХ дослідження по визначенню ПАМ і М2-подібних макрофагів проведено за допомогою стрептавідин-пероксидазного методу. Дослідження дозволило встановити, що кількісне представництво CD68+ та CD163+ Мф дуже різнилося від пацієнтки до пацієнтки, а також в межах зразку, що залежить зокрема від морфологічних особливостей РМЗ, охопленого біопсією. Щільність інфільтрації CD163+ макрофагами вогнища РМЗ негативно корелювала з післяопераційним виживанням, але не достовірно, проте це вкладається у загальну концепцію про негативний прогноз інфільтрації М2-подібними макрофагами. Потрібна більша кількість досліджень для підтвердження негативного значення щільності інфільтрації ПАМ первинного вогнища РМЗ для післяопераційного прогнозу. Не виключена захисна роль повноцінних М1-подібних ПАМ вогнища первинного ураження при РМЗ на рівні персоналізованого підходу. Перспективною є розробка диференційної діагностики і підходу до лікування РМЗ з урахуванням рівнів його інфільтрації субпопуляціями ПАМ.*

**Ключові слова:** рак/карцинома молочної залози, пухлино-асоційовані макрофаги, молекулярно-біологічні типи РМЗ, післяопераційний прогноз виживання.

*Tumor-associated macrophages (TAM) of the M2-type dominate in tumors and produce molecules, favorable for their growth, stimulating tumor growth. However, changing the M2-type for M1 can slow down or arrest this growth. For realization of the M1 / M2 modulation direction in the treatment of carcinoma / breast cancer (BC), a substantiated diagnosis and confirmation of the TAM negative prognosis is necessary. Therefore, the aim of the study was to evaluate the relation of tumor associated macrophages to the postoperative prognosis / survival of patients with 5 molecular-biological types of breast carcinoma. Materials of the study were intraoperative tissues of tumors and ipsilateral lymph nodes in radically removed mammary glands. Pathomorphological study of lymph nodes was conducted to clarify the diagnosis in relation to N0/1. The density of TAM infiltration was determined by immunohistochemical staining of CD68 and CD163 in 30 samples of five molecular biological types of breast cancer (three clinical cases of each type). Immunohistochemical (IHC) studies for the determination of TAM and M2-like macrophages were conducted using streptavidin-peroxidase method. The quantitative representation of CD68 + and CD163 + Mph is very different from patient to patient and also within one sample, which depends, in particular, on the morphological characteristics of breast cancer, studied by the biopsy. The density of infiltration by CD163 + macrophages of the BC focus negatively correlated with postoperative survival, which did not reach statistical significance, but is included in the general concept of a negative prognosis of infiltration by M2-like macrophages. Further research is needed to confirm the negative significance of the TAM infiltration density in the BC primary focus for postoperative prognosis. Promising is the development of differential diagnosis and approach to the treatment of breast cancer, taking into account the levels of its infiltration by subpopulations of TAM.*

**Key words:** cancer / carcinoma of the mammary gland, tumor associated macrophages, molecular-biological types of. breast cancer, postoperative survival prediction

*To cite this English version: Aikian A.Z., Shynkevych V.I., Kaidashev I.P. Analysis of association between the density of infiltration in primary carcinoma of the mammary gland by tumor-associated macrophages and postoperative prognosis. // The Medical and ecological problems. - 2019. - Vol 23, № 1-2. - P. 3-7.*

## Introduction

Tumor-associated macrophages (TAM) of the M2-type dominate in tumors and produce molecules, favorable for their growth, stimulating tumor growth. However, changing the M2-type for M1 can slow down or arrest this growth. Such an effect is mediated by the direct activity of M1 and their ability to stimulate Th1-type cytotoxic T cells and other effector cells [1]. For realization of the M1 / M2 modulation direction in the treatment of carcinoma / breast cancer (BC), a substantiated diagnosis and confirmation of the TAM negative prognosis is necessary. Therefore, the aim of the study was to evaluate the relation of tumor associated macrophages to the postoperative prognosis / survival of patients with 5 molecular-biological types of breast carcinoma.

## Materials and methods

Tissue samples. Biopsy samples and clinical data were obtained from patients undergoing treatment at Poltava Regional Clinical Dispensary. The study was approved by the Ethics Commission of UMSA. The average age of patients was 60 years, ranging from 30 to 79 years.

Materials of the study were intraoperative tissues of tumors and ipsilateral lymph nodes in radically removed mammary glands. Pathomorphological study of lymph nodes was conducted to clarify the diagnosis in relation to N0/1. Immunohistochemical (IHC) characteristics of the removed tumors (HER2, ER, PR, Ki67) were used to determine the molecular-biological subtype of BC in order to balance the study groups. IHC and pathologic findings were obtained at the diagnostic and advisory center CSD Health care, Kyiv).

Immunohistochemistry and antibodies. For IHC detection of TAM that infiltrated the primary focus of BC, we used the CD68 marker; to determine the M2-like TAM – the CD163 marker [2].

IHC studies for the determination of TAM and M2-like macrophages were conducted using streptavidin-peroxidase method. Paraffin sections, 2-3 μm thick, obtained by standard technique of the automated cycle of the pathologic-anatomical laboratory, were deparaffined, dehydrated, antigens were restored in citrate buffer (pH 6.0) in a microwave oven (at a power of ≈600 W, 3 cycles of 7 minutes with a break for 1 min), cooled for 20 min, washed in disodium and phosphate buffered saline (PBS, pH 7.2-7.4) for 2 min, blocked endogenous peroxidase with a reagent from the PolyVue HRP / DAB Detection System (For Mouse & Rabbit Primary Antibodies, Diagnostic BioSystems, USA), washed in PBS for 3 min. The slices were then incubated at 4˚C overnight with mouse anti-CD68 monoclonal antibodies (clone PG-M1, REF PD M065-S, Diagnostic BioSystems, USA) and anti-CD163 (clone 10D6, REF Mob460-01, in dilution 1: 100 in Antibody Diluent Buffer for DTP, Antibody Diluent, Dako, USA). Further, the sections were treated in two steps with the Mouse / Rabbit PolyVueTM HRP / DAB Detection System (Diagnostic BioSystems, USA), a detector system for visualizing the chromosomal DAB response, the nuclei were bleached with hematoxylin Meyer and enclosed with a cedar balm under the cover glass. Antibody Diluent buffer was used instead of the primary antibodies as the negative control, the lymph nodes tissues – as the positive one.

Assessment of immunohistochemical staining. We conducted assessment by counting $CD68^+$ TAM and $CD163^+$ M2-like TAM under the light microscope (Biolam, LOMO, Russia: lens×40, eyepiece $K7^×$, magnification ×280, field diameter of the field of view 18 mm) in 7-10 consecutive fields of view for the IHC-reaction of each section, calculating the arithmetic mean, within the tumor nests and tumor stroma. The count included immunopositive cells with macrophage morphology. Microphotographs were obtained using a Microscope Leica DM500, Leica, Germany, lens × 40).

4-year follow-up. Patients were regularly observed during visits to the clinic or over the telephone for up to 4 years (or more precisely, up to 58 months maximum), or until the time of death. Overall survival was used for prognostic analysis.

Statistical analysis. All calculations were conducted using GraphPad Prism 5. The proportions were compared using the $\chi^2$ test or Fischer's exact criterion. Overall survival was estimated using the Kaplan-Mayer method. The values of $p \leq 0.05$ were considered as statistically significant for all analyzes.

## Results

The study included 6 patients with 5 immunohistochemical types of BC, equal in terms of metastases in the ipsilateral axillary lymph nodes, namely: 6 persons with non-luminal $HER2^+$, 3 N0 and N1; 6 persons with luminal A, 3 N0 and N1, 6 persons with luminal B $HER2^-$, 3 N0 and N1; 6 persons with luminal B $HER2^+$, 3 N0 and N1; and 6 persons with triple negative BC, also 3 N0 and N1, 30 patients in total.

The primary results of the calculation of immunopositive Mph are given in Table 1.

*Table 1*
*Quantitative characteristics of tumor-associated macrophages, which infiltrate the primary focus of BC in each patient (average number in the field of view mg.×280)*

| IHC BC type | N0 | | N1 |
|---|---|---|---|
| | $CD68^+$ TAM | $CD163^+$M2-like TAM | $CD68^+$ TAM |
| Non-luminal $HER2^+$ | 10.7 | 3.1 | 21.6 |
| | 3.6 | 1.9 | 32.1 |
| | 5.8 | 4.8 | 16.2 |
| Luminal A | 16.5 | 7.4 | 9.2 |
| | 8.8 | 4.3 | 23.1 |
| | 14.1 | 4.9 | 8 |
| Luminal B $HER^-$ | 5.5 | 1 | 6.7 |
| | 16.6 | 1 | 4.3 |
| | 19.6 | 1 | 8 |
| Luminal B $HER^+$ | 4.6 | 1 | 8.5 |
| | 6 | 1 | 2.5 |
| | 5.6 | 1 | 4.3 |
| TNBC | 12.5 | 5.1 | 23.5 |
| | 11.4 | 6.0 | 22.3 |
| | 12.5 | 1.0 | 26.6 |

In general, the quantitative characteristics of TAM showed that in all cases, the number of $SD68^+$ Mph exceeded $CD163^+$ Mph. All cells usually formed larger or smaller foci or clusters, which depended on the expressiveness of the stroma, the density of the tumor nests, the presence of necrosis centers and other morphological, very individual characteristics of the samples (Fig. 1, 2) [3].

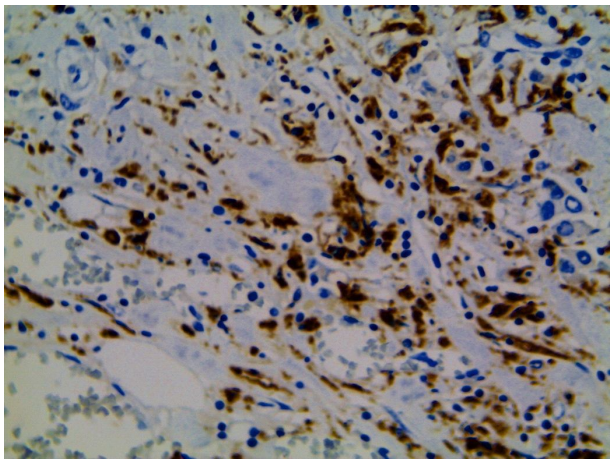

*Fig. 1. Dense infiltration of the CD68 + TAM sample i n triple negative BC N1.*

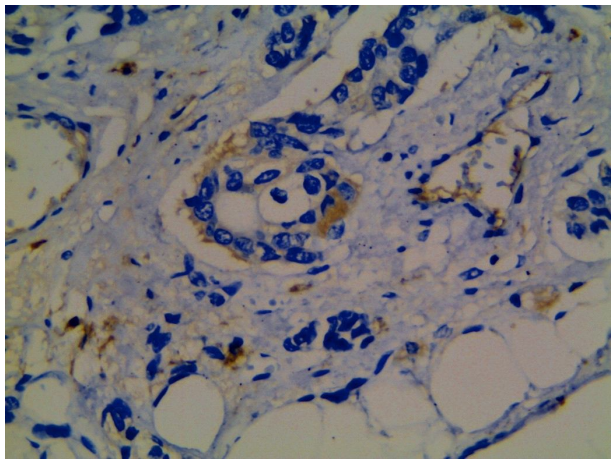

*Fig. 2. Field of view with moderate density of infiltration of the CD163 + macrophages of luminal BC A N0.*

For statistic analysis of levels of TAM infiltration and postoperative survival, the participants of the study were divided into 2 subgroups, by conditionally high (≥15 cells in the field of view mg. × 280) and low (<15) infiltration density of the primary focus of BC $CD68^+$ TAM. In the subgroup with a high level of infiltration, one person died, survival was 89%, in the subgroup with a low – two patients (83% survival), the difference was not reliable (Fig. 3, p = 0.81, log-rank test). With comparable proportions of 1/10 and 2/20, cumulative mortality in the subgroups is the same.

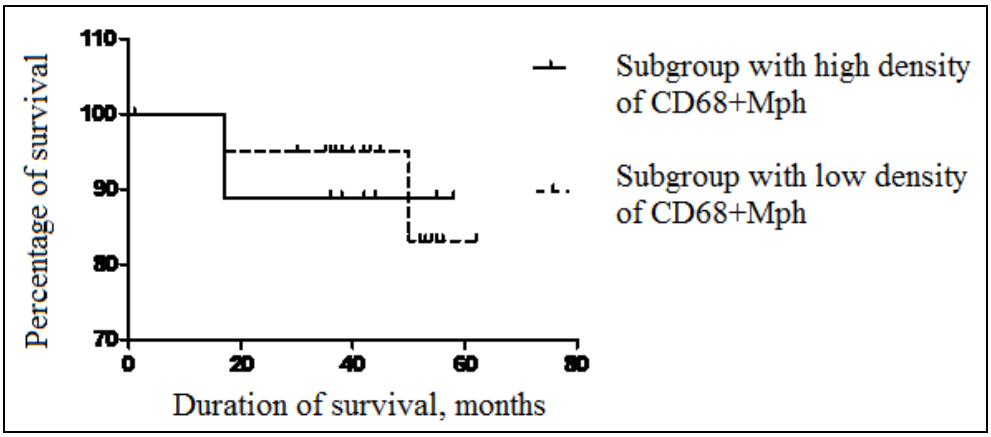


*Fig. 3. Survival proportions and infiltration level of CD68 + TAM (Kaplan-Mayer Test).*

To find out the relationship between the density of infiltration of BC $CD163^+$ M2-like TAM and patients survival, the conditional division into two subgroups was conducted as follows: a moderate level of infiltration:> 3 cells in the field of view mg. × 280, the low level is 0-3. In a subgroup with a moderate level of infiltration, two persons died (survival was 81%), in the subgroup with low – one patient (survival - 91%), which did not reach statistical significance, when compared (p = 0.94, log-rank test, Fig. 4) and needs further observations for the conclusion, but in principle coincides with the concept that relatively higher infiltration of the primary focus with M2-like Mph is negative [1].

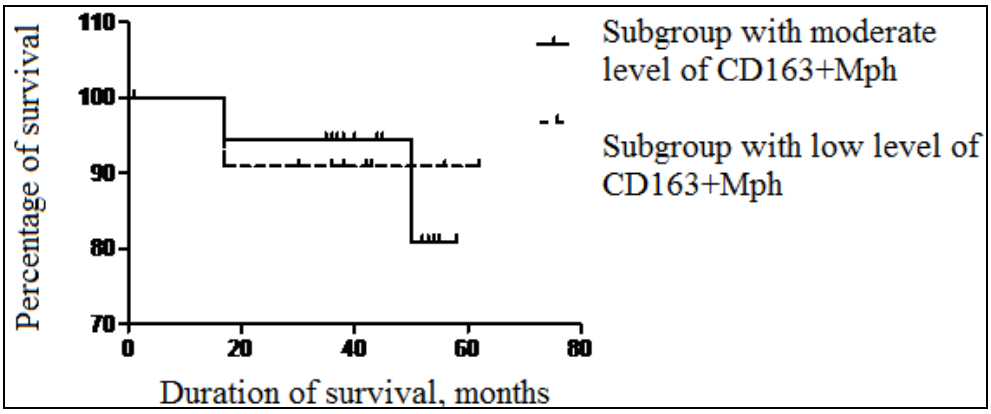


*Fig. 4. Proportions of survival, depending on the level of infiltration of CD163 + M2 (Kaplan-Mayer test)*

When comparing proportions of cumulative mortality: 2/18 (11%) (the subgroup with moderate infiltration density) versus 1/12 (8%) (the subgroup with low infiltration density), the differences also did not reach statistical significance, p = 0.8.

## Discussion

The study presents the results of analysis of possible correlations between the infiltration density of separately M1-like TAM, which are conventionally considered $CD68^+$ Mph, and M2-like - according to the widely used marker CD163, the primary focus of breast cancer: the relatively higher infiltration level by precisely $CD163^+$ macrophages correlated with a decrease in survival (81% ), which did not reach the reliability, however, is included in the general concept of the negative prognosis of tumor infiltration precisely with M2-like Mph [1].

The study is organized as a cross-sectional, balanced by immunohistochemical characteristics of tumors (HER2, ER, PR, Ki67) (or the surrogate classification for molecular-biological subtypes), pilot, and a small number of participants is a limitation of this study.

Previous studies have shown that increased macrophage density in biopsy samples of patients with breast cancer before treatment correlates with a decrease in non-recurrent and general survival [6]. The literature discusses the evidence that both M1 and M2 Mph have tumoral properties: in the early stages of transformation, M1-like TAM, due to the production of reactive oxygen and nitrogen forms, can potentially increase the rate of mutations in the epithelial cells and thus accelerate the tumor process; in the developed tumors, Mph demonstrate alternatively activated M2 functions, including the production of immunosuppressive factors (IL-10 and TGF-β) which can actively suppress the anti-tumoral immune response, produce growth factors and rebuild the matrix, supporting the growth of tumor cells and intensifying the invasion [4]. Nevertheless, in a study devoted to the research of macrophages localization in the human breast tumors, the high representation of $CD68^+$ Mph in the gaps of the ductal tumors correlated with a decrease in metastases in the lymph nodes [5], reflecting some resistance to the tumor process.

Other studies, with a larger number of participants (100), found that high levels of $CD68^+$ TAM infiltration of the tumor tissue were reliably associated with a worse prognosis compared with a relatively low level of infiltration [6], but these results were not correlated with molecular biological varieties of breast cancer

In its own study, we observed that both $CD68^+$ and $CD163^+$ Mph were grouped in different tumor sites and their localization was highly dependent on connective tissue structures of the tumor. By the way, almost all samples of breast cancer, in particular luminal, were characterized by a distinct desmoplastic response [3].

Probably, TAM is an important regulator of the development and remodeling of the intercellular matrix in the microenvironment of tumors, which has been studied less, but is a direct reflection of the standard functions of Mph [4,7]. This provision is indirectly confirmed by the data that the histological localization of TAM in different regions of breast cancer is correlated with the risks of metastasis and prognosis [8, 9].

According to earlier literature data, both M1 and M2 TAM in BC can suppress the proliferation of T cells, showing immunosuppression and anti-tumor efficacy [10]. Other data indicate that not all TAMs have the ability to inhibit proliferation of CD8 T cells [11]. Consequently, some of the TAM functions are likely to be universal (recruitment, localization, matrix remodeling), whereas other properties (specific interactions with other infiltration cells) may depend on the tumor model under study [4]. These findings may explain the findings of negative effects of various, but not all, TAM subpopulations on the prognosis of breast cancer, and therefore leave an assumption about the protective role of some of them, in combination with individual tumor characteristics.

In our study, the average quantitative indices of $CD68^+$ and $CD163^+$ Mph were lower than in other researches, due to the count of immunopositive cells in *successive* tumor fields, and possibly due to the balanced representation of 5 molecular genetic types of carcinoma. The mean values of the amounts of $CD68^+$ and $CD163^+$ Mph were very different from patient to patient and also within one sample. In other studies, the authors showed a higher number of $CD68^+$ TAM in breast cancer: an average of 61.14 ± 23.76 cells in the field of view of the total of × 400, but the count of these cells was performed in “intensive reaction areas” and patients were not balanced by the molecular- biological types of breast cancer [6].

The prospect for research is the development of differential diagnosis and treatment of breast cancer, taking into account the levels of its infiltration by TAM subpopulations. Regarding the direction of “repolarization” of Mph within the microenvironment of the tumor to the M1 phenotype, it is necessary to take into account their potential tumoral properties [4].

## Conclusions

1. The density of infiltration by $CD163^+$ macrophages of the BC focus negatively correlated with postoperative survival, which did not reach statistical significance, but is included in the general concept of a negative prognosis of infiltration by M2-like macrophages. Further research is needed to confirm the negative significance of the TAM infiltration density in the BC primary focus for postoperative prognosis.

2. The protective role of full-rate M1-like TAM of the primary focus in breast cancer at the level of personalized approach is not excluded.

3. The quantitative representation of $CD68^+$ and $CD163^+$ Mph is very different from patient to patient and also within one sample, which depends, in particular, on the morphological characteristics of breast cancer, studied by the biopsy.

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*Матеріал надійшов до редакції 24.01.2019 р.*

UDC 616.831-005.1:547.587.1-08

DOI https://doi.org/10.31718/mep.2019.23.1-2.02

# EFFECTIVENESS OF ACETYLSALICYLIC ACID IN CORRECTION OF POST-STROKE FATIGUE DURING ACUTE CEREBROVASCULAR EVENTS

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*Постінсультна втома (ПІВ) – поширений і часто виснажливий наслідок інсультів, який спостерігається більше, ніж у третини хворих на інсульт. Недавні дослідження виявили етіологічну та патогенетичну гетерогенність ПІВ залежно від часу виникнення після гострої цереброваскулярної патології (ГЦП). ПІВ, яка виникає під час гострого інсульту, пов'язана переважно з біологічними факторами, включаючи інсульт-індуковані імунні та запальні реакції. Зокрема, нами виявлені значні зв'язки між клінічними ознаками ПІВ та певними закономірностями рівнів С-реактивного білка (СРБ) та інтерлейкіну (ІЛ) -1β у сироватці крові протягом перших 3 місяців після появи ГЦП (робота у друку). Враховуючи те, що для ПІВ не існує чітко визначеної етіології, не існує раціонально обґрунтованих втручань. Якщо ж дисрегуляція імунної відповіді є важливим фактором, що сприяє ПІВ, втручання, які зменшують запалення, є відповідними стратегіями лікування. Було б доцільно розглянути АСК, хоча і з більш високою дозою, ніж це зазвичай використовується для профілактики вторинного інсульту, як лікування ПІВ. Таким чином, доцільно вивчати вплив АСК у протизапальній дозі (300 мг на добу) на маркери системного запалення і на клінічний перебіг ПІВ протягом перших 3 місяців після появи ГЦП. Метою дослідження стало вивчення впливу ацетилсаліцилової кислоти (АСК)в протизапальній дозі (300 мг на добу) на клінічний перебіг ПІВ та маркери системного запалення протягом перших 3 місяців після появи гострої цереброваскулярної продії (ГЦП). Матеріал і методи. У дослідження включено 39 хворих на ішемічні інсульти та транзиторні ішемічні атаки (ТІА), які потребували прийому АСК. У всіх пацієнтів діагностували ПІВ протягом перших 3 днів після появи ГЦП. ПІВ діагностували за допомогою анкети – шкали оцінки втоми (FAS). Ми сформували дві групи пацієнтів. Першу (контрольну групу ПІВ) складали 24 пацієнти, які використовували АСК відповідно до «Єдиного клінічного протоколу медичної допомоги. Ішемічний інсульт (невідкладна, первинна, вторинна (спеціалізована) медична допомога, медична реабілітація)». Їм після виключення геморагічного інсульту нейровізуалізацією було розпочато прийом АСК в дозах 150-300 мг на день під час перебування в стаціонарі з наступним прийомом 150 мг на добу (профілактична доза) безперервно після виписки з лікарні. Друга група (група ПІВ АСК) складалася з 15 пацієнтів, які почали використовувати АСК, тільки після виключення геморагічного інсульту, в дозі 300 мг на добу протягом 3 місяців, з подальшим зменшенням дози до 75-150 мг на добу (профілактична доза). Діагностика присутності / відсутності ПІВ, вимірювання вираженості ПІВ та одночасного визначення системних маркерів запалення в сироватці крові проводилися в певні моменти часу після початку ГЦП: у перші 3 дні, через 1 місяць і через 3 місяці. Концентрації СРБ, ІЛ-1β та ІЛ-6 у сироватці крові визначали за допомогою імуноферментного аналізу. Висновки. 1. Застосування АСК вдозі 300 мг на добу протягом 3 місяців у пацієнтів, яким протягом перших днів після виникнення ГЦП діагностували ПІВ, пов'язане зі значним зниженням інтенсивності ПІВ згідно FAS,порівняно з використанням профілактичної дози АСК. 2. Використання АСК в дозі 300 мг на добу протягом 3 місяців після появи ГЦП пов'язане зі значною модифікацією запальної реакції після інсульту у вигляді змін рівня СРП та ІЛ-1β.*

**Ключові слова:** інсульт, втома, С-реактивний білок, інтерлейкін, ацетилсаліцилова кислота.

*Post-stroke fatigue (PSF) is a common and often debilitating sequela of strokes that affects more than one third of stroke patients. Recent investigations revealed etiologic and pathogenetic heterogeneity of PSF depending on the time after acute cerebrovascular event (ACE). PSF that occur during acute stroke is associated predominantly with biological factors, including stroke-inducing immune and inflammatory reactions. In particular, we found significant associations between clinical features of PSF and certain regularities of C-reactive protein (CRP) and interleukin (IL)-1β levels in blood serum during the first 3 months after ACE occurrence. Given that there is not a clearly defined etiology for PSF, there are no rationally informed interventions. If dysregulation of the immune response isan important contributing factor to PSF, interventions that lessen inflammation would be appropriate treatment strategies. It would be reasonable to consider ASA, albeit at a higher dose than is normally used for secondary stroke prevention, as a treatment for PSF. Thus, it is advisable to study effects of ASA at the anti-inflammatory dose (300 mg a day) on markers of system inflammation an don PSF clinical course during the first 3 months after ACE occurrence. Objective: to study effectiveness of ASA at the anti-inflammatory dose (300 mg a day) on PSF clinical course and ASA effects on markers of system inflammation during the first 3 months after acute cerebrovascular event (ACE) occurrence. We recruited in the study 39 in hospital patients with ischemic strokes and transient ischemic attacks (TIA) who needed to take acetylsalicylic acid (ASA). All patients had been diagnosed with PSF within the first 3 days after ACE onset. PSF was diagnosed by use of questionnaire – Fatigue Assessment Scale (FAS). We formed two groups of patients. The first group (control PSF group) consisted of 24 patients who used ASA according to «Unified clinical protocol for medical care. Ischemic stroke (emergency, primary, secondary (specialized) medical aid, medical rehabilitation)» - after excluding hemorrhagic stroke by neuroimaging it was started ASA intake in the doses of 150-300 mg a day enterally during hospital stay with subsequent intake of 75-150 mg a day (prophylactic dose) continuously after hospital discharge. The second group (ASA PSF group)*

*To cite this English version: Delva I.I. Effectiveness of acetylsalicylic acid in correction of post-stroke fatigue during acute cerebrovascular events. // The Medical and ecological problems. - 2019. - Vol 23, № 1-2. - P. 8-12.*

*had 15 patients who started to use ASA just after excluding hemorrhagic stroke in the dosage of 300 mg a day for 3 months with subsequent dose reduction to 75-150 mg a day (prophylactic dosage) continuously. Diagnosis of PSF presence/absence, measurement of PSF severity and simultaneous measurement of systemic inflammatory markers in blood serum were carried out at the certain time points after ACE onset: at the first 3 days, at 1 month and at 3 months. Concentrations of CRP, IL-1β andIL-6 in blood serum were measured by enzyme-linked immunosorbent assay. The use of ASA in the dose of 300 mg a day during 3 months in patients who had been diagnosed with PSF within the first days after ACE occurrence is associated with significant decreasing of PSF intensity due to FAS in comparison with using of preventive ASA doses. The use of ASA in the dose of 300 mg a day during 3 months after ACE occurrence is associated with significant modification of post-stroke inflammatory response in form of CRP and IL-1β blood level changes.*

**Key words:** stroke, fatigue, C-reactive protein, interleukin, acetylsalicylic acid.

Post-stroke fatigue (PSF) is a common and often debilitating sequela of strokes that affects more than one third of stroke patients [1].

Recent investigations revealed etiologic and pathogenetic heterogeneity of PSFdepending on the time after acute cerebrovascular event (ACE) [2, 3, 4]. PSF that occur during acute stroke is associated predominantly with biological factors [4], including stroke-inducing immune and inflammatory reactions [5, 6, 7].In particular, we found significant associations between clinical features of PSF and certain regularities of C-reactive protein (CRP) and interleukin (IL)-1β levels in blood serum during the first 3 months after ACE occurrence (the paper in print).

Given that there is not a clearly defined etiology for PSF, there are no rationally informed interventions. To date, very few trials have been done to address therapies for PSF. In fact, the most recent Cochrane review concluded that "there is insufficient evidence available to guide the management of fatigue after stroke" [8]. However, on the other hand, if dysregulation of the immune response is an important contributing factor to PSF, interventions that lessen inflammation would be appropriate treatment strategies [9]. Based on the studies that show a decrease in multiple sclerosis related fatigue with acetylsalicylic acid (ASA) at doses 500 mg a day and more [10, 11], and as ASA is prescribed to virtually all patients with ischemic stroke (who do not need to be anticoagulated), it would be reasonable to consider ASA, albeit at a higher dose than is normally used for secondary stroke prevention, as a treatment for PSF [9]. For anti-inflammatory purposes ASA is used at the dosage of 300 mg a day or more [12], for example, at doses of 300 mg a day, ASA effectively decreases plasma concentrations of pro-inflammatory substances (including also CRP and pro-inflammatory cytokines) [13, 14].

Thus, taking into account all above-mentioned facts, it is advisable to study effects of ASA at the anti-inflammatory dose (300 mg a day) on markers of system inflammation and on PSF clinical course during the first 3 months after ACE occurrence.

Objective: to study effectiveness of ASA at the anti-inflammatory dose (300 mg a day) on PSF clinical course and ASA effects on markers of system inflammation during the first 3 months after ACE occurrence.

## Material and methods

We enrolled in the study 39 in hospital patients with ischemic strokes and transient ischemic attacks (TIA) who needed to take ASA according to «Unified clinical protocol for medical care. Ischemic stroke (emergency, primary, secondary (specialized) medical aid, medical rehabilitation)». All patients had been diagnosed with PSF within the first 3 days after ACE onset. Patients were included in the study if they agreed to participate and were able to provide informed consent. Exclusion criteria were major medical illness that could cause secondary fatigue (oncological, hematological diseases, cardiac, liver, kidney and respiratory insufficiency, progressive angina pectoris, acute myocardial infarction), diseases with systemic inflammatory reactions (post-stroke infectious complications, pyrexia, concomitant chronic infectious and autoimmune diseases), alcohol abuse, consciousness impairments, insufficient cognitive ability (Mini-Mental State Examination scores less than 24), depressive and anxious disorders (Hospital Anxiety and Depression Scale scores more than 10 for both pathologies), impaired speech function to participate (severe dysphasia or dysarthria), impaired language or written ability to complete the study questionnaire, severe functional disabilities (modified Rankin scale scores ≥4).

PSF was diagnosed by use of questionnaire – Fatigue Assessment Scale (FAS). FAS consist of 10 questions: 5 questions about mental fatigue and 5 questions about physical fatigue.Each question has 5 answer options. The range of possible FAS values varies from 10 to 50 points.FAS scores 22 or more mean fatigue [15].

We formed two groups of patients. The first group (control PSF group) consisted of 24 patients who used ASA according to «Unified clinical protocol for medical care. Ischemic stroke (emergency, primary, secondary (specialized) medical aid, medical rehabilitation)» - after excluding hemorrhagic stroke by neuroimaging it was started ASA intake in the doses of 150-300 mg a day enterally during hospital stay with subsequent intake of 75-150 mg a day (prophylactic dose) continuously after hospital discharge [15]. The second group (ASA PSF group) had 15 patients who started to useASA just after excluding hemorrhagic stroke in the dosage of 300 mg a day for 3 months with subsequent dose reduction to 75-150 mg a day (prophylactic dosage) continuously.

Diagnosis of PSF presence/absence, measurement of PSF severity and simultaneous measurement ofsystemic inflammatory markers in blood serum were carried out at the certain time points after ACE onset: at the first 3 days, at 1 month and at 3 months.

Concentrations of CRP, IL-1β and IL-6 in blood serum were measured by enzyme-linked immunosorbent assayin Research Institute for Genetics and Immunological Grounds of Pathology and Pharmacogenetics at Ukrainian medical stomatological academy.IL-1β and IL-6 levels were studied using test systems of «Vector-Best» company (Russia), CRP level was evaluated using test system of the «Хема»company (Russia) according to the enclosed instructions.

Categorical data were represented by number (n) and percentage (%).Normality of the quantitative data was checked by Kolmogorov-Smirnov test. Variables with normal distribution were represented as mean (M) and standard deviation (SD).Variables with non-normal distribution were expressed as mediana (Me) and interquartile (25%-75%) range (Q1-Q3). Differences in categorical variables were compared using Fisher exact test.Non-

parametric data were evaluated using Mann-Whitney U test (for two independent groups) or using Friedman test and subsequent post-hoc Newman-Keuls analysis (for three dependent groups). A p-value <0,05 was considered statistically significant.

## Results and discussion

As can be seen from Table 1, two patients groups were not critically distinct from each other (even mean age and NIHSS scores) due to relatively small numbers of observations.

*Table 1*
*Characteristics of the baseline study sample*

| Characteristics | | Group of patients | |
|---|---|---|---|
| | | control PSF | ASA PSF |
| mean age (years), M±SD | | 62,0±12,4 | 70,1±6,5 |
| males, n (%) | | 9 (38%) | 6 (40%) |
| ACE | ischemic stroke, n (%) | 22 (92%) | 12 (80%) |
| | TIA, n (%) | 2 (8%) | 3 (20%) |
| NIHSSscore at hospital admission (points), M±SD | | 5,6±4,8 | 7,5±3,5 |

Table 2 demonstrates that in the ASA PSF group there was statistically significant reduction in PSF intensity at 3 months after ACE compared to the initial PSF scores at hospital stay, whereas in the control PSF group there were no statistically significant changes in PSF severity during the whole observation period. It is important that the rates of PSF were almost similar in all three observations in the two patients groups.PSF as pathological entity has different natural clinical course and can be spontaneously disappeared within the first post-stroke months [16]. On the ground of the time-based PSF characteristics (because the most cases of PSF spontaneous self-resolution occur just within the first 3 post-stroke months) we conditionally divided all PSF cases during acute ACE as early PSF (manifested within the first month after ACE occurrence with subsequent self-resolution not later than at 3 months time-point obseravation) and persistent PSF (manifested within the first post-stroke month and was still present at 3 months time-point observation) [16]. Thus, it is likely that ASA in the dosage of 300 mg a day has delayed effect (that appears only after 3 months) on PSF intensity, when early PSF had already spontaneously self-resolved; or ASA has influence exclusively on severity of persistent PSF but not on severity of early PSF (this hypothetic explanation can be indirect proof of etiological and pathogenetic distinctions of PSF depending on its time duration).

*Table 2*
*PSF characteristics according to FAS*

| Time-point after ACE onset | Group of patients | | | |
|---|---|---|---|---|
| | control PSF | | ASA PSF | |
| | n (%) | Me (Q1-Q3) | n (%) | Me (Q1-Q3) |
| 3 days | 24 (100%) | 34,0 (27,8-44,0) | 15 (100%) | 41,0 (37,0-45,0) |
| 1 month | 19 (79%) | 39,0 (29,5-41,5) | 9 (60%) | 36,0 (35,0-40,0) |
| 3 months | 12 (50%) | 29,0 (26,0-34,8) | 8 (53%) | 32,5 (30,5-35,0)* |

** -significant differences (p<0,05), according to Friedman test and post-hoc Newman-Keuls analysis, in comparison with the first 3 days results in ASA PSF group.*

According to Table 3, the control PSF group had significant reduction of CRP blood concentrations only at 3 months time-point observation compared with the first 3 days values.In the ASA PSF group there was dramatic reduction of CRP blood level already at 1 month time-point observation in comparison with the first 3 days results and this significant reductionof CRP concentration remained practically unchanged also at 3 months time-point observation. In addition,the degree of CRP reduction in the ASA PSF group was much more pronounced than in the control PSF group in two time-points – at 1 month and at 3 months after ACE occurrence.

*Table 3*
*CRP concentration in blood serum (mg/ml), Me (Q1-Q3)*

| Time-point after ACE onset | Group of patients | |
|---|---|---|
| | control PSF | ASA PSF |
| 3 days | 28,8 (27,1-29,9) | 28,5 (21,3-30,4) |
| 1 month | 30,3 (26,0-32,5) | 9,9 (6,7-22,2)** *** |
| 3 months | 24,0 (15,4-29,8)* | 13,7 (8,7-23,4)** *** |

**- significant differences (p<0,05), according to Friedman test and post-hoc Newman-Keuls analysis, in comparison with the first 3 days results in the control PSF group;*

*** - significant differences (p<0,05), according to Friedman test and post-hoc Newman-Keuls analysis, in comparison with the first 3 days results in the ASA PSF group;*

**** - significant differences (p<0,05), according to Mann-Whitney U test, in comparison with results of the control PSF group in the same time point after ACE onset.*

Table 4 shows statistical changes in IL-1β blood level in the control PSF group in form of significant increasing at 1 month time-point observation compared with the first 3 days results followed by the subsequent reduction to

the initial values at 3 months after ACE. While in the ASA PSF group, IL-1β level was virtually unchanged in all three time-point observations. Moreover, in the ASA PSF group IL-1β blood serum concentration was significantly lower at 1 month after ACE compared to the control PSF group. So, ASA use in the dose of 300 mg a day for 3 months was associated with the smoothing of IL-1β blood serum peak at 1 month after ACE occurrence which is typical for PSF patient during acute ACE.

*Table 4*
*IL-1β concentration in blood serum (pg/ml), Me (Q1-Q3)*

| Time-point after ACE Onset | Group of patients | |
|---|---|---|
| | control PSF | ASA PSF |
| 3 days | 18,5 (17,0-19,3)* | 17,0 (17,0-21,0) |
| 1 month | 23,5 (20,8-26,0) | 18,0 (17,5-20,5)** |
| 3 months | 18,5 (15,0-22,3)* | 17,0 (15,0-21,5) |

** - significant differences (p<0,05), according to Friedman test and post-hoc Newman-Keuls analysis, in comparison with 1 month results in the control PSF group;*

*** - significant differences (p<0,05), according to Mann-Whitney U test, in comparison with results of the control PSF group at 1 month after ACE onset.*

Table 5 shows that serum IL-6 levels during the whole 3 months observation period were constant in both patients groups.

*Table 5*
*IL-6concentration in blood serum (pg/ml), Me (Q1-Q3)*

| Time-point after ACE onset | Group of patients | |
|---|---|---|
| | control PSF | ASA PSF |
| 3 days | 13,5 (6,0-17,5) | 13,9 (9,5-17,9) |
| 1 month | 12,8 (12,0-14,2) | 12,0 (10,5-13,3) |
| 3 months | 12,1 (11,2-13,6) | 13,1 (12,3-13,7) |

As is known, interactions of cytokines are extremely complex with multidirectional, multilevel regulations. So it's quite difficult to explain the found phenomenon of CPR and IL-1β statistical changes whereas IL-6 levels were stable. May be this phenomenon can be considered as a peculiarity of post-stroke inflammatory response only in PSF patients with acute ACE. Anyway this issue requires further study and is beyond the scope of our research.

Summing up, we found statistically significant reduction of PSF intensity at 3 months after ACE onset in the ASA PSF group that takes place together with statistically significant modification of systemic inflammatory manifestations (in the form ofa more rapid and more pronounced decreasing of CRP blood level and smoothing of IL-1β elevationin blood within the first 3 months after ACE occurrence). Overlapping in time clinical and laboratory phenomena it can be assumed that decreasing of PSF intensity in the ASA PSF group at least partly may be due to ASA anti-inflammatory properties (at dose of 300 mg a day) through modifying post-stroke inflammatory reactions.

According to recent researches, inflammation may be a significant factor in the development of fatigue. A crucial mechanism by which cytokines modulate neuronal functions is through modifications of monoaminergic neurotransmission, specifically by activating enzymes interfering with dopamine and serotonin biosynthesis [17,18], as well as through modification of glutamate neurotransmission [19]. These alterations in neurotransmitter systems ultimately lead to modifications in neuronal functions, which in turn induce behavioral changes collectively so-called «sickness behavior» that includes fatigue, reduced activity, altered mood state, changes in cognitive functions, so on [20].

Probably, ASA in the dose of 300 mg a day since the first days after ACE and further for next 3 months significantly reduces PSF intensity according to FAS through the ASA anti-inflammatory activity and suppression of post-stroke inflammatory response.

From the practical point of view, it is probably useful to take ASA in the dose of 300 mg a day for 3 months by patients who had been diagnosed with PSF already within the first days after ACE occurrence.However further investigations are needed to elaborate these findings.

## Conclusions

1. The use of ASA in the dose of 300 mg a day during 3 months in patients who had been diagnosed with PSF within the first days after ACE occurrence is associated with significant decreasing of PSF intensity due to FAS in comparison with using of preventive ASA doses.

2. The use of ASA in the dose of 300 mg a day during 3 months after ACE occurrence is associated with significant modification of post-stroke inflammatory response in form of CRP and IL-1β blood level changes.

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*Матеріал надійшов до редакції 12.02.2019 р.*

# ПРОБЛЕМИ СТОМАТОЛОГІЇ


UDK 616.314.2-089.28 / 29-633-76: 51-7

DOI https://doi.org/10.31718/mep.2019.23.1-2.03

## MATHEMATICAL SUBSTANTIATION OF THE USE OF DIFFERENT TYPES OF CLAMMERS DURING THE RESTORATION OF THE DENTITION WITH DOUBLE-SIDED DISTAL DEFECTS AND THE DEFECT INCLUDED IN THE FRONTAL AREA BY REMOVABLE PROSTHETICS

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*До основних умов, які забезпечують хороші функціональні якості і довговічність протезних конструкцій з точки зору біомеханіки, безсумнівно, відноситься оптимальний спосіб фіксації часткового знімного протеза, який полягає в правильному виборі системи кламерів, що забезпечують надійну фіксацію протеза в заданому положенні і в той же час завдає мінімум шкоди опорним зубам, а також найбільш раціональний розподіл оклюзійних навантажень що передаються на опорні тканини, так як саме ці фактори визначають довготривалість функціонування знімних протезів. Метою виконуваних біомеханічних досліджень є аналіз напружено-деформованого стану опорних зубів часткових знімних протезів, що заміщають включений і кінцеві дефекти зубного ряду при фіксації протезів на опорних зубах за допомогою утримуючих та опорно-утримуючих кламерів. Дослідження виконувалися на моделі нижньої щелепи з габаритними розмірами поперечного перерізу, відповідним деяким усередненим розмірами. Перша об'ємна кінцево-елементна модель розроблена для дослідження напружено-деформованого стану зв'язок періодонта опорних зубів і опорних тканин ясен при заміщенні включеного і двостороннього кінцевого дефектів зубного ряду (1 клас 1 підклас за Кеннеді) нижньої щелепи знімним протезом з фіксацією утримуючими кламерами на іклах і перших премолярах. Друга об'ємна кінцево-елементна модель розроблена для дослідження напружено-деформованого стану зв'язок періодонта опорних зубів і опорних тканин ясен при заміщенні включеного і двостороннього кінцевих дефектів зубного ряду нижньої щелепи знімним бюгельним протезом з фіксацією опорно-утримуючими кламерами на іклах і перших премолярах з опорними плечима кламерів з дистальних сторін премолярів. В результаті виконаних досліджень можна зробити наступні висновки: В обох розглянутих варіантах фіксації знімних бюгельних протезів навантажених максимальними значеннями еквівалентних напружень в періодонті опорних зубів локалізовані у верхівки альвеолярного гребеня, що свідчить про передачу бюгельним протезом саме "розхитуючих" опорні зуби зусиль. Максимальні значення еквівалентних напружень в періодонті опорних зубів при фіксації знімного бюгельного протеза опорно-утримуючими кламерами на іклах і перших премолярах перевищують відповідні значення еквівалентних напружень на відміну від випадку фіксації протеза утримуючими кламерами на 30 - 140%! Зі збільшенням піддатливості ясен, значення еквівалентних напружень в періодонті опорних зубів при фіксації знімного бюгельного протеза утримуючими кламерами змінюються несуттєво в межах 25%, а збільшення відповідних еквівалентних напружень при використанні опорно-утримуючих кламерів може становити 65%. З метою забезпечення максимального щадного режиму тканин періодонта опорних зубів і як наслідок - можливість найбільш тривалого користування протезом без розхитування опорних зубів в даній клінічній ситуації більш доцільним видається віддавати перевагу застосуванню бюгельних протезів з фіксацією саме утримуючими кламерами.*

**Ключові слова:** Кламмер, дефект зубного ряду, математичне моделювання, ортопедична стоматологія

*The basic conditions that ensure good functional qualities and durability of prosthetic structures from the point of view of biomechanics, undoubtedly include an optimal way of fixing a partial removable prosthesis, which consists in the correct choice of the clamping system that ensures reliable fixation of the prosthesis in the given position and at the same time causing a minimum of harm supporting teeth, as well as the most rational distribution of occlusal loads transmitted to the supporting tissues, since it is these factors that determine the durability functioning dentures. The purpose of biomechanical studies is to analyze the stress-strain state of the supporting teeth of partial removable prostheses replacing the included and terminal defects of the dentition when fixing the prosthesis on the supporting teeth with the help of holding*

*To cite this English version Kozak R.V., Korol D.M. Mathematical substantiation of the use of different types of clammers during the restoration of the dentition with double-sided distal defects and the defect included in the frontal area by removable prosthetics. // The Medical and ecological problems. - 2019. - Vol 23, № 1-2. - P. 13-16.*

*and supporting-retaining clamps. The studies were performed on a model of the mandible with overall dimensions of the cross section corresponding to some average dimensions. The first volumetric finite element model was developed to study the stress-strain state of periodontal ligaments of supporting teeth and supporting gingival tissues when the incomplete and bilaterial end defects of the dentition were replaced (1 class 1 subclass by Kennedy) of the mandible by a removable denture with fixation by retaining clamps on the canines and the first premolars. The second volumetric finite element model is designed to study the stress-strain state of periodontal ligaments of supporting teeth and supporting gum tissues when the included and two-sided end defects of the dentition of the lower jaw are replaced by a removable clasp prosthesis with fixation of support-retaining clamps on canines and first premolars with support arms of clamps from the distal sides of premolars. As a result of the studies performed, the following conclusions can be drawn: In both considered variants fixation of removable clasp prostheses loaded with maximum values of equivalent stresses in the periodontium of the supporting teeth is localized at the apex of the alveolar ridge, which indicates the transfer of the "clutching" supporting teeth of the clasp prosthesis. The maximum values f equivalent stresses in the periodontitis of the supporting teeth during fixation of the removable clasp prosthesis by the support-retaining clamps on the canines and the first premolars exceed the corresponding values of the equivalent stresses for the case of fixing the prosthesis with retention clamps by 30-140%! With an increase in gum compliance, the values of equivalent stresses in the periodontitis of the supporting teeth during fixation of the removable clasp prosthesis by the retaining clasps vary insignificantly within 25%, and an increase in the corresponding equivalent stresses with the use of support-holding clasps may be 65%. In order to ensure the maximum sparing regimen of periodontal tissues of supporting teeth and as a result - the possibility of the most prolonged use of the prosthesis without loosening of the supporting teeth, it seems more expedient to give preference to the use of clasp prostheses with the fixation of the retaining clasps.*

**Key words:** Clammer, dentition defect, mathematical modeling, prosthetic dentistry

The main conditions ensuring good functional qualities and durability of prosthetic structures from the point of view of biomechanics undoubtedly include the optimal method of fixing a partial denture, which is the correct choice of a system of clasps [1, 3] ensuring reliable fixation of the prosthesis in a predetermined position and same time causing minimal damage to the supporting teeth, as well as the most rational distribution of occlusal power transmitted to the supporting tissue, since these factors determine the duration of the functioning of removable prostheses [2] .

The purpose of the biomechanical studies is the analysis of stress-deformed state of abutment teeth partial dentures, and the second substitute terminal included defects in dentition during fixation prostheses on the abutment teeth by means of retaining and supporting-retaining clasps.

## Materials and methods

The primary criterion when compared fixing denture clasps for different designs can receive force (stress $\sigma_{экв}^{max}$ ) in the periodontal abutment teeth, arising from the impact on the prosthesis functional loads.

The study of the stress-strain state of abutment teeth, which undertake some of the functional loads transmitted through the clasp prosthesis, is most expediently performed using a finite-element analysis, which is promising for solving various biomechanical problems in contemporary orthopedic dentistry and has been recently increasingly confirmed in the works of both foreign and domestic authors.

Mathematical modeling was performed using the well-known NASTRAN modeling and finite element analysis package, designed for implementation in a Windows environment on a personal computer. The package with which the considered models are constructed and analyzed on the basis of a finite-element procedure determines the displacement of each node of the final element along three coordinate axes, normal and tangential stresses, as well as equivalent Huber-Mises stresses.

The studies were performed using elastic volumetric models of partial removable arc dentures replacing the distal and included defects of the mandibular tooth row.

We developed three-dimensional finite-element models of the lower jaw for the analysis of the stress-strain state., They contain all main structural components: the alveolar bone, which includes both the cortical layer and the spongy substance; soft gum tissue; ligamentous apparatus of the periodontium; teeth consisting of a crown with enamel and neck, and the root part. The main dimensions used in modeling the profiles of the abutment and prosthetic teeth are taken according to the recommended dimensions for modeling [1].

Studies were performed on the model of the lower jaw with the overall dimensions of the cross section corresponding to some averaged sizes. The consolidation of the three-dimensional model of the lower jaw was carried out in the nodes of the final elements located in the areas of the temporal mandibular joints and places of attachment of masticatory muscles Fig. 1.

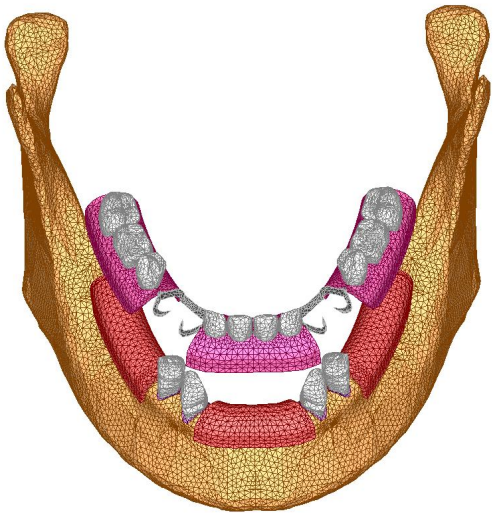

*Fig. 1. Volumetric finite element model of the lower jaw with partial removable arc denture fixation with retaining clasps*

The main dimensions used in modeling profiles of supporting canines and premolars are taken according to the recommended sizes for modeling. The width of the periodontal gap of the abutment teeth is within 0.15 - 0.3 mm, and the thickness of the soft tissues of the gums above the top of the alveolar process is up to 2.5 mm. The physicomechanical characteristics of the structural components of the ultimate element model of the lower

jaw and the partial denture are presented in Table. 1, according to the values given in [2] and [3].

The use of contact elements (working only under compression) in the finite element model between the lower surface of the prosthesis base and the upper surface of the gum tissues, as well as the surfaces of clasps and abutment teeth, prevents the transfer of forces from the clutch prosthesis to the supporting tissues of the dentary segment in the absence of contact between the contacting surfaces.

*Table 1.*
*Physico-mechanical characteristics of the structural components of the finite element model of the mandible fragment used in the calculations*

| Material | The modulus of elasticity E, M p and | Poisson's ratio | Strength $\sigma_{in,}$ MPa |
|---|---|---|---|
| Cortical bone layer | $2 \cdot 10^4$ | 0,3 | 45 |
| Spongy bone | $5 \cdot 10^3$ | 0,3 | 15 |
| Periodontium | 0,5 – 50 | 0,45 | 3,8 |
| Gum | 0,75–75 | 0,25 | – |
| Dentine | $2 \cdot 10^3$ | 0,3 | 24 |
| Enamel tooth crown | $4{,}0 \cdot 10^4$ | 0,3 | 34 |
| Plastic prosthetic seat | $2{,}5 \cdot 10^3$ | 0,3 | 50 |
| Alloy metal | $2{,}2 \cdot 10^5$ | 0,32 | 800 |

When creating a mathematical model as functional loads (occurring during chewing of the food) taken load applied to the occlusal surfaces of teeth crowns and denture substitutable directed along the axes of the respective teeth. To eliminate the stress concentrations observed at the points of application of forces, the load was evenly distributed over the occlusal surfaces of the replacement teeth of the removable denture. The absolute values of the load have little influence on the solution of the problem (since any reference load value can be used to compare the maximum values of the equivalent stresses in the periodontium using different shaped molded clasps of removable clasp dentures).

The first volumetric finite element model is designed to study the stress-strain state of the periodontal ligaments. The abutment teeth and the supporting gum tissue in the substitution included and double terminal defects dentition (1 Class 1 subclass by Kennedy) mandibular denture retaining latching clasps in canines and a first premolar.

Under a load of a removable prosthesis, all possible options for the localization of the food lump were considered along the length of the prosthesis saddle. As can be seen from the Table. 2.

*Table 2.*
*The results of calculations of equivalent stresses in the periodontal teeth of the lower jaw with fixation of a removable prosthesis with retaining and supporting-retaining clasps the maximum values of the equivalent stresses in the periodontal teeth of the abutment teeth occur when placing the food lateral incisor and over the second molar and are respectively 1,408 MPa and 1,315 MPa.*

| Clammer view when fixing the prosthesis | Maximum equivalent stresses in periodontal (MPa) at localization of the functional load on | | | | |
|---|---|---|---|---|---|
| | Central incisor | Lateral incisor | The second premolar | The first molar | The second molar |
| | The modulus of elasticity of the gums E = 7.5 MPa | | | | |
| holding | 1,209 | 1,408 | 1,103 | 1,19 | 1,315 |
| support-holding | 1,374 | 1,736 | 1,822 | 1,215 | 1,323 |
| | The modulus of elasticity of the gums E=5 MPa | | | | |
| holding | 1,359 | 1,691 | 0,982 | 1,122 | 1,329 |
| support-holding | 1,623 | 2,139 | 2,187 | 1,184 | 1,33 |
| | The modulus of elasticity of the gums E=2,5 MPa | | | | |
| holding | 1,723 | 1,132 | 1,279 | 0,996 | 1,382 |
| support-holding | 2,305 | 2,989 | 2,817 | 1,527 | 1,374 |

The second volumetric finite element model was developed to study the stress-strain state ligaments periodontal supporting teeth and the supporting gum tissue in the substitution included and double end s defective s dentition mandibular detachable clasp prosthesis with the fixation support-retaining clasps for canines and first premolars to the support clumps shoulders from the distal sides of the premolars.

The results of the calculation of equivalent stresses in the tissues of the periodontal abutment teeth are presented in Table. 2 (second line). As can be seen from the table. 2, bolus for all localization equivalent embodiments is a strain periodontal supporting teeth during fixation of the prosthesis-supporting retaining clasps exceed the corresponding values of x are equivalent strain minutes arising during fixation of the prosthesis retaining clasps. Maximum Feed M values of the equivalent stresses in the periodontal supporting teeth arise when bolus and in the vicinity of the supporting teeth on the lateral incisor and the first premolar respectively equal to 1,736 MPa and 1,822 MPa.

For greater clarity in Fig. 2, the fields of equivalent stresses of the periodontal abutment teeth are presented for cases of fixation of the clasp prosthesis with retaining and supporting-retaining clamps.

The maximum values of the equivalent stresses in the supporting periodontium of teeth on their partial denture fixation cases arise at the top of the alveolar crest. This distribution of stresses indicates the transfer of the efforts that are loosening the abutment teeth to the cusp, since with the application of the axial component of the functional load directly to the abutment teeth, the equivalent stresses in the periodontal are distributed more evenly and their maximum values are observed at the apex of the tooth root.

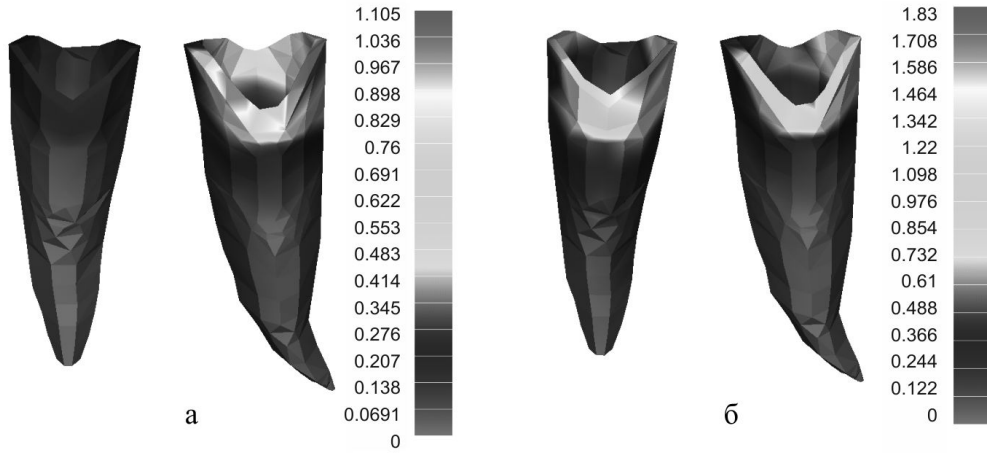


*Fig. 2. The fields of equivalent stresses of the periodontal abutment teeth are presented for cases of fixation of the clasp prosthesis with retaining and supporting-retaining clamps.*

Further studies I performed with varying Hovhan the values of the elastic modulus of the gum tissue (determining the gingival compliance) for the cases of fixation of the clasp prosthesis with retaining and supporting-retaining clasps. The maximum equivalent stresses in the periodontal teeth of the abutment, taking into account the different compliance of the gums for the case of fixation of removable clasp prostheses with retaining and supporting-retaining clasps on the canines and the first premolars are presented in Table. 2 (lines 3-6).

## Conclusions

1. In both these embodiments fixation removable denture clasp s loaded with maximum values of equivalent voltages at the periodontal abutment teeth are localized at the apex of the alveolar ridge, which indicates the transfer of the efforts that are “loosening” the abutment teeth by the clutch prosthesis.

2. The maximum values of equivalent stresses in the periodontal teeth of the supporting teeth when fixing a removable clasp prosthesis with supporting-holding clasps on the canines and first premolars exceed the corresponding values of equivalent stresses for the case of fixation of the denture by clasps by 30 - 140%!

3. With an increase in the gingival compliance, the values of equivalent stresses in the periodontal teeth of the abutment teeth when fixing a removable clasp prosthesis with retaining clips do not change significantly within 25%, and an increase in the corresponding equivalent stresses when using support-holding clasps can be 65%.

4. In order to ensure maximum gentle treatment of periodontal tissues of the supporting teeth and, as a result, the possibility of the most long-term use of the prosthesis without loosening of the supporting teeth, it is more appropriate to give preference to the use of the clasp prostheses with fixation of precisely the retainings clasp.

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*Матеріал надійшов до редакції 14.02.2019 р.*

UDC 616.311-06:616-056.52]-053.81

DOI https://doi.org/10.31718/mep.2019.23.1-2.04

# CONDITIONS OF THE ORAL CAVITY STATUS IN YOUTH WITH ALIMENTARY-CONSTITUTIONAL FORM OF OBESITY

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*Поширеність ожиріння значно зросла, особливо серед молодих людей, що обумовлено певним способом життя, якістю їжі та харчовою поведінкою. Ожиріння призводить до розвитку великого числа супутніх захворювань, таких як артеріальна гіпертонія, серцевий інсульт, артрит та інші захворювання. Ми провели стандартне клінічне обстеження порожнини рота 154 молодих пацієнтів (18-21 року) - всі вони були студентами Української медичної стоматологічної академії. Було встановлено, що більшість пацієнтів з ІМТ> 30 кг/см$^2$ мають обтяжену спадковість у сім'ї, в 66,2% випадків у одного з батьків обстеженого з ожирінням було ожиріння, а у 32,43% пацієнтів обоє батьків мали ожиріння, яке значно вище, у порівнянні з людьми із нормальним ІМТ і надмірною вагою. Була висока поширеність захворювань пародонту близько 74% і каріозних уражень зубів - 97,4% серед усіх студентів, незважаючи на вік і контингент обстежених пацієнтів - студенти-медики. У пацієнтів з ІМТ> 30 кг/см$^2$ поширеність генералізованих форм гінгівіту і пародонтиту була в три рази вище у порівнянні з особами із нормальним ІМТ. Значення гігієнічних індексів порожнини рота були низькими у всіх дослідних групах, але їх значення були трохи нижче у пацієнтів з нормальним ІМТ в порівнянні з пацієнтами з надлишковою вагою та ожирінням. Поширеність запальних змін в яснах була вище в осіб з ожирінням, усі вони мали легкий ступінь ураження тканин пародонта. Запальні процеси у яснах були найбільш інтенсивними у пацієнтів з другим ступенем ожиріння. За результатами дослідження наявність ожиріння першого та другого ступеня слід розглядати як фактор ризику, що провокує захворювання тканин пародонту. Для осіб з ІМТ> 30 кг/см$^2$ при захворюваннях пародонту слід проводити заходи з вторинної профілактики запальних та запально-дистрофічних захворювань пародонту, а у осіб без захворювань пародонту на тлі ожиріння слід проводити первинну профілактику захворювань пародонту.*

**Ключові слова:** захворювання пародонту, ожиріння, профілактика, надмірна вага, пародонтит,карієс, гінгівіт.

*Obesity prevalence has significantly increased especially in young adults, which is caused by a particular lifestyle, food quality and dietary behavior. Obesity leads to development of huge array of comorbid conditions such as arterial hypertonia, heart stroke, arthritis and other diseases. We conducted standard clinical examination of oral cavity of 154 young patients (18-21 years old) – all of them were students of Ukrainian Medical Stomatological Academy. It has been determined that the majority of patients with BMI >30 kg/cm$^2$ have aggravated family heredity, in 66,2% cases one parent of obese students had obesity and 32,43% patients have both parents with obesity that is significantly higher compared with persons with normal BMI and overweight. There was a high prevalence of periodontal diseases about 74% and carious lesions of teeth - 97.4% among young people despite the age and contingent of examined patients - medical students. In patients with BMI >30 kg/cm$^2$ prevalence of generalized forms of gingivitis and periodontitis was by three times higher compared with individuals with normal BMI. The values of oral hygienic indexes were poor in all examined groups, but their values were slightly lower in patients with normal BMI than in those with overweight and obesity. The prevalence of inflammatory changes in gums was higher in persons with obesity: all of them had a mild degree of lesions in periodontal tissues. Inflammatory processes in the gums were the most intense in patients with the second degree of obesity. According to the results of the study, the presence of the first and the second degree of obesity should be considered as a risk factor triggering periodontal tissues diseases. For persons with BMI >30 kg/cm$^2$ with periodontal disease measures for the secondary prevention of inflammatory and inflammatory dystrophic periodontal diseases should be carried out and in persons without periodontal disease on the background of obesity measures primary prevention should be done.*

**Key words:** periodontal diseases, obesity, prevention, overweight, periodontitis, caries, gingivitis.

## Introduction

Obesity acquires the status of non-infection pandemic disease for the last 40 years. According to WHO worldwide obesity has nearly tripled since 1975. In 2016 more than 1.9 billion adults 18 years and older were overweight. Out of these people over 650 million were obese. 39% of adults aged a ged 18 years and over were overweight in 2016 and 13% were obese [15]. There are many well-known obesity-related diseases such as insulin resistance, type 2 diabetes mellitus, and cardiovascular disease and other. Obesity can induce the development of heart stroke, arterial hypertension, cancer, diseases of musculoskeletal system and diseases of periodontal tissues.

There is periodontal and systemic immune response of overweight hosts to periodontitis. The most relevant linker is systemic inflammation and impaired immune response by which obesity might trigger aberrant periodontal inflammation and exacerbated alveolar bone loss [8]. Through test carried out on laboratory mice with induced obesity researchers explore significant decrease of alveolar bone level and increase of periodontal osteoclast, leukocyte, and macrophage number in obese mice with periodontitis compared with normal weight mice with induced periodontitis [16]. In periodontal tissues of

*To cite this English version: Skrypnyk M., Petrushanko T., Kryvoruchko T., Neporada K. Conditions of the oral cavity status in youth with alimentary-constitutional form of obesity. // The Medical and ecological problems. - 2019. - Vol 23, № 1-2. - P. 17-21.*

monosodium glutamate-induced obesity rats activation of oxidative stress and nitrosative stress alteration and depolymerization of main connective tissues biopolymers were detected compared with rats without obesity [1].

There is a significant association between variety of body mass index (BMI) and waist-hip ratio (WHR) used in the assessment of body composition as indicators of obesity and risk of body fat distribution have an influence on the quality of life for aged individuals [9]. In young adults abdominal obesity is associated with poorer scores of emotional, school and psychosocial abilities and total life quality scores [12]. Due to mentioned above facts, overweight and obesity are not only medical, but global social problems needed to be tackled.

The mechanism of pathological changes development in periodontal tissues are not clear enough and it needs to be studied in depth in order to provide such group of patients with effective etiological and pathogenetic therapy.

The aim of research. The aim of our research was to explore the structure of oral cavity pathology in patients with diverse values of BMI in order to detect diseases comorbid with overweight in oral cavity.

## Materials and methods

We conducted standard clinical examination of oral cavity in 154 young patients (18-21 years old) – all of them were students of the Ukrainian Medical Stomatological Academy. All participants were informed about using of their personal information in the material of research and signed a written agreement for further examination. Index assessment of oral status entails the determination of caries intensity by DMFT index [11], estimation of oral hygiene (Green-Vermillion index) [5], periodontal tissues (index Rateitchak) [14], PMA (Parma) [13], CPI (complex periodontal index) (Leus) [3], PBI (Saxer and Muhlemann)[14] and Winkel Tongue Coating index (WTC) [7]. For all examined patients periodontograma was completed. Periodontal diagnose made due to M.F. Danilevsky classification [3], alteration in oral mucosa and lips diseases were classified according to P.T. Maksymenko [10], occlusion pathology was estimated with classification by L.P. Grigoryeva [6].

In all patients, we determine the values of anthropometrical measures: body mass, height, waist and hip measures and hand dynamometry. Severity of obesity was estimated with WHO classification of obesity due to body mass index Ketle (BMI). Values of BMI were the criteria for group distribution: 1) patients with normal weight (BMI – 18,5 – 24,99 kg/cm$^2$) n=31; 2) patients with overweight (BMI - 25 – 29,99 kg/cm$^2$) n=49; 3) patients with the first degree obesity (BMI - 30 – 34,99 kg/cm$^2$) n=34 ; 4) patients with the second degree obesity (BMI - 35 – 39,99 kg/cm$^2$) n=40.

Inclusion and exclusion criteria. Inclusion criteria were patients of both gender aged from 18 to 21, Ukrainian origins, presence of constitutional or acquired forms of obesity in those who were obese.

The exclusion criteria were pregnant or lactating women; patients with endocrine forms of obesity; patients of non-Ukrainian race; presence of non-removable orthodontic appliances.

Statistical methods**.** SPSS 11.5 software was used for statistical analysis, and data were presented as mean ± standard deviation ($\bar{x} \pm s$). Intra-group comparisons were conducted with a paired-sample t test, and the analysis of the correlation between the indicators was performed by Pearson's correlation analysis. $P < 0.05$ was considered statistically significant.

## Results and discussion

The average students of BMI in the first group of patients was 22,3 ± 0,2 kg/cm$^2$, in the second group – 27,84±0,2 kg/cm$^2$, in the 3rd group – 32,3 ± 0,38 kg/cm$^2$, in the fourth group – 38,54 ± 0,84 kg/cm$^2$. Among the examined students in the first group 64,5% were residents of large cities, 35,5% were residents of villages and small cities, the second group – 40,8% and 59,2%, the third group – 64,7% and 35,3%, the fourth - 60% and 40% respectively.

Most of the examined with the first and the second degree of obesity severity were female. In the third group, the percentage of women was 76,5%, in the 4th – 55,0%, the ratio between obesity prevalence in women and men in the 1st and 2nd groups was almost the same. Our obtaining data about the prevalence of obesity among people of different sexes is confirmed with the results of some epidemiological studies [4].

According to WHO, one of the criteria of metabolic syndrome is waist value 102 cm and higher for men and 88 cm for women. The excess of these measurements found in 76,5% of the patients of the third group and in 100% of the patients in the fourth group. The average value of waist and hip in patients of the first group was 75,67±1,45 cm and 98,33±1,42 cm, respectively, in persons of the 2nd group – 83,3 ± 1,85 cm and 103 ± 2,25 cm, the third one group – 91,82±1,92 cm and 114,58±1,3 cm, in the fourth group – 108,23±2,3 cm and 121,95±2,24 cm, respectively. One of the criteria for abdominal obesity is a waist-hip ratio, for men it is ≤1,0, for women ≦0,85[4]. According to anthropometric data only 6,2% patients in the second group had abdominal obesity, in the third group - 11,77% and 30% in the 4th group had abdominal obesity.

In 32,5% out of all examined patients we observed the presence of allergy mostly to medicines and food products. Prevalence of allergy was the highest in patients with the second degree of obesity up to 40% with prevalence of polyvalent allergy. The presence of allergic reactions in persons with normal body mass was about 22,5%, in persons with overweight (second group) an allergic anamnesis was complicated in 32,6% of patients, allergic reactions on food products were predominant in both groups. In the group with the 1st degree of obesity the presence of allergic reactions was found in 26.5%.

The majority of obese students had aggravated with heredity (Table 1) or there were numerous exogenous factors such as unhealthy lifestyle, lack of food culture in the family and disturbance in diet behavior as evidenced by the higher prevalence of obesity among the parents of patients with obesity. In the fourth group in 35% of patients both parents suffered from obesity and in 75% one of the parents was obese. In the third group, the prevalence of obesity in both parents was up to 29,41% and one of the parents was obese in 55,88%. The prevalence of obesity in parents of examined students in the first and the second group is shown in Table 1. about 15% of parents of patients with the first and the second degree of obesity had Type 2 diabetes mellitus, which is significantly higher than in patients with normal body weight and overweight. The prevalence of cardiovascular system diseases and atopic diseases in the parents of examined patients had no definite liaison with the presence of obesity in examined individuals.

*Table 1.*
*Presence of some diseases in parents of examined patients.*

| Diseases, % \ BMT | 18,5-24,99 $cm^2$/kg | 25-29,99 $cm^2$/kg | 30-34,99 $cm^2$/kg | 35-39,99 $cm^2$/kg |
|---|---|---|---|---|
| Obesity in one of parents | 23,53 | 48,98 | 55,88 | 75 |
| Obesity in both parents | 6,45 | 18,37 | 29,41 | 35 |
| Diabetes mellitus type II | 8,82 | 10,2 | 14,7 | 15 |
| Cardiovascular diseases | 14,7 | 18,37 | 17,64 | 10 |
| Atopic diseases | 12,9 | 6,12 | 0 | 5 |

The parameters of hand dynamometry were as follows: in the first group among men - 47,5 ± 2,2 H, among women - 25,63 ± 1,23 H, in the second group - men - 49,95 ± 2,29 H, women - 29,23 ± 2,1H, in the third group - men 52,5 ± 2,4H, women - 25,84 ± 1,7H, in the fourth group - men 47,7 ± 2,4H, women 26,22 ± 1,56H.

The presence of papular and pustular skin rash was detected in 32,26% of students with normal BMI, in 53,18% of persons with overweight, the highest prevalence of skin rash was in students with 1 degree of obesity - 70, 59%, and 65% persons with second degree of obesity had skin lesions.

The presence of bad habits (smoking) was noted in 29% of people with normal weight, their age of smoking was 2,4 ± 0,54 years, the number of cigarettes per day was 5,4 ± 0,8 sig/day. In the group with overweight the percentage of smokers was 32,65%, the duration of smoking – 2,3 ± 0,56 years, the intensity of smoking was 5,88 ± 0,76 sig/day. In the third group, the percentage of smokers was 29.41%, the duration of smoking 2,6 ± 0,74 years, the intensity – 5,4 ± 1,05 sig/day. In persons with the second degree of obesity the percentage of smokers was up to 30%, the duration of smoking was 2,3 ± 0,42 years, the frequency was 10,8 ± 2,1 sig/day. The initial age of regular smoking for vast majority was between 17 and 18 years, which is associated with stress factors like graduation at school and entering to university. There was nodifference between sex of smokers.

Regular checking-up at dentist (twice a year and more often) did 41,9% of patients in the first group, 57,1% in the second group, 41,2% in the third groups and 75% in the fourth group. 45,2% of patients of the first group, 28,6% in the second group made checking-up at dentist once a year, 47,1% in the third group and 25% in the fourth group visit. Not regularly (with a presence of acute pain) to the dentist went 13% patients with normal BMI, 14,3% in the second group, 11,7% patients of the third group.

The prevalence of caries in examined groups was on average 97,4%, which corresponds to the results of a number of researchers. The intensity of the carious process in the investigated groups is given in Table 2.

*Table 2.*
*Prevalence of dental caries in patients with diverse BMI value. DMFT (permanent teeth) – decayed, missing and filled teeth.*

| Group | Decay | Missing | Filled | Mean DMFT |
|---|---|---|---|---|
| IBM 18,5-24,99 $kg/cm^2$ | 2,66±0,37 | 0,1±0,05 | 3,12±0,3 | 5,88±0,67 |
| IBM 25 –29,99 $kg/cm^2$ | 3,35±0,4 | 0,24±0,11 | 3,24±0,49 | 6,84±0,58 |
| IBM 30 – 34,99 $kg/cm^2$ | 2,71±0,59 | 0,53±0,2 | 3,88±0,88 | 7,11±1,07 |
| IBM 35 – 39,99 $kg/cm^2$ | 4,25±0,85 | 0,1±0,06 | 1,6±0,42 | 5,96±0,84 |
| Total | 3,47±0,33 | 0,26±0,08 | 3,01±0,35 | 6,74±0,43 |
| | $p_{1-2}$ >0,05<br>$p_{1-3}$ >0,05<br>$p_{1-4}$ >0,05<br>$p_{2-3}$ >0,05<br>$p_{2-4}$ >0,05<br>$p_{3-4}$>0,05 | $p_{1-2}$>0,05<br>$p_{1-3}$ >0,05<br>$p_{1-4}$ >0,05<br>$p_{2-3}$ >0,05<br>$p_{2-4}$ >0,05<br>$p_{3-4}$<0,05 | $p_{1-2}$ >0,05<br>$p_{1-3}$ >0,05<br>$p_{1-4}$ <0,05<br>$p_{2-3}$ >0,05<br>$p_{2-4}$ <0,05<br>$p_{3-4}$<0,05 | $p_{1-2}$ >0,05<br>$p_{1-3}$ >0,05<br>$p_{1-4}$ >0,05<br>$p_{2-3}$ >0,05<br>$p_{2-4}$ >0,05<br>$p_{3-4}$>0,05 |

*Note: $p_{1-2}$ - the level of significance is obtained when comparing groups of patients with normal weight and overweight patients;*
*$p_{1-3}$ - the level of significance is obtained when comparing groups patients with normal weight and patients with the first degree obesity;*
*$p_{1-4}$ - the level of significance is obtained when comparing groups patients with normal weight and patients with the second degree obesity;*
*$p_{2-3}$ - the level of significance is obtained when comparing groups of overweight patients and patients with the first degree obesity;*
*$p_{2-4}$ - the level of significance is obtained when comparing groups of overweight patients and patients with the second degree obesity;*
*$p_{3-4}$ - the level of significance is obtained when comparing groups patients with the first degree obesity and patients with the second degree obesity.*

The prevalence of malocclusion was found in 48,1% patients in the first group, 43% in the second group, 53% in the third group and 60% in the fourth group.

Anomalies of soft tissues development in oral cavity (truncated frenulum of the tongue, small vestibulum of mouth) were found in 6,45% students in the first group,

12,24% of the second group, 11.76% of the third group and 10% in the fourth group.

The prevalence of periodontal diseases was up to 74%, chronic generalized catarrhal gingivitis and chronic localized catarrhal gingivitis were predominant forms of diseases. Chronic generalized periodontitis was diagnosed only in 4,55% of all cases (all - representatives of the third and the fourth group). The prevalence of periodontal disease with BMI is shown in Fig. 1. The frequency of pathological lesions in periodontal tissues increased with the rise of the BMI value, as well as the prevalence of generalized forms of periodontal diseases.

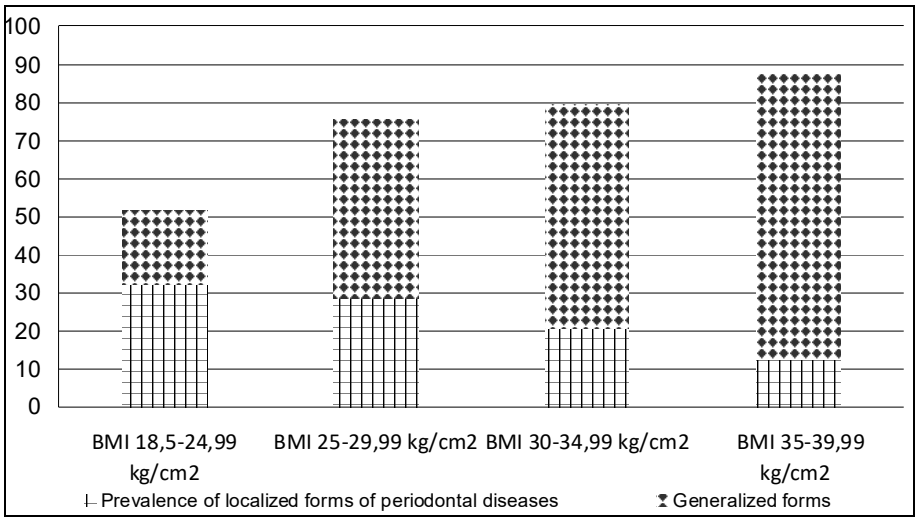


*Fig. 1. Prevalence of generalized and localized forms of periodontal diseases among youth with diverse BMI values, is shown in percentage.*

Index assessment of periodontal status is presented in Table 3.

*Table 3.*
*Values of oral hygiene indexes in patients with diverse BMI.*

| Index \ Group (BMI) | 18,5-24,99 kg/cm$^2$ (n=31) | 25-29,99 kg/cm$^2$ (n=49) | 30-34,99 kg/cm$^2$ (n=34) | 35-39,99 kg/cm$^2$ (n=40) |
|---|---|---|---|---|
| OHI | 0,54±0,11 | 1,34±0,07 | 1,42±0,12 | 1,4±0,11 |
| $p_{1-2}$ < 0,001 $p_{1-3}$ <0,001 $p_{1-4}$ <0,001 $p_{2-3}$ >0,05 $p_{2-4}$ >0,05 $p_{3-4}$>0,05 | | | | |
| API, % (Rateitchak) | 16,53±2,3 | 11,4±3,2 | 6,67±1,2 | 10,7±3,1 |
| $p_{1-2}$ > 0,05 $p_{1-3}$ <0,001 $p_{1-4}$ >0,001 $p_{2-3}$ >0,05 $p_{2-4}$ >0,05 $p_{3-4}$>0,05 | | | | |
| PMA, % | 6,1±1,5 | 10,3±1,3 | 14,7±1,64 | 16,8±1,5 |
| $p_{1-2}$ < 0,05 $p_{1-3}$ <0,001 $p_{1-4}$ <0,001 $p_{2-3}$ <0,05 $p_{2-4}$ <0,001 $p_{3-4}$>0,05 | | | | |
| CPI (Leus) | 1,38±0,15 | 1,52±0,11 | 1,7±0,1 | 1,66±0,15 |
| $p_{1-2}$ > 0,05 $p_{1-3}$ >0,05 $p_{1-4}$ >0,05 $p_{2-3}$ >0,05 $p_{2-4}$ >0,05 $p_{3-4}$>0,05 | | | | |
| PBI, % (Saxer, Muhlemann) | 6,4±0,9 | 15,6±2,4 | 15,7±2,3 | 22,9±2,6 |
| $p_{1-2}$ < 0,001 $p_{1-3}$ <0,001 $p_{1-4}$ <0,001 $p_{2-3}$ >0,05 $p_{2-4}$ <0,05 $p_{3-4}$>0,05 | | | | |
| WTC index | 3,04±0,32 | 3,29±0,35 | 3,65±0,66 | 3,5±0,47 |
| $p_{1-2}$ > 0,05 $p_{1-3}$ >0,05 $p_{1-4}$ >0,05 $p_{2-3}$ >0,05 $p_{2-4}$ >0,05 $p_{3-4}$>0,05 | | | | |

*Note: $p_{1-2}$ - the level of significance is obtained when comparing groups of patients with normal weight and overweight patients;*
*$p_{1-3}$ - the level of significance is obtained when comparing groups patients with normal weight and patients with the first degree obesity;*
*$p_{1-4}$ - the level of significance is obtained when comparing groups patients with normal weight and patients with the second degree obesity;*
*$p_{2-3}$ - the level of significance is obtained when comparing groups of overweight patients and patients with the first degree obesity;*
*$p_{2-4}$ - the level of significance is obtained when comparing groups of overweight patients and patients with the second degree obesity;*
*$p_{3-4}$ - the level of significance is obtained when comparing groups patients with the first degree obesity and patients with the second degree obesity.*

The prevalence of oral mucosa and lips diseases was 22,07 %. The prevalence of oral mucosa and lips diseases in the first group was 19,35%, in the second - 24,49%, in the third - 17,64%, in the 4th - 25,0%. Among patients of the first and the second groups, traumatic lesions of mucosa dominate in the 3-rd and 4-th group with a high frequently of chronic recurrent aphthous stomatitis up to - 40%, which is evidenced by the presence of undiagnosed enterocolitis according to [2].

## Conclusions

Thus, there is a significant prevalence of obesity among young females compared with male gender. The vast majority of all obese patients with BMI >30kg/cm$^2$ in 62,2% cases were residents of large cities. In our opinion the fact is caused by a particular lifestyle - hypodynamia, irregular diet and the presence of strong social risk factors for the development of emotional stress compared with residents of rural area. It has been determined that the majority of patients with BMI >30 kg/cm$^2$ have a heavy family heredity, in 66,2% cases one parent of

obese students had obesity and 32,43% patients have both parents with obesity that is significantly higher compared with persons with normal BMI and overweight. All patients had aggravated allergic history, the presence of allergic reactions was detected in 32,5% of all students, the highest prevalence was in individuals with BMI 35-40 kg/cm$^2$ - 40%. Papular and pustular skin rash was detected in the 3rd and the 4th group in 2 and 2.3 times frequently than in patients with normal BMI.

There is a high prevalence of periodontal diseases about 74% and carious lesions of teeth - 97.4% among young people despite the age and contingent of examined patients - medical students. This indicates the absence of effective primary and secondary prevention measures of dental pathology on the state, group and individual levels.

We did not find any link between the intensity of the carious process and the BMI. Although the highest value of the component of the DMFT-D index (non-filling carious cavities) was in the fourth group up to – 4,25 ± 0,85 which, in our opinion, indicates that there is no motivation in the dental health care in patients with the 2 degree of obesity. The prevalence of mucous lesions was the highest in persons with the 2 degree of obesity - 25%, with a domination of chronic recurrent aphthous stomatitis, which indicates the presence of digestive system organs disease in this group of individuals. The tongue hygiene indicator was clearly correlated with the value of BMI of patients. The tongue was the most coated in individuals with the second degree of obesity. Coating on the tongue is a sign of a presence of digestive system pathology in this group of individuals.

The presence of malocclusion was the highest in individuals of the fourth group, and was up to 60%. In the structure of morbidity, chronic generalized catarrhal gingivitis was prevalent – 44,8%; the share of chronic generalized periodontitis was 4,55%, all individuals of the 3th and the 4th groups. We found a direct correlation between the prevalence of periodontal disease and the BMI in patients, as well as the increase of percentage of generalized forms of periodontal diseases. The values of oral hygienic indexes were poor in all examined groups, but their values were slightly lower in patients with normal BMI than in those with overweight and obesity. The prevalence of inflammatory changes in the gums was higher in persons of the 3th and the 4th groups PMA =14,5±1,8 and 16,8±1,5%, respectively. All persons with overweight and obesity had a mild degree of lesions in periodontal tissues with a CPI> 1,5, compared with individuals with normal weight where the CPI value was 0,54±0,11. Inflammatory processes in the gums were the most intense in patients with the second degree of obesity, where the PBI was 22,9±2,8%, that is by 1,5 times higher than in the second and third group and by 4 times higher than in the first group.

In our opinion, the development of inflammatory changes in the gums in patients with the second degree of obesity is not caused by local factors. The key role in development of inflammation process is systemic - proinflammatory adipocytocins that are secreted into blood by adipose tissue in excess. We would like to sum up that systemic factors play a crucial role in the development of periodontal tissues alteration in obese individuals, because there is no significance between local factors in all group of individuals where dental deposits quantity was the same. Overweight leads to the disturbance of compensatory and adaptive processes in the whole body by forming chronic systemic mild inflammation in the body.

According to the results of the study, the presence of the first and the second degree of obesity should be considered as a risk factor triggering periodontal tissues diseases. For persons with BMI >30 kg/cm$^2$ with periodontal disease measures for the secondary prevention of inflammatory and inflammatory dystrophic periodontal diseases should be carried out and in persons without periodontal disease against the background of obesity, measures of primary prevention should be done.

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*Матеріал надійшов до редакції 22.01.2019 р.*

UDC 616.313:616-07:616.711-007.5

DOI https://doi.org/10.31718/mep.2019.23.1-2.05

# THE CORRESPONDENCE OF THE TONGUE CENTRAL LINE DEVIATION WITH THE PATHOLOGY OF SPINAL CURVATURE

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*Наукові дослідження вказують на неправильну поставу як фактор, що впливає на виникнення аномалії оклюзії та їх ускладнення. Поверхня язика може відображати порушення травлення, серцевої діяльності, стану нервової і ендокринної систем. У літературі є дані про те, що язик може допомогти в діагностиці багатьох захворювань, адже зовнішній вигляд язика в значній мірі пов'язаний з його анатомічними особливостями, а рецептори слизової оболонки мають широку кореляцію з центральною нервовою, серцево-судинною, ендокринною та, навіть, з опорно-руховою системами. Мета. Визначення відповідності розташуванням серединної лінії язика ступеню викривлення хребта на різних рівнях у дітей хворих на сколіоз. Матеріали і методи. Було обстежено 56 дітей (30 дівчат, 26 хлопців) у віці 11-14 років, з тривалістю захворювання опорно-рухового апарату 1-3 роки. Кожному пацієнту було проведено огляд власне порожнини рота, язика для визначення положення серединної лінії язика. Проаналізовано рентгенологічні знімки хребта хворих досліджуваних груп. Результати. У дітей, де давність захворювання становить 1 рік порушення серединної лінії язика виявлено в 25,02% випадків, у дітей з давністю захворювання 2 роки - в 65,52% випадків, при тривалості захворювання 3 роки 86,66% пацієнтів мають викривлення серединної лінії язика. Висновок. Проведене дослідження дозволяє рекомендувати лікарям - стоматологам під час проведення огляду порожнини рота звертати увагу не тільки на розмір, колір, стан сосочків язика, а й на положення середньої лінії, викривлення якої може інформувати про викривлення хребта в різних відділах. Необхідні подальші дослідження для виявлення механізмів впливу стану хребта і впливу скелетних м'язів на м'язи щелепно-лицевої ділянки, які беруть участь у формуванні оклюзії і функції органів порожнини рота.*

**Ключові слова:** осанка, сколіоз, викривлення серединної лінії язика, захворювання опорно-рухового апарату.

*Scientific researches suggest posture problems as a factor affecting the occurrence of occlusion anomalies as well as their complications. The surface of the tongue may reflect disorders in digestion, cardiac activity, nervous and endocrine systems. In the literature, there is data that the tongue can help in the diagnosis of many diseases, because its general appearance is in a significant relation with its anatomical features. Receptors of its mucous membrane are closely related to the central nervous, cardiovascular, endocrine and locomotor systems. Aim. The purpose of the study was to determine the correspondence between the location of the tongue median line and the degree of distortion of the vertebral column at different levels in children with scoliosis. Materials and methods. Totally 56 children (30 girls, 26 boys) aged 11-14 years were examined, with a duration of musculoskeletal disorders 1-3 years. For each participant, the oral cavity examination was performed, the position of the middle line of the tongue was determined. X-ray images of vertebral column of patients from investigated groups were analyzed. Results. In the group with 1 year duration of the disease 25.02% patients had distorted median line of the tongue, in the group with 2 years of duration of the disease - 65,52% participants had the deviation of the medial line of the tongue, in the group with 3 years of duration of the disease 86.66% of patients had the deviation of the median line of the tongue. Conclusions. This research allows us to recommend paying attention not only to the size, color, state of the papillae of the tongue, but also to the position of the middle line, whose distortion may indicate the deviation of the vertebral column in different portions. Further research is needed to detect the mechanisms of influence of the spine and the influence of skeletal muscles on muscles of the maxillofacial area, which are involved in the formation of occlusion and function of the organs of the oral cavity.*

**Key words:** posture, scoliosis, central line of the tongue deviation, musculoskeletal disorders

## Introduction

Until recent years, pathologies of organs of the oral cavity and the mucouse membrane were considered as local pathological processes associated only with poor hygienic condition of the oral cavity and the negative influence of pathogenic factors. However, modern researches and experimental models of the diseases allow us to establish that the oral cavity has a close anatomical and physiological relationship with various systems of the organism [1, 2, 3].

Scientific researches suggest posture problems as a factor affecting the occurrence of occlusion anomalies as well as their complications. In case of pathological occlusion (usually distal occlusion) there is an increased load on the constantly tensed occipital, scalenus, thoracic-clavicular-osseous muscles due to displacement of the center of gravity of the head augmentation. [4, 5]. Taking into consideration the high mobility of the cervical portion of the vertebral column some preconditions for disfunction of all systems and organs appear; especially for those located in the maxillo-facial area and exercising on the facial skull, cranial and spinal nerves, autonomic

*To cite this English version: Shundryk M.A., Marchenko I.Y., Tkachenko I.M., Vodoriz Y.Y., Boyko V.V. The correspondence of the tongue central line deviation with the pathology of spinal curvature. // The Medical and ecological problems. - 2019. - Vol 23, № 1-2. - P. 22-25.*

ganglia, vascular bundles, different muscles of the mentioned area, including mylohyoid and hyoglossus muscles, which cooperate with proper longitudinal and vertical muscles of the tongue.

Even ancient physicians used the tongue to decipher diagnostic symptoms of almost any human disease from its surface. Indeed, it can be stated that disorders of various organs are usually manifested with changes in the surface of the tongue even before their pathological symptoms appear. Even with the disappearance of symptoms, the tongue may indicate that the etiology of the disease remains unresolved and the full recovery did not occur.

Increasing sensitivity and discoloration of certain parts of the tongue reflect the disturbance of those organs associated with corresponding areas on its surface.

The localization of the plaque which covers the tongue indicates the accumulation of toxins in the stomach and small intestine (if the plaque is localized in the middle part of the tongue), in the large intestine (if the plaque is localized in the distal third of the tongue).

The surface of the tongue may reflect disorders in digestion, cardiac activity, nervous and endocrine systems.

In the scientific literature, there is data that the tongue can help in the diagnosis of many diseases, because its general appearance is in a significant relation with its anatomical features. Receptors of its mucous membrane are closely related with the central nervous, cardiovascular, endocrine and locomotor systems. [3, 5]. The line which goes in the middle of it, accurately reflects the state of the human backbone. The curvature of the line on the tip of the tongue indicates the curvature of the vertebral column in the cervical portion, the one in the middle signs about problems in the thoracic part, at the root deviation corresponds to the curvature of the vertebrae in the lumbar section.

For the human spine, three moderately pronounced physiological bends are natural. Cervical spine has a curve, convex forward, that begins at the axis at the apex of the odontoid process and ends at the middle of the second thoracic vertebra (lordosis). The thoracic curve, concave forward, begins at the middle of the second and ends at the middle of the twelfth thoracic vertebra (kyphosis). The lumbar curve begins at the middle of the last thoracic vertebra and ends at the sacrovertebral angle (lordosis).

Scoliosis is a pathological lateral curvature of the vertebral column with the obligatory rotation of the vertebral bodies. The characteristic feature of which is a progression of the disease, associated with age and growth of the child.

Unfortunately, nowadays disorders of the backbone are very common among children and adolescents; especially scoliosis, which is 5-6 times more likely to occur in girls than in boys and also affects the reproductive function which makes it is a socially significant disease [6, 7].

Whereas the spine deformation reflects can affect the median fissure of the tongue appearance, dentists as first-line doctors communicating with wide sections of the population during examinations in kindergartens, schools or in clinics can easily suspect the pathology and inform the patient or his/her parents about it, thereby to prevent further development of the disease.

Aim

The purpose of the study was to determine the correspondence between the location of the tongue median line and the degree of distortion of the vertebral column at different levels in children with scoliosis.

## Materials and methods

In order to achieve this goal, we performed our research on the basis of Poltava regional sanatorium for children with musculoskeletal disorders. Totally 56 children (30 girls, 26 boys) aged 11-14 years were examined, with duration of musculoskeletal disorders 1-3 years (Tab 1). The comparison of the location of the median line of the tongue (photos) with the position of the spine (X-ray) in different departments - cervical, thoracic, and lumbar, was also performed.

*Table 1*
*Dispersion of patients regarding to their age and duration of the disease*

| Age | N of patients | | Duration of the disease (years) | | |
|---|---|---|---|---|---|
| | n = 56 | % | 1 | 2 | 3 |
| 11 | 11 | 19.64 | 4 | 7 | 0 |
| 12 | 11 | 19.64 | 4 | 6 | 1 |
| 13 | 15 | 26.74 | 3 | 7 | 5 |
| 14 | 19 | 33.98 | 1 | 9 | 9 |

## Results and discussion

According to the obtained data, in can be stated that the largest number of patients were children with duration of the disease for 2 years – 29 patients (51.80%), for 1 year -12 patients (21.42%), 3 years - 15 patients (26.78%).

In the group with 1year duration of the disease, 25,02% patients had the disrupted median line of the tongue. In the group with 2 years of duration of the disease, 65,52% participants had the deviation of the median line of the tongue. In the group with 3 years of duration of the disease, 86.66% of patients had the deviation of the median line of the tongue (Tab. 2).

*Table 2*
*Dispersion of the patients with the central line of the tongue deviation*

| Duration of the disease (years) | N of patients | N of patients with the central line of the tongue deviation | |
|---|---|---|---|
| | | n | % |
| 1 | 12 | 3 | 25.02 |
| 2 | 29 | 19 | 65.52 |
| 3 | 15 | 13 | 86.66 |

The curvature of the spine in the cervical region corresponded to the bend of the median line of the tongue in the tip region, the bend of the spine in the thoracic region corresponded to the bend of the median line of the tongue in the middle part, the bend of the spine in the lumbar region corresponded to the bend of the medianl line of the tongue in the root area (Fig.1,2,3, 4).

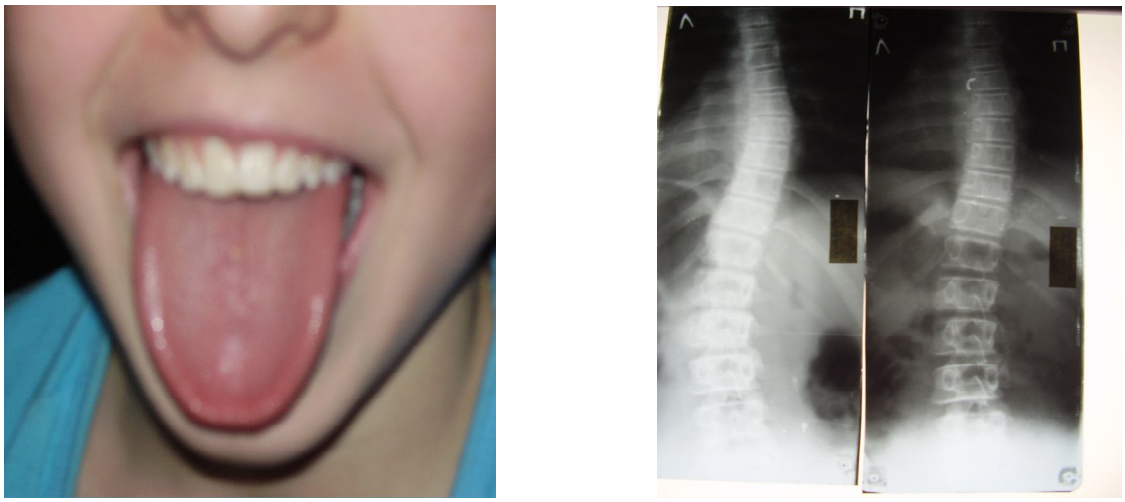

*Fig. 1. Patient N., 11 years. Deviation of the middle line of the tongue in the tip corresponds to the curvature of the vertebral column in the cervical region.*

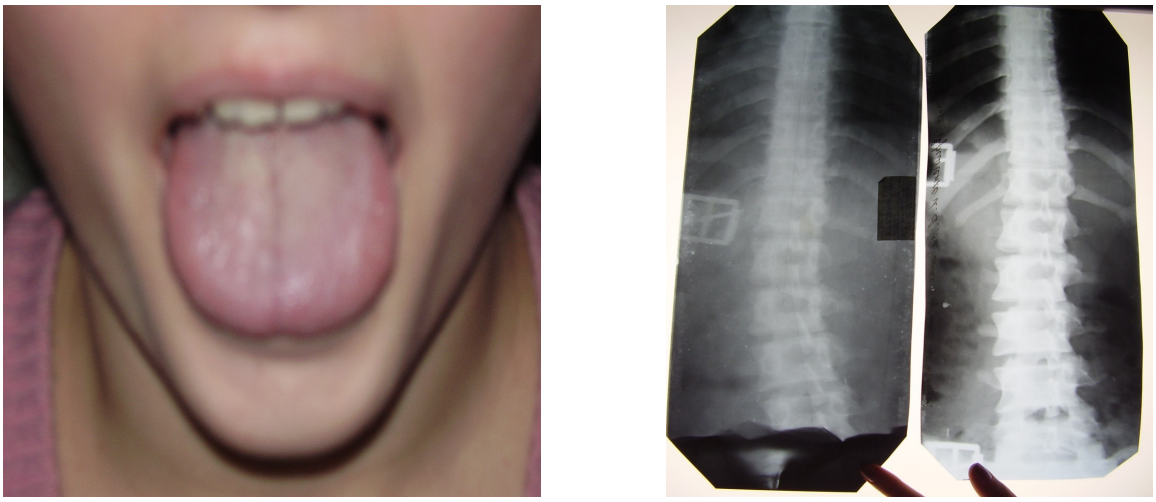

*Fig. 2. Patient M., 12 years. Distortion of the middle line of the tongue in the middle portion corresponds to the distortion of the spine in the thoracic region.*

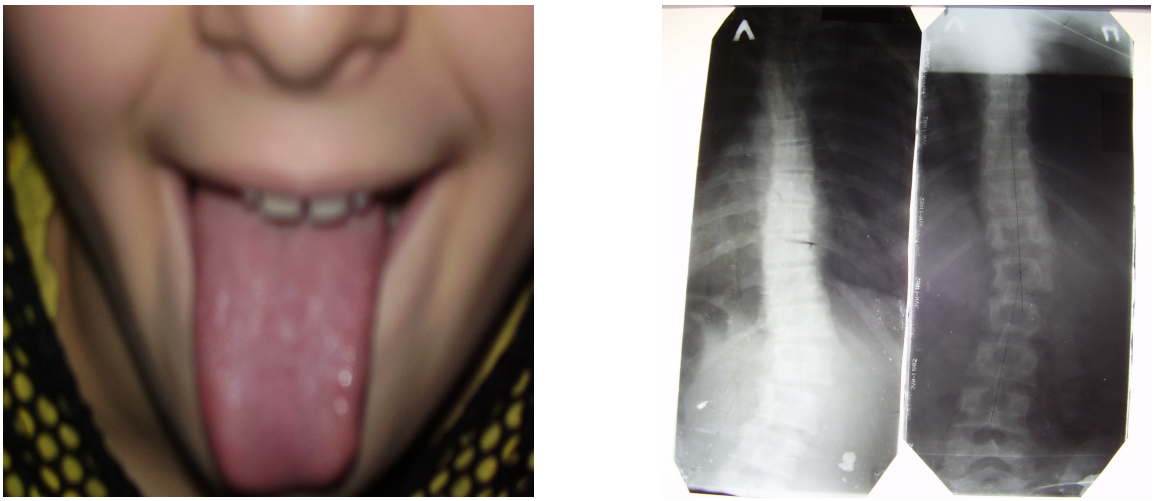

*Fig. 3. Patient K., 10 years. Distortion of the middle line of the tongue in its upper third area corresponds to the distortion of the spine in the lumbar region.*

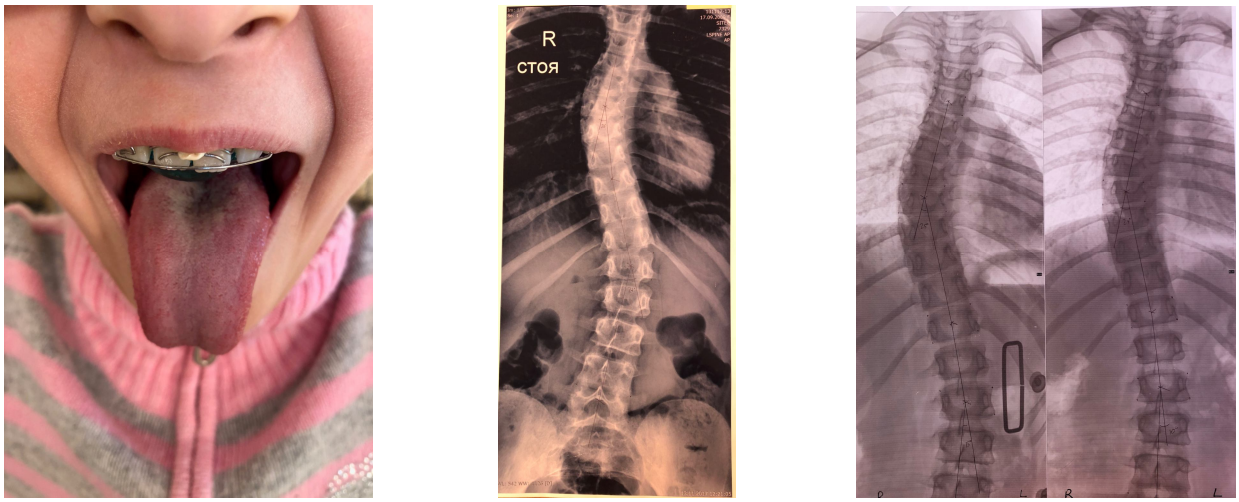


*Fig. 4. Patient Y., 11 years. S-shaped distortion of the middle line of the tongue corresponds to the diagnosis: idiopathic S-shaped right-side thoracic, left-sided lumbar scoliosis of the second degree.*

## Conclusions

Observation data give us the right to conclude that dentists can suspect the presence of spinal deformation without general examination and X-ray examination in patients with the disease duration period of at least 2-3 years during the preventive examination of children and adolescents.

This research allows us to recommend paying attention not only to the size, color, state of the papillae of the tongue, but also to the position of the middle line, whose distortion may indicate the deviation of the vertebral column in different portions.

This study suggests further elucidation of the mechanism of influence of the vertebral column condition and skeletal muscles influence on the muscles of the maxillofacial area, which are involved in the formation of occlusion and function of oral cavity organs.

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*Матеріал надійшов до редакції 05.02.2019 р.*

# ЕКСПЕРИМЕНТАЛЬНА МЕДИЦИНА


UDC: 615.282:582.28

DOI https://doi.org/10.31718/mep.2019.23.1-2.06

## SUSCEPTIBILITY OF PLANKTONIC AND FILM FORMS OF *CANDIDA GLABRATA* AND *CANDIDA ALBICANS* TO CATIONIC SURFACTANT ANTISEPTICS

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*Дослідити чутливість планктонних і плівкових форм C. albicans та C. glabrata до антисептиків на основі катіонних поверхнево-активних речовин (ПАР). Матеріали та методи дослідження. Об'єктом дослідження стали 20 клінічних штамів C. albicans та 15 C. glabrata, виділених від хворих хірургічних відділень. Чутливість планктонних форм досліджуваних штамів мікроорганізмів до антисептичних засобів вивчали за допомогою кількісного макрометоду подвійних серійних розведень у рідкому поживному середовищі Сабуро. Біоплівкоутворюючі властивості досліджуваних клінічних штамів визначали за допомогою спектрофотометричного методу за G.D. Christensen (MtP-test). Вплив антисептиків на плівкові форми Candida spp. визначали шляхом відтворення біоплівок за вищеописаною методикою з додаванням суббактеріостатичних концентрацій антисептиків. В дослідженні використовували антисептики на основі катіонних ПАР хлоргексидину біглюконат 0,05 (Хлоргексидин-КР, виробництва ПАТ «Хімфармзавод «Червона зірка», м. Харків, Україна (ХГ)) та декаметоксин 0,2 (Декасан, виробництва ТОВ «Юрія-Фарм», м. Київ, Україна (ДКМ)). Результати. За результатами досліджень встановлено нижчу чутливість штамів C. glabrata до ХГ, у порівнянні з чутливістю штамів C. albicans. Поряд з цим, активність ДКМ щодо досліджуваних представників Candida spp. достовірно не відрізнялась. Клінічні штами C. glabrata виявилися більш чутливими до ДКМ порівняно з їх чутливістю до ХГ. Штами C. albicans проявили середні плівкоутворюючі властивості, в той час як C. glabrata – високі. Досліджувані антисептики на основі катіонних ПАР володіли в однаковій мірі потужною активністю щодо плівкоутворення клінічних штамів Candida spp. Висновки. Антисептики на основі катіонних ПАР (ХГ та ДКМ) володіють протигрибковою активністю щодо планктонних і плівкових форм клінічних штамів C. albicans та C. glabrata.*

**Ключові слова:** *Candida,* катіонні поверхнево-активні речовини, чутливість, біоплівки

*The aim of the study was to investigate the sensitivity of planktonic and film forms of C. albicans and C. glabrata to cationic surfactant antiseptics. Materials and methods. The study was based on investigating 20 clinical strains of C. albicans and 15 C. glabrata isolated from surgical inpatientsю. The sensitivity of planktonic forms of investigated strains to antiseptic agents was quantitatively evaluated by two-fold serial dilutions (macrodilution) in Sabouraud liquid nutrient medium. Biofilm-forming properties of clinical strains C. albicans and C. glabrata were assessed by using the Christensen's spectrophotometric method (MtP-test "microtiter plate test"). The influence of the antiseptics on C. albicans and C. glabrata film forms was assessed by the reproduction of the biofilms according to the above-described procedure with adding antiseptics in sub-bacteriostatic concentrations and the subsequent spectrophotometric ODU assessment. In the study we used antiseptics based on cationic surfactants, chlorhexidine digluconate 0.05 (Chlorhexidine-KR, manufactured by PJSC "Khimfarmzavod "Chervona zirka"", Kharkiv, Ukraine (CHH)) and decamethoxin 0.2 (Decasan, produced by Yuria-Farm LLC ", Kyiv, Ukraine (DCM)). Results. According to the research results, lower sensitivity of C. glabrata strains to CHH was found, compared to the sensitivity of C. albicans strains. In addition, the activity of DCM in the investigated representatives of Candida spp. did not differ significantly. Clinical strains of C. glabrata were more susceptible to DCM compared to their susceptibility to CHH. C. albicans strains showed medium film-forming properties, while C. glabrata - high. The investigated cationic surfactant antiseptics possessed the same degree of activity on the film-forming properties of clinical strains of Candida spp. Conclusions. Cationic surfactant antiseptics (CHH and DCM) possess antifungal activity against planktonic and film forms of C. albicans and C. glabrata.*

**Key words:** *Candida,* cationic surfactant antiseptics, sensitivity, biofilm.

### Introduction

Yeast-like fungi that belong to the genus Candida are known to be an inseparable component of human microbiota. However, immune deficiency conditions or / and

*To cite this English version Faustova M.O., Loban' G.A., Nazarchuk O.A., Ananieva M.M. Susceptibility of planktonic and film forms of candida glabrata and candida albicans to cationic surfactant antiseptics. // The Medical and ecological problems. - 2019. - Vol 23, № 1-2. - P.26-29.*

impaired homeostasis can contribute to the development of candidiases [1].

According to the relevant literature, until the beginning of the 21st century yeast fungi caused about 3% of post-operative infectious complications [2]. Lately, however, *Candida spp*. has been reported to as a rapidly growing cause of infectious post-operative complications. Fungal infections have been found out to result in high mortality rate, which sometimes reaches the rates of deaths due to tuberculosis and malaria [3]. Fungi of the genus *Candida* rank the sixth place among the main nosocomial pathogens and the fourth place among the dominant causative agents of nosocomial bacteria. This is due to the decline in community immunity among the population, alterations in the biological properties of pathogens and the rapid development of microbial resistance to antimicrobial agents [2, 4]. In order to improve the situation it is of great importance to reconsider and update existing treatment standards.

In the past, *C. albicans* was known as the most prevalent species of *Candida spp.* throughout the world and described as a causative agent of about two thirds of all cases of invasive candidiasis [5]. However, today there are some changes within the spectrum of etiological factors of candidiasis infection that indicate a growing shift towards the so-called non-albicans species (*C. glabrata, C. krusei, C. tropicalis*) [5-7].

While *C. albicans, C. krusei, C. tropicalis* are genetically related, *C. glabrata* does not have a close phylogenetic relationship with other members of the genus. This determines the existence of significant differences in their morphology, pathogenicity factors, sensitivity to medications and thus the pathogenesis of mycoses caused by this causative agent [8]. This requires a comprehensive in-depth study of the biological properties of *Candida spp.* within the boundaries of the genus and the search for medicines that would have the same equal antifungal effect on different representatives of the genus.

The aim

of the study was to investigate the sensitivity of planktonic and film forms of *C. albicans* and *C. glabrata* to cationic surfactant antiseptics.

## Materials and methods

The study was based on investigating 20 clinical strains of *C. albicans* and 15 *C. glabrata* isolated from surgical inpatients staying at M. Sklifosovskiy Poltava Regional Clinical Hospital and M. Pyrogov Vinnytsia Regional Clinical Hospital. The research has been carried out according to Helsinki Declaration on the ethical principles for medical research involving human subjects and approved by the Bioethics Committee of M. Pyrogov Vinnytsia National Medical University and the Commission on Ethical Issues and Bioethics of Ukrainian Medical Stomatological Academy.

To investigate the microflora, the material was taken from the surface of the infected surgical wound, followed by cultivation on Sabouraud culture medium for 48 hours. The final identification was carried out in accordance with the standard methodology for morphological, tinctorial and biochemical properties and by applying automatic bacteriological analyzer Vitec - 2compact bioMérieux (France).

The sensitivity of planktonic forms of investigated strains to antiseptic agents was quantitatively evaluated by two-fold serial dilutions (macrodilution) in Sabouraud liquid nutrient medium in accordance with the Order of the Ministry of Public Health of Ukraine No. 167, April 5, 2007 "On Approval of Methodological Instructions on "Assessment of Sensitivity of Microorganisms to Antibacterial Drugs "[9]. For this purpose, a microbial suspension (inoculum) equivalent to 1.0 was prepared according to the McFarland standards, and diluted 100-fold in a saline, after that the microbial concentration obtained was 3x1010 CFU / $cm^3$. Then, inoculums prepared *ex tempore* and taken in a dose of 1.0 ml were applied into each test tube with a two-fold dilution of antiseptic solution and into one test tube containing 1.0 ml of nutrient broth ("negative" control). All test tubes with the exception of "negative" control were incubated in the standard atmosphere at 35 ° C for a day. The "negative" control test tube was kept at a temperature 4 ° C until the results were recorded.

Biofilm-forming properties of clinical strains *C. albicans* and *C. glabrata* were assessed by using the Christensen's spectrophotometric method (MtP-test "microtiter plate test"). Biofilms were modelled in Sabouraud liquid nutrient medium in wells of a sterile, flat-bottom 96-well polystyrol tablet (Corning, USA) and stained with 1% solution of crystalline violet. The properties of microorganisms to form a biofilm were measured by absorbance of the dye in units of optical density (ODU) using a spectrophotometer with a wavelength of 570 nm. The ability of microorganisms to form biofilms was evaluated as low (ODU <0.120), average (ODU = 0.121-0.239) and high (ODU > 0.240) [10].

The influence of the antiseptics on *C. albicans* and *C. glabrata* film forms was assessed by the reproduction of the biofilms according to the above-described procedure with adding antiseptics in sub-bacteriostatic concentrations and the subsequent spectrophotometric ODU assessment.

In the study we used antiseptics based on cationic surfactants, chlorhexidine digluconate 0.05 (Chlorhexidine-KR, manufactured by PJSC "Khimfarmzavod "Chervona zirka"", Kharkiv, Ukraine (CHH)) and decamethoxin 0.2 (Decasan, produced by Yuria-Farm LLC ", Kyiv, Ukraine (DCM)).

The statistical analysis of the obtained findings was performed by using the standard SPSS Statistics 23 and Microsoft Excel 2010 software packages. We calculated the arithmetic mean (M), the mean error of the arithmetic mean (± m), and the criterion of the reliability of the differences (p). The differences between the indicators studied were evaluated according to Student's t-test.

## Results and discussion

The obtained findings demonstrated that CHH and DCM were found to produce varying antifungal effect on investigated clinical strains of yeast fungi (Table 1). The minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFcC) of CHH for *C. glabrata* significantly exceeded the MIC and MFcC showed by DCM for *C. albicans* in 1.6 times ($p < 0.05$). This indicated a lower sensitivity of *C. glabrata* strains to CHH when compared with the *C. albicans* strain sensitivity. In addition, the activity of DCM against the investigated representatives of *Candida spp.* did not differ significantly ($p < 0.05$). However, it should be noted that MFcC demonstrated by DCM against clinical strains of *C. glabrata* was 1.7 times lower than MFcC of CHH against the relevant strain ($p < 0.05$). This pointed out a significantly higher sensitivity of *C. glabrata* to DCM than to CHH.

*Table 1*
*Susceptibility of Candida spp clinical strains to antiseptics, $10^{-3}$ mg/ml, M±m*

| Microorganism | N | Chlorhexidine digluconate | | Decamethoxin | |
|---|---|---|---|---|---|
| | | MIC | MFcC | MIC | MFcC |
| *C. albicans* | 20 | 3,02±1,48 | 4,39±2,18 | 3,51±1,75 | 5,55±3,38 |
| *C. glabrata* | 15 | 4,93±2,20* | 7,29±3,87** | 3,13±1,77 | 4,38±1,89§ |

***Note:*** ** - the reliability of the difference between the chlorhexidine digluconate MIC values relative to C. glabrata and the chlorhexidine digluconate MIC values relative to C. albicans, p <0,05;*
*** - the reliability of the difference between the chlorhexidine MFcC digluconate values relative to C. glabrata to the MFTSC values of chlorhexidine digluconate relative to C. albicans, p <0,05; §- the reliability of the difference between the decamethoxin MFcC values relative to C. glabrata and the chlorhexidine digluconate MFcC values relative to C. glabrata, p <0.05.*

The study showed the representatives of the genus *Candida* differed in their ability to biofilm formation (Fig. 1). The ability to absorb the dye by *C. glabrata* (0,251 ± 0,04 ODU) exceeded the *C. albicans* values (0,203 ± 0,05 ODU) in 1,2 times (p = 0,004), i.e. the clinical strains of *C. albicans* demonstrated average film-forming properties, while those of *C. glabrata* strains were higher.

It should be noted that the cationic surfactants antiseptics studied had a strong activity against the film formation by *Candida spp*. clinical strains. CHH contributed to a decrease in the optical density of the biofilms of the investigated fungi species in 1,1 times (p = 0,001), compared with their indices without the presence of the antiseptics. Similarly, there was a decrease in the film-forming properties by *C. albicans* and *C. glabrata* in the presence of DCM in a 1.1 time and 1.3 times respectively, compared with baseline (p = 0.001).

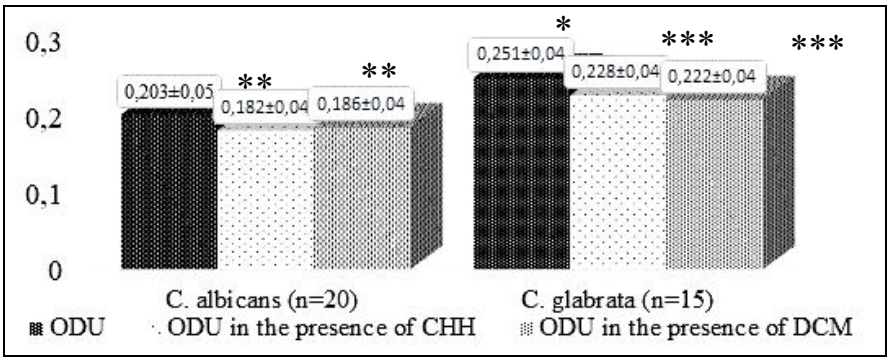


*Fig. 1. Characteristics of biofilm-forming properties of Candida spp. clinical strains in the presence of antiseptics*

*(* - the reliability of the differences between the ODU value of C. glabrata biofilms and the ODU value of C. albicans biofilms, p <0.001;*
*** - the reliability of the differences between the ODU value of C. albicans biofilms in the incidence of CHH and DCM and the ODU values demonstrated by biofilms without antiseptics, p <0.001;*
**** - the reliability of the difference of the ODU value of the C. glabrata biofilms in the presence of CHH and DCM to the index of ODU values demonstrated by biofilms without antiseptics , p <0.001).*

The biofilm formation by *C. albicans* is known to start by the microorganism adhesion to the artificial surface with the subsequent production of the extracellular matrix. Cells embedded into this matrix multiply, forming a biofilm. In turn, the ability of *C. glabrata* to form a similar extracellular matrix has not been experimentally proven. Accordingly, the powerful film-forming properties of the latter are genetically determined and associated with the presence of a number of surface adhesins involved in the process of biofilm formation. Recently, the genome of representatives of the *Candida* species is being actively studied, establishing a connection between their strains' properties of biofilm formation and the presence of surperficial Epa6, Epa7 and other proteins [11].

Cationic surfactant antiseptics used in the study have been found out to reliably suppress the biofilm-forming properties of both *C. albicans* clinical strains and *C. glabrata* strains, which are different from *C. albicans* by their biological properties. The mechanism of action of the drugs of this group consists in the ability to change the surface tension of the cell of the microorganism. It impacts the surface structure of the cell and stimulates the disruption of its osmotic balance, resulting in "osmotic shock" and the death of the microorganism [12].

The obtained results point out the high efficacy of cationic surfactant antiseptics on planktonic and film forms of *Candida spp*. clinical strains. The equally potent anti-fungal action of CHH and DCM against both *C. albicans* and *C. glabrata* strains makes the medicines studied appropriate in the treatment of candidiasis in view of the significant differences in their antimycotic effects to microbes within the genus.

## Conclusions

Cationic surfactants antiseptics (CHH and DCM) possess antifungal activity against clinical strains of *Candida spp*. Clinical strains of *C. albicans* demonstrate higher susceptibility to CHH, compared with the clinical strains of *C. glabrata*. In turn, DCM has a significantly higher anti-fungal effect against *C. glabrata*, compared with CHH. *C. albicans* clinical strains show average biofilm properties, while *C. glabrata* strains have high biofilm-forming properties. CHH and DCM significantly inhibit the ability to form biofilms by clinical strains of *Candida spp*.

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*Матеріал надійшов до редакції 31.01.2019 р.*

# ОГЛЯДИ ЛІТЕРАТУРИ


УДК:159.942.3-043.3-056.22:616-02

DOI https://doi.org/10.31718/mep.2019.23.1-2.07

## ALEXITHYMIA IN HEALTHY PEOPLE AND ITS ROLE IN DEVELOPMENT OF DIFFERENT DISORDERS

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*Знижену здатність або ускладненість усно висловити, назвати власні емоційні стани або почуття інших людей, прийнято називати алекситимією. В даний час проводяться дослідження, в яких з'ясовують, чи є алекситимія специфічною властивістю особистості або ж вона може призводити до психосоматичних захворювань, будучи їх предиктором. Деякі дослідники вважають, що алекситимія сама по собі не є захворюванням і являє собою ряд характеристик, властивих певним індивідам. Алекситимія чітко виражена в складі особистості пацієнтів із серцево-судинними захворюваннями і являє собою окремий фактор в цій структурі. В цілому дослідження показують, що пацієнти з алекситимією гіперчутливі як до внутрішніх соматичних неприємних відчуттів, так і до зовнішніх больових стимулів, проте описати відмінності різних видів болю у них не виходить. Алекситимію можна розглядати як один з преморбідних особистісних факторів, що знижують компенсаторні можливості психологічного захисту при люцидному алкоголізмі. Алекситимія виявлена у багатьох пацієнтів з наркоманіями. Замість того, щоб просто звільнитися від болісних, нестерпних або гнітючих почуттів, люди, які зловживають хімічними речовинами, можуть використовувати їх для управління афектами, особливо коли ці афекти виявляється важко вловити, розрізнити і дати їм назву. У той же час недостатньо вивчені відмінності в проявах алекситимії при алкогольних і наркотичних залежностях, в тому числі її вплив на розвиток залежної поведінки. Вивчення алекситимії є актуальним питанням сучасної психіатрії та наркології, оскільки дозволить застосовувати персоніфікований підхід до пацієнта та вдосконалити сучасні лікувально-реабілітаційні заходи.*

**Ключові слова:** алекситимія, афективні розлади, лікування психічних розладів, психосоматичн захворювання, ликувально-реабилитацийни заходи.

*The reduced ability or difficulty to express verbally, to name one's own emotional states or feelings of other people, is commonly called alexithymia. Currently, studies are being conducted in which they ascertain whether alexithymia is a specific property of a person or whether it can only predispose to psychosomatic diseases, being their predictor. Some researchers believe that alexithymia itself is not a disease and is a series of characteristics characteristic of certain individuals. Alexithymia is clearly expressed in the personality structure of patients with cardiovascular diseases and represents a separate factor in this structure. In general, studies show that patients with alexithymia are hypersensitive to both internal somatic unpleasant sensations and external pain stimuli, but they cannot describe the differences between different types of pain. Alexithymia can be considered as one of the premorbid personality factors that reduce the compensatory psychological defense capabilities in lucid alcoholism. Alexithymia is found in many drug addicts. Instead of simply getting rid of painful, intolerable, or overwhelming feelings, people who abuse chemicals can use them to control affects, especially when these affects are hard to grasp, distinguish, and give them a name. At the same time, the differences in the manifestations of alexithymia in alcohol and drug addiction, including its influence on the development of addictive behavior, are not well understood. The study of alexithymia is a topical issue of modern psychiatry and narcology, which allows us to apply a personified approach to the patient and to improve modern therapeutic and rehabilitation measures.*

**Keywords:** alexithymia, affective disorder, treatment of mental disorders, psychosomatic diseases, therapeutic and rehabilitation measures.

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The first problem with alexithymia was identified by P.

** To cite this English version: Skrypnikov A.M., Zhyvotovska L.V., Herasymenko L.O., Boiko D.I. Alexithymia in healthy people and its role in development of different disorders. // The Medical and ecological problems. - 2019. - Vol 23, № 1-2. - P. 30-33.*

Sifneos. Further research in this area has been and is being conducted very intensively, and today alexithymia is one of the significant problems of neurology and neuropsychology. Over the past two decades, alexithymia problems have been widely and extensively discussed in the medical literature. The main results of these studies are discussed in the present review [21].

Currently, studies are being conducted in order to ascertain whether alexithymia is a specific property of a person or whether can only predispose to psychosomatic diseases, being their predictor. Some researchers believe that alexithymia itself is not a disease and is a series of characteristics of certain individuals [22].

Currently, alexithymia is understood as a psychological characteristic of an individual, characterized by difficulty or complete inability of a person to accurately describe their own emotional experiences and understand the feelings of another person, difficulties in determining differences between feelings and bodily sensations, fixation on external events to the detriment of internal experiences. Aleximics are characterized by an endless description of physical sensations, often without connection with a particular disease. Neglect of one's internal mental and physical well-being is combined with a limited ability to regulate the internal state. Internal sensations are described as boredom, emptiness, fatigue, tension, arousal. The main defect in the affect area of alexithymics is the inability to differentiate emotions and sensations of unspecified physiological disorder. There is also a feature such as limited use of symbols, as evidenced by the poverty of fantasy and imagination. In the alexithymical personality, the listed features may appear equally or one of them may prevail [3].

Recent progress in neuroimaging has provided important information regarding emotional experience and the presence of alexithymia. Hence, from the point of view of social and affective processing, people with alexithymia show lower reactivity in areas of the brain associated with emotions. Many studies have reported reduced activation in the limbic areas (for example, the cingulate cortex, anterior insular part, the amygdala) and the prefrontal cortex, when people with alexithymia try to feel other people's feelings or outlive their own emotions, compared to those with no alexithymia [14].

The results of studies using functional MRI show that in patients with alexithymia, the activity of the left dorsolateral prefrontal cortex, the dorsal part of the bridge, the cerebellum is reduced, and in the pain matrix - the left caudal front part of the cingulate. They also showed increased activity in the zone of the Reil island on the right and in the lower frontal gyrus. During the test for recognition of emotions, there is a high activity in the anterior part of the cingulate gyrus in people with a low level of alexithymia and, on the contrary, low activity in this area in people with a high level of alexithymia. The relationship between alexithymia and the concentration of neurotransmitters in the zone of the insula and the anterior part of the cingulate gyrus is studied [15,16,19].

As for primitive emotional reactions, such as the response to pain, people with alexithymia have increased activity in areas of the brain that are involved in physical sensation. Moreover, in difficult social situations, people with alexithymia may not be able to use feelings to behave accordingly. Thus, a defect in the development of emotional nerve structures can lead to increased sensitivity to bodily sensations and unhealthy behavior, which, in turn, can be a possible mechanism that links alexithymia and psychosomatic disorders [17].

One can distinguish primary and secondary alexithymia. Primary alexithymia is a constitutional feature of the personality, and the secondary one is the result of disorders of different pathogenesis (organic lesions of the central nervous system, depression, some chronic diseases, emotional-volitional disorders, etc.) [18].

Primary alexithymia is caused by defects in the formation of the brain in the fetal development, the consequences of postpartum trauma, postpartum complications and has been observed since childhood. Secondary alexithymia develops as a result of psychotrauma, but more often on the background of lung neurological disorders or minimal cerebral dysfunction. It can also be a manifestation of latent depression, increased anxiety, post-traumatic stress disorder, and the like. The development of secondary alexithymia is also affected by improper parenting (denying, controversial, hyperopia, hypopoiesis), the situation in the family. Secondary alexithymia can be both a stable personality trait and a temporary reaction to depression or anxiety [10].

From the standpoint of modern psychiatry, it is considered that the primary allexity is practically not subject to correction. Psychotherapy of secondary alexithymia in most cases can be successful, but to consolidate the positive results is desirable and medication therapy. In relation to aleximics is conducted psychotherapy, aimed at reducing the level of anxiety and psycho-emotional stress. In addition, the use of special techniques that help the patient learn to recognize and describe the words of their emotions, overcome the lack of emotional reactions, develop intercostal relationships and the ability to include imagination [6].

To explain the alexithymia syndrome and its role in the formation of psychosomatic disorders, J. S. Nemiah identifies two models of "denial" and "deficit". The model of "denial" implies a global inhibition of affects. If the negation is regarded as a psychological defense, then theoretically it is possible to allow the reversibility of the protective process and the subsequent disappearance of the alexithymia syndrome and somatic symptoms. In this case, we can speak of "secondary alexithymia," that is, a condition that is found in some patients who have suffered severe injuries, and in patients with psychosomatic diseases who, after psychotherapy, acquire feelings and fantasies that were previously so amazingly absent. However, as clinical experience shows, in many patients with psychosomatic disorders, alexithymic manifestations are irreversible, despite the long, intensive and skillful deep psychotherapy. Such patients remain totally incapable of affect and fantasy. For them, the deficit model seems more acceptable. In accordance with this point of view, there is no inhibition, but the absence of functions and the underlying mental apparatus. In the deficit model, emphasis is placed on the disorder of instinct, which, bypassing mental processing due to its reduced ability to symbolize instinctive needs and fantasize, directly affects somatic with adverse consequences [4].

Specific interpersonal problems are associated with alexithymia, especially the problem of cold, distanced or aggressive behavior in society, typical of some patients with alexithymia, inability to build relationships with loved ones, inability to make friends, inability to empathy, emotional intimacy. Helicophobia (fear of being ridiculed by other people) and the level of emotional intelligence are

also closely associated with alexithymia, but researchers studying this connection come to the conclusion that these conditions are not combined [5].

At the same time, it was found that alexithymia is not associated the disrupted ability of cognitive empathy in the form of a change of position when performing cognitive activity, suggesting a communicative orientation on another person. When performing the cognitive task of defining concepts, taking into account the position of another person, subjects with high alexithymia scores showed the ability to take this position into account, not differing from the abilities of the subjects without signs of pronounced alexithymia. Thus, alexithymia is associated with impaired ability to decentration only during the processing of emotional stimuli. There is a lack of ability to understand the feelings of others, empathize with them and respond differentially to their emotional states. At the same time, such a parameter of alexithymia as “poverty of fantasy” is positively connected with the willingness to “get involved in the problems of others”, which can be interpreted as evidence of the desire to compensate for the poverty of one’s own inner life by intensive participation in the problems of other people [8].

We revealed a statistically greater severity of alexithymia in boys compared with girls. This psychological information is explained, firstly, by some gender characteristics (greater emotional sensitivity and psychological flexibility of girls, their tendency toward emotional identification, sincere sympathy, less hostility and greater emotional variation); secondly, with indisputable specificity of the education of boys in society. The existing stereotype of upbringing "male" behavior eliminates the orientation towards feelings and is aimed at forming a strong personal and social position. In this case, inflexible, schematized mechanisms of behavior are formed, which are accompanied by socially approved aggressiveness, which contributes to the accumulation of alexithymic manifestations.

The relationship between the severity of alexithymia and the level of education in the absence of health disorders has been proven. The highest rates of alexithymia are characteristic of subjects with a low level of education. This psychological information corresponds to the logic of the study. The presence of a high level of education is due to greater erudition, cognitive activity, an understanding of the versatility of the world, the ability to respond flexibly to its changes and readiness for tolerant interaction. Education also implies mastering the most constructive patterns of behavior and the ability to choose from them the most appropriate situation and the emotional state of the communication partner. The obtained psychological information indicates the need to pay special attention to persons with a low level of education in the aspect of accumulating alexithymic traits as needing special psychological support technologies [1].

As a result of modern research, it has also been established that alexithymia is clearly expressed in the personality structure of patients with cardiovascular diseases and is a separate factor in this structure. Alexithymia takes part in the formation of a specific life activity: excessive concentration on the performance of professional duties, the constant desire to achieve success and the need for recognition creates the basis for common emotional incompetence, inability to build constructive empathic relationships with the world. The rhythm of life of such patients leaves no time for emotional manifestations, and they are subject to repression. This displacement of emotions and serves as the basis for the formation of somatic disease, which is accompanied by ignoring the painful symptoms. The presence of the disease is perceived by the patient as an obstacle to the implementation of professional activities. Pronounced disorders lead to the need to distinguish between emotional and physiological manifestations, but this is hampered by pronounced alexithymia [2].

Studies in the field of algology are of particular relevance: the specificity of sensations in patients with pain syndromes of various etiologies has been revealed, the level of alexithymicity in those who complain of pain is studied. In general, studies show that patients with alexithymia are hypersensitive to both internal somatic unpleasant sensations and external pain stimuli, but they cannot describe the differences between different types of pain. Questionnaires for studying the nature and intensity of pain do not always help, because patients often do not understand the meanings of the words suggested in the questionnaires [20].

Based on the results of psychometric studies, we can conclude that alexithymia cannot be considered, as a number of authors consider, only as a predictor or a sign of personality psychosomatisation, since it is clearly detected in practically healthy individuals of different age groups. The relatively constant detection rate of alexithymia in individuals of different age groups, as well as its association with a number of indicators of the emotional-personal sphere, suggest that most likely alexithymia can be considered as one of the components of the integral personality characteristic. The evidence of the consistency of this assumption is the data on the age characteristics of the nature of the correlation of alexithymia with anxiety and depression, as well as its relationship with indicators such as intraversion, emotionally unstable and external personality types [11].

Alexithymia can be considered as one of the premorbid personality factors that reduce the compensatory psychological defense capabilities in lucid alcoholism (in the early stages of alcohol dependence) and complicate the structure of alcohol anosognosia in patients (in the later stages of the disease) due to its components, such as alcohol anozygnosis due to insufficient awareness and due to an alcoholic decline in personality. “Secondary alexithymia” is layered on the stable personality characteristics described by the “congenital deficit” model, which can be regarded as a risk factor for the development of alcohol dependence [12].

While conducting clinical work with victims of alcoholism, Henry Crystal noted that some of his patients are unable to differentiate their feelings (for example, they cannot distinguish anxiety from depression), are prone to somatisation of affect and cannot express their feelings with words. All these people, answering the question about their condition, cannot identify various emotional states in themselves, for example, tell them whether they are sick, tired or hungry, are experiencing sadness or anger. They may briefly display brilliant mental abilities, but ultimately show that their reactions are caused more by events and facts, but are almost unrelated to emotions [7].

The lack of control in the emotional sphere leads to the need to use external means that can affect the emotional state, including to the use of psychoactive substances. Alexithymia is found in many drug addicts. The high severity level of the alexithymic in the personality structure of psychoactive drug users contributes to a decrease in their adaptive capacities and is one of the psy-

chological factors for the development of the addiction syndrome. Unhealthy, repetitive aspects of substance use are attempts to work out painful affective states for which words, memories, or other symbolic representations do not exist. Instead of simply getting rid of painful, intolerable, or overwhelming feelings, people who abuse chemicals can use them to control affects, especially when these affects are hard to grasp, distinguish, and give them a name [9].

Individuals with alexithymic traits are often characterized by a specific imbalance, in which emotions are mainly manifested in the context of dependence, and not in real life. If the context of addiction is saturated with emotions, then reality, on the contrary, becomes emotionally impoverished, in real social relations the intensity of the emotional background decreases. This is especially noticeable when the attitude of others to the addict changes in a negative way. His detachment begins to perform a defensive reaction. Alexithymia is one of the risk factors for addiction among young people. The relationship between alexithymia and addictions is found as a result of the realization of various forms of addictive behavior, as well as the transformation of a person's personality. At the same time, the specificity of alexithymia in young people with non-chemical dependencies consists in total self-isolation of a person from the surrounding real world. The relationship of alexithymia with addictions among young people is manifested in the lack of stability of the emotional-volitional sphere, as well as a low level of self-control [13].

**Conclusions:** Despite the fact that more and more researchers are paying attention to the phenomenon of alexithymia, there are still quite a few studies in the literature on this phenomenon in different categories of patients. Thus, the study of manifestations of alexithymia is a topical issue of modern psychiatry, which will improve existing rehab programs, enhance quality of life and return patients to full social functioning.

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*Матеріал надійшов до редакції 25.01.2019 р.*

# ІНФОРМАЦІЯ ДЛЯ АВТОРІВ

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У якості невід'ємної частини процесу публікації, автори, рецензенти і редактори повинні повідомити про будь-які конфлікти інтересів і надати детальну інформацію, підписавши форму Заяви про Службову Етику та надіславши її на адресу редакції журналу. Автори рукописів зобов'язані поважати право приватності пацієнта. Перед початком дослідження пацієнт повинен заповнити і розписатися у формі Заяви про Інформовану Згоду. До статті додається акт експертної комісії про відсутність конфіденційної інформації та направлення установи. В направленні засвідчується, що жодна частина рукопису не була опублікована і не прийнята до друку іншими виданнями.

Статті публікуються українською, російською або англійською мовами. Авторський оригінал подається у двох примірниках, що складаються із основного тексту (стаття – 15 сторінок, огляд – 20 сторінок, коротке повідомлення – 7 сторінок); списку літератури (статті – до 20, огляди – до 50, короткі повідомлення – до 15 джерел); таблиць; ілюстрацій (не більше 4); назв рисунків; анотацій українською, російською та англійською мовами (орієнтовно 250 слів), що повинні містити обґрунтування мети, матеріалів та методів, результати дослідження.

На першій сторінці зазначаються: шифр УДК; прізвища авторів, ініціали, наукові ступені та звання; назва статті; установи, де працюють автори, місто; ключові слова – від 5 до 10 слів або словосполучень, що розкривають зміст статті. Назва статті російською, українською та англійською мовами повинна бути стислою і не перевищувати 120 символів. Підзаголовок є прийнятним. Текст статті повинен бути структурований наступним чином: вступ, мета, матеріал і методи, результати та висновок. На останній сторінці тексту власноручні підписи всіх авторів: прізвище, ім'я та по-батькові, поштова адреса, номери телефонів (службовий, домашній), за якими редакція буде контактувати із авторами. Подаючи статтю до редакції, автори тим самим підтверджують оригінальність роботи. Це означає, що авторські права або будь-які інші права власності третіх осіб не порушуються. Підписами автори засвідчують, що жодна частина рукопису не була опублікована і не прийнята до друку іншими виданнями. Текст друкується шрифтом не менше 2,8 мм на білому папері через два інтервали, на аркушах формату А4 (210×297 мм), поля з усіх боків по 20 мм. Крім двох роздрукованих копій, матеріал потрібно надати на компакт-диску, текст статті повинен бути у форматі Microsoft Word. Латинські терміни, іншомовні слова повинні бути надруковані курсивом. Тільки загальновживані скорочення можуть подаватися без пояснення. Скорочення у назві статті не є прийнятними. Всі величини приводяться в одиницях СІ, однак допустимими є й інші загальновживані позначення та одиниці вимірювання (I, min., h, C, Da, cal). Ілюстрації (рисунки, фотографії) повинні бути пронумеровані. Назви рисунків повинні бути надруковані на окремій сторінці. Малюнки повинні бути виконані з використанням інструментів, доступних у текстових редакторах або в Excel. Фотографії повинні бути високоякісними. Таблиці розміщуються на окремих аркушах, нумеруються послідовно, кожна сторінка супроводжується коротким заголовком. Рисунки є доповненням до тексту статті і не повинні повторювати інформації, поданої у рукописі. На звороті рисунків олівцем ставлять їхні порядкові номери, зазначають прізвище першого автора, скорочену назву статті. Список літератури оформлюється на окремих сторінках без скорочень. Автори подаються за абеткою, спочатку джерела кирилицею, потім латиницею. Посилання у тексті позначаються цифрами у [квадратних] дужках. Порядок оформлення списку літератури: для монографій – Прізвище, ініціали. Назва книги. Місце видання: видавництво, рік видання. Кількість сторінок; для журналів – Прізвище, ініціали. Назва статті. Назва журналу. Том, номер. Рік: сторінки, на яких вміщено статтю.

Одночасно, автори надають повний переклад тексту, підрисуночних підписів і табличних матеріалів англійською мовою. У переліку використаної літератури посилання, наведені кирилицею, транслітеруються із застосуванням програми "Trans 1.02" або подібних програм.

Усі рукописи журналу рецензовані незалежними експертами. Процедура рецензування включає перевірку статті протягом двох тижнів двома спеціалістами, призначеними редакційною радою. Рукопис із рецензією надсилається автору для внесення коректив перед остаточним поданням статті до редакції журналу.

Після публікації статті автори передають авторські права редакції журналу. Редакція залишає за собою право змінювати і виправляти рукопис, однак внесені корективи не повинні змінювати загального змісту та наукового значення статті.

Залучаючи до дослідження пацієнтів, автори несуть відповідальність за виконання етичних стандартів Гельсінкської декларації 1975 із поправками 2005 року. Рукопис повинен містити наступний пункт: "Ми заявляємо, що під час дослідження права пацієнтів були враховані у відповідності до вимог Гельсінкської конвенції". При виникненні сумнівів щодо відповідності рукопису до вимог Гельсінкської декларації, автори будуть зобов'язані відзвітуватися про сумнівні аспекти дослідження і обґрунтувати підстави свого підходу.

Якщо дослідження виконується без залучення лабораторних тварин, рукопис повинен містити наступний пункт: "Ми заявляємо, що ми не проводимо досліджень на тваринах". Дослідження, які проводяться на тваринах, повинні відбуватися у відповідності із встановленими інституціональними нормами використання лабораторних тварин. Науковці повинні керуватися принципами гуманного ставлення до тварин, що використовуються в дослідах. Необхідно подати наступну інформацію: вид тварин, генетичний статус: лінія (згідно правил стандартного позначення ліній лабораторних тварин); категорія лабораторних тварин або їх мікробіологічний статус; маса та вік тварин на початку експерименту; карантин або тривалість періоду акліматизації під час перевезення тварин на великі відстані; утримання тварин під час експерименту (параметри мікроклімату, температура, вологість, об'єм повітря, світловий режим, тип клітки, тип підстилки). Автори повинні підтвердити відповідність нормативам утримання та годування тварин (Європейська конвенція по захисту хребтових тварин, що використовуються з експериментальною або іншою метою. – Страсбург, 1986), наявність сертифікату якості, а також повідомити джерело набуття тварин. Необхідно описати всі процедури, які виконуються на тварині, дози препаратів, що вводилися, хірургічні втручання та інші дії, а також відмітити використання при цьому методів анестезії (див. інформацію про Права Людини і Тварини).

Ці правила поширюються на всі види рукописів, у тому числі статті, короткі доповіді, коментарі до клінічних випробувань. Рукописи, які не відповідають цим вимогам, будуть повернені авторам для корекції.

# INFORMATION FOR AUTHORS

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