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Glycemic-dependent Changes of Skin Autofluorescence Level in Children and Adolescents with Type 1 Diabetes Mellitus

Proskurina M.V.¹, Kiseleva N.G.¹, Salmin V.V.^{2,3,4}, Taranushenko T.E.¹

¹ Prof. V.F. Voino-Yasenetsky Krasnoyarsk State Medical University (KrasSMU)

1 Partizan Zheleznyak St., 660022 Krasnoyarsk, Russian Federation

² Moscow Institute of Physics and Technology (National Research University, MIPT)

1A Bldg. 1 Kerchenskaya St., 117303 Moscow, Russian Federation

³ Bauman Moscow State Technical University (National Research University, BMSTU)

5 Bldg. 1 2-ya Baumanskaya St., 105005 Moscow, Russian Federation

⁴ National Research Nuclear University Moscow Engineering Physics Institute (MEPHI)

31 Kashirskoye Rd., 115409 Moscow, Russian Federation

ABSTRACT

Aim. To study the effect of glycated hemoglobin level, average daily glycemia and its variability on UV-induced skin autofluorescence in children and adolescents with type 1 diabetes.

Materials and methods. The study included 47 children and adolescents with type 1 diabetes living in a restricted-access administrative and territorial unit. The autofluorescence spectra of the skin from the inner surface of the shoulder and nails of patients were recorded using an original compact spectrofluorometer based on STS-VIS OCEAN OPTICS © USA microspectrometer with UVA excitation. The statistical analysis was performed using Statsoft Statistica 12.0 software. The fluorescence spectra were normalized to the average value of the UV LED signal and the moving average smoothed using a 10 nm window. Then, the renormalization of spectra was carried out, minimizing their spread from the average sample spectrum.

Results. The study revealed the most changeable regions of UV-induced skin autofluorescence spectrum with variations in the level of glycated hemoglobin, average daily glycemia, and glycemic variability.

Conclusion. The study confirms the prospects of using skin autofluorescence measurements as a non-invasive tool for assessing the state of carbohydrate metabolism.

Keywords: skin autofluorescence, type 1 diabetes mellitus, children, adolescents, glycated hemoglobin, average daily glycemia, glycemic variability

Conflict of interest. The authors declare the absence of obvious or potential conflicts of interest related to the publication of this article.

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✉ Proskurina Margarita V., prmargov@rambler.ru

Изменение уровня аутофлуоресценции кожи детей и подростков с сахарным диабетом 1-го типа в зависимости от гликемических показателей

Проскурина М.В.¹, Киселёва Н.Г.¹, Салмин В.В.^{2,3,4}, Таранушенко Т.Е.¹

¹ Красноярский государственный медицинский университет (КрасГМУ) им. проф. В.Ф. Войно-Ясенецкого Россия, 660022, г. Красноярск, ул. Партизана Железняка, 1

² Московский физико-технический институт (национальный исследовательский университет) (МФТИ) Россия, 117303, г. Москва, ул. Керченская, 1а, корп. 1

³ Московский государственный технический университет им. Н.Э. Баумана (национальный исследовательский университет) (МГТУ) Россия, 105005, г. Москва, ул. 2-я Бауманская, 5, стр. 1

⁴ Национальный исследовательский ядерный университет «Московский инженерно-физический институт» (НИЯУ МИФИ) Россия, 115409, г. Москва, Каширское шоссе, 31

РЕЗЮМЕ

Цель: исследовать влияние уровня гликированного гемоглобина, среднесуточной гликемии и ее вариабельности на УФ-индуцированную аутофлуоресценцию кожи у детей и подростков, страдающих сахарным диабетом 1-го типа.

Материалы и методы. В исследование включены 47 детей и подростков с сахарным диабетом 1-го типа, проживающих на территории закрытого административно-территориального образования. Проведена регистрация спектров аутофлуоресценции кожи с внутренней поверхности плеча и ногтя пациентов с помощью оригинального компактного спектрофлуориметра на базе микроспектрометра STS-VIS OCEAN OPTICS © USA с UVA-возбуждением. Статистический анализ проводился с помощью программного обеспечения Statsoft Statistica 12.0. При выполнении анализа выполнялась нормировка спектров флуоресценции на среднее значение сигнала УФ светодиода и сглаживание методом скользящего среднего с окном 10 нм. Затем проводилась перенормировка спектров, минимизирующая разброс спектров от среднего спектра по выборке.

Результаты. В ходе исследования выявлены наиболее изменчивые области спектра УФ-индуцированной аутофлуоресценции кожи при вариации уровня гликированного гемоглобина, среднесуточной гликемии и вариабельности гликемии.

Заключение. Исследование подтверждает перспективность использования измерения аутофлуоресценции кожи в качестве неинвазивного инструмента оценки состояния углеводного обмена.

Ключевые слова: аутофлуоресценция кожи, сахарный диабет 1-го типа, дети, подростки, гликированный гемоглобин, среднесуточная гликемия, вариабельность гликемии

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено этическим комитетом ФГБОУ ВО КрасГМУ им. проф. В.Ф. Войно-Ясенецкого Минздрава России (протокол № 114 от 05.10.2022).

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INTRODUCTION

According to the definition of clinical guidelines, type 1 diabetes mellitus (T1DM) is a disease that occurs as a result of autoimmune destruction of insulin-producing β -cells of the pancreas, with subsequent development of absolute insulin deficiency. Studying this disease is relevant due to the early development of irreversible vascular complications and disability. Considering the severity of manifestation and the lability of the disease course in children and adolescents, early diagnosis and dynamic monitoring of pathology development are of no less importance.

The glycated hemoglobin (HbA1c) indicator has been recommended by the World Health Organization (WHO) since 2011 [1] and has been used for more than 25 years as a diagnostic criterion for carbohydrate metabolism disorders [2].

HbA1c shows the average blood sugar level over the past 90 days, so new methods are required to understand changes in the glycemic profile better.

With the introduction of continuous glucose monitoring (CGM) methods into clinical practice by diabetologists, the term glycemic variability appeared, which shows fluctuations in the average blood sugar level and is considered an independent predictor of diabetes complications due to the impact on target organs through oxidative stress, glycation, low-grade chronic inflammation, endothelial dysfunction, platelet activation, impaired angiogenesis, and renal fibrosis [3].

Modern CGM systems include a sensor that measures glucose levels in the interstitial fluid at intervals of 1 to 5 min, collects, and transmits them to the third component (receiver) in real time. The use of this technology provides information on glycemia at the time of the study, dynamics in glucose levels, its current direction, and the rate of change, which facilitates timely decision-making on glycemic correction [4]. To simplify the interpretation of the large amount of glycemic data obtained through CGM, the following percentage values were identified:

1. Average glucose level.

2. Glucose Management Index (GMI) is a calculated score that was developed based on the observed differences between average CGM glucose levels and laboratory-measured HbA1c. GMI is calculated using a formula that was developed and validated based on a regression line of a graph with glucose concentration on the x-axis and simultaneous HbA1c measurement

on the y-axis: $GMI (\%) = 3.31 + 0.02392 \times (\text{average glucose, mg/dL})$.

3. TIR is time in range which in this case is 70–180 mg/dL (within normal limits): target > 70%. TBR is time below range < 70 mg/dL (level 1 hypoglycemia): target < 4%. Time below range < 54 mg/dL (level 2 hypoglycemia): target < 1%.

4. TAR is the time above range > 180 mg/dL: target < 25%.

5. CV is coefficient of variation for glucose levels calculated as (standard deviation of glucose / average glucose value) $\times 100$ and includes duration, frequency, and amplitude of shifts in blood glucose levels between low and high levels, target $\leq 36\%$ [5, 6].

Despite the undeniable advantages of existing methods of glycemic control, they are still invasive, which leads to low compliance and insufficient glycemic control in patients.

In this regard, the search for non-invasive diagnostic methods of the listed parameters of carbohydrate metabolism is undoubtedly an urgent task. Therefore, methods based on optical spectroscopy of the patient's skin are of great interest. In recent years, many studies have been published on this topic, confirming the feasibility of the method [7, 8]. However, in each case this approach solves only one diagnostic problem. There are currently no ideas for developing a multi-task method for non-invasive diagnostics of the biochemical parameters of carbohydrate metabolism in patients with diabetes mellitus.

The aim of our work was to study UV-induced skin autofluorescence spectra in children and adolescents with type 1 diabetes mellitus and to assess the correlations of these spectra with glycated hemoglobin, average daily glycemia, and variability.

MATERIALS AND METHODS

The study was conducted at Clinical Hospital No. 51, a branch of the Federal Siberian Research Clinical Center Clinical Hospital No. 42. The skin autofluorescence test was conducted in 47 patients with T1DM. The group of children included 29 individuals (61.7%), and the group of adolescents included 18 individuals (38.3%). The majority of the study group were boys – 57.4%. The average duration of the disease in patients at the time of the examination was 4.47 years, minimum and maximum levels of HbA1c were 6.0 and 18.7%, respectively.

All children were on constant insulin replacement therapy from the moment when the disease was detected: 10 patients (21.2%) used continuous

subcutaneous insulin infusion (CSII) and 37 individuals (78.7%) used a syringe pen. All patients observed during the study underwent continuous glucose monitoring (CGM), with a predominance of Libre flash monitoring. Patients with T1DM and diseases affecting the accuracy of HbA1c were excluded from the study. During the study, the patients were divided into groups that are traditionally accepted in pediatric practice. Depending on the level of glycated hemoglobin: group 1 with glycated hemoglobin $\leq 7.0\%$ ($n = 2$), group 2 with glycated hemoglobin of $7.1\text{--}10\%$ ($n = 18$), and group 3 with its level $\geq 10.1\%$ ($n = 27$). Considering the small number, group 1 was combined with group 2. According to the average daily glycemia, we identified the following groups of patients: 1. ≤ 10 mmol/l; ($n = 23$); 2. ≥ 10 mmol/l; ($n = 24$). Based on the coefficient of variability, two subgroups of patients were identified: 1. $\leq 36\%$ ($n = 16$); 2. $\geq 36\%$ ($n = 41$).

Autofluorescence spectra were collected from the inner surface of the shoulder for 30 seconds using an original compact spectrofluorometer based on the STS-VIS OCEAN OPTICS © USA microspectrometer with UVA excitation generated by a light-emitting diode (375 nm) [9].

The skin fluorescence spectra obtained using the device comprise two wide contours. The first contour in the range of 400–700 nm represents, in fact, skin autofluorescence, and the second contour in the range of 700–820 nm shows the spectrum of the UV light-emitting diode excitation at 375 nm, in the second diffraction order of the diffraction grating (Fig. 1).

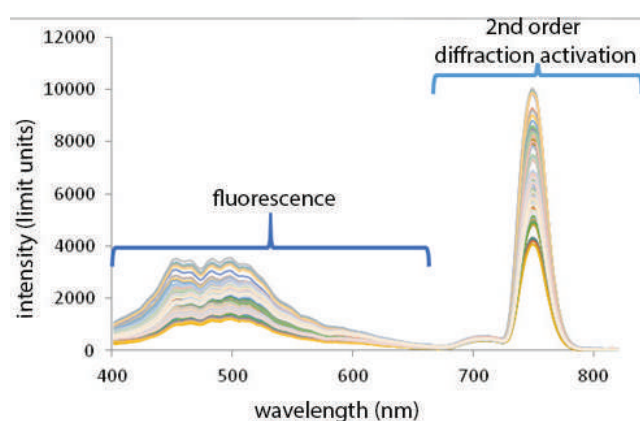


Fig. 1. Skin fluorescence spectra of patients with diabetes mellitus obtained directly using a spectrofluorometer with LED UV excitation (375 nm).

For further analysis, the fluorescence spectra were normalized to the average value of the UV LED signal and leveled using the moving average method with a

10 nm window. This spectra normalization method is further referred to as D-normalization (Fig. 2, a).

Additional normalization was used to compare the shape of the spectra. For this purpose, the average spectrum was calculated for the entire group of patients $F(\lambda)$ and for each spectrum $F_i(\lambda)$, the linear regression coefficients a_i, b_i were calculated using the least squares method so that after subsequent renormalization, these spectra were as close as possible to the average. Then, the spectra were renormalized by taking into account the coefficients obtained:

$$f_i(\lambda) = \frac{F_i(\lambda) - b_i}{a_i}$$

The result of applying additional normalization is a decrease in the standard deviation (Fig. 2, b). Such normalization is called I-normalization.

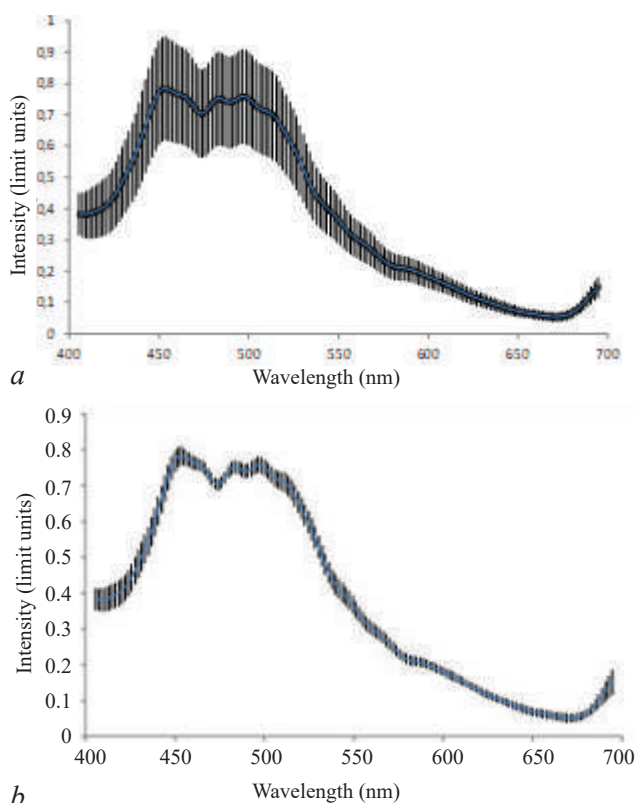


Fig. 2. Skin autofluorescence spectra of a group of patients with T1DM using different normalization methods. a – D; b – I

Statistical analysis was performed using the Statsoft Statistica 12.0 software package and Microsoft Excel. The spectra were processed using normalization and leveling algorithms. Data analysis was performed using descriptive and nonparametric statistics. The Mann–Whitney test (data were presented as a spectrum of Z-evaluation) was used for the paired comparison

of the fluorescence spectra. The main differences in the points of the spectrum are presented by the median value of fluorescence intensity and interquartile range $Me [Q_1; Q_3]$.

RESULTS

The results of using D- and I-normalization with subsequent comparison of autofluorescence spectra in groups of patients with T1DM depending on the level of glycated hemoglobin are presented in the graph (Fig. 3). Significant differences are observed in both methods of spectrum normalization. However, I-normalization led to more significant differences in groups 1 and 2. The greatest difference in the spectrum was observed in the region of the Soret band of hemoglobin at 433 nm ($p < 10^{-4}$) and the region of the NADH peak at 487 ($p < 0.005$) isosbestic point of the alpha band of oxy and deoxyhemoglobin at 592 nm ($p < 0.001$).

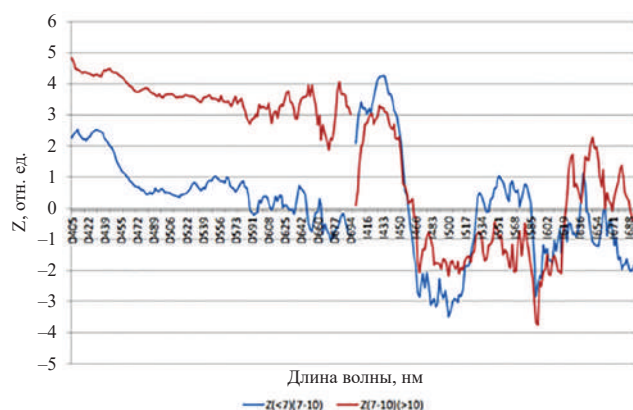


Fig. 3. Spectra of pairwise Z-scores based on the level of glycated hemoglobin

Table 1

Significant Differences in Fluorescence Intensity at Specified Wavelengths According to Mann-Whitney Test for Different Levels of Glycated Hemoglobin			
Wave-length	Comparison group, $Me [Q_1; Q_3]$		p (based on Mann-Whitney test)
	HbA1c < 7,0	HbA1c = 7,1–10,0	
I433	0.540 [0.512; 0.551]	0.514 [0.498; 0.531]	$<10^{-4}$
I487	0.740 [0.730; 0.750]	0.748 [0.739; 0.760]	0.002
I592	0.197 [0.191; 0.204]	0.202 [0.194; 0.206]	0.014
Wave-length	Comparison group, $Me [Q_1; Q_3]$		p (based on Mann-Whitney test)
	HbA1c = 7.1–10.0	HbA1c > 10.1	
I433	0.514 [0.498; 0.531]	0.534 [0.518; 0.557]	0.001
I592	0.202 [0.194; 0.206]	0.194 [0.186; 0.199]	$<10^{-3}$

Note. Differences were considered statistically significant at $p < 0.05$ (here and in Tables 2, 3).

The graph in Fig. 4 shows D-normalization, indicating significant differences in autofluorescence spectra at different values of average daily glycemia.



Fig. 4. Spectra of Z-evaluation when comparing the average daily glycemia with different methods of normalization

Table 2

Significant Differences in Fluorescence Intensity at Specified Wavelengths According to Mann-Whitney Test for Average Daily Glycemia			
Wave-length	Average daily glycemia, $Me [Q_1; Q_3]$		p (based on Mann-Whitney test)
	<10 mmol/L	≥ 10 mmol/L	
D470	0.771 [0.656; 0.826]	0.701 [0.618; 0.764]	0.004
D652	0.073 [0.064; 0.081]	0.066 [0.058; 0.074]	0.002

Significant regions of the spectrum that distinguish groups of glycemic variability are present in both normalization methods. The graph of the spectrum of Z-scores for comparing glycemic variability is shown in Fig. 5. As shown in the graph, a finer structure of the spectrum when using I-normalization allows analysis of changes in individual metabolites at different levels of glycemic variability. Thus, the most significant sections of the spectrum are represented by the Soret band of hemoglobin at 427 nm ($p < 0.005$), the peak of NADH fluorescence at 485 nm ($p < 0.005$), the peaks of β and α bands of oxyhemoglobin at 539 nm ($p < 0.01$) and 581 nm ($p < 0.001$), respectively, as well as the region of porphyrin fluorescence at 660 nm ($p < 0.01$).

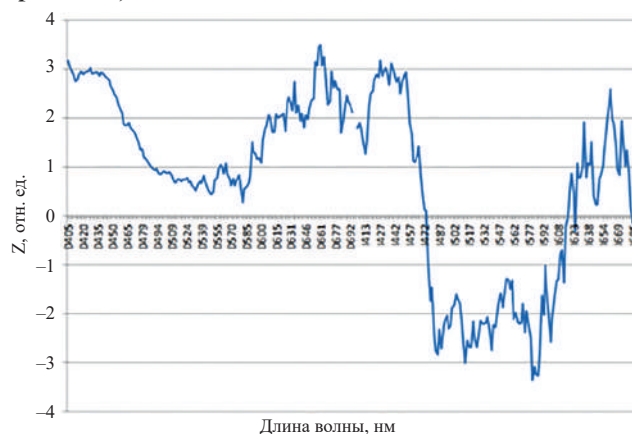


Fig. 5. Spectra of Z-scores for comparing the glycemic variability

Table 3

Significant Differences in Fluorescence Intensity at Specified Wavelengths According to Mann-Whitney Test for Glycemic Variability			
Wave-length	Glycemic variability, $Me [Q_1; Q_3]$		p (based on Mann-Whitney test)
	<36%	\Rightarrow 36%	
I427	0.481 [0.468; 0.494]	0.460 [0.441; 0.481]	0.001
I485	0.748 [0.730; 0.750]	0.750 [0.742; 0.760]	0.004
I539	0.417 [0.397; 0.431]	0.430 [0.415; 0.440]	0.006
I581	0.203 [0.196; 0.217]	0.216 [0.210; 0.223]	$<10^{-3}$
I661	0.063 [0.061; 0.069]	0.060 [0.053; 0.066]	0.01

DISCUSSION

Although glycated hemoglobin is spectrally indistinguishable from other hemoglobin derivatives, its presence in the systemic bloodstream obviously causes hypoxic changes in peripheral tissues, which can be recorded using the spectrofluorometric method. The majority of studies devoted to the relationship between autofluorescence level and HbA1c in patients with diabetes mellitus indicate a correlation between these indicators, both in children and adults [10–12].

According to the results of the study, the overall reflection level (scattering) of UV radiation from the skin was the most significant parameter, depending on average daily glycemia.

As the study shows, an increase in average daily glycation decreases fluorescence in the entire spectral range in relation to the reflected excitation radiation. This may be due to an increase in the reflection coefficient and not a simultaneous change in all metabolites. An increase in the reflection coefficient, in turn, is associated with a change in the refractive index of blood plasma with an increase in the concentration of glucose in it. This finding is consistent to a certain extent with data on changes in the refractive index of the skin with an increase in glycemia [13, 14].

Moreover, when assessing the relationship between autofluorescence spectra and glycemia variability indices, it was found that a finer spectrum structure at I-normalization apparently allows analysis of changes in individual metabolites at different levels of glycemia variability. The results showed a statistical relationship between skin autofluorescence spectra and glycemia variability. Furthermore, a comparison of autofluorescence spectra revealed not only hypoxic shifts in the case of high variability, which is expected in severe diabetes mellitus, but also an increase in the pool of porphyrins.

In 1949, Sterling et al. first reported an association between the development of diabetes mellitus and porphyria. In the studies conducted, marked increase in serum glucose and insulin levels were observed in patients with porphyria.

However, despite numerous studies, the exact mechanism by which patients with porphyria, especially asymptomatic patients, experience increased insulinemia remains unknown [15]. In the present study, the cause of the increase in porphyrins can be presumably associated with a decrease in the insulin response during adolescence, when hormonal changes are observed, mainly due to the level of growth hormone and sex hormones [16].

CONCLUSION

When changing laboratory parameters of glycated hemoglobin level, average daily glycemia, and glycemic variability, there appear significant changes in the spectrum of UV-induced fluorescence of the skin in children with type 1 diabetes mellitus.

It was revealed that significant differences in the skin autofluorescence spectra under UV excitation were detected both in the overall signal level and at wavelengths coinciding with the absorption peaks of hemoglobin in the Soret band region, alpha and beta bands, isosbestic points of oxy and deoxy hemoglobin, the fluorescence peak of NADH, and porphyrins. In this regard, the discovered relationship of skin autofluorescence in the region of the porphyrin peak at 660 nm with the degree of glycemic variability in children with T1DM is poorly understood.

The results obtained in the study enable to conclude that it is possible to create a non-invasive method for monitoring various metabolic changes in diabetes mellitus based on UV-induced autofluorescence spectroscopy of the skin, which simultaneously solves the problems of such diagnostic methods as determining the level of glycated hemoglobin, average daily glycemia, and glycemia variability. This possibility can be realized through metabolic connections of the indicated clinical indicators with endogenous chromophores and fluorophores of the skin.

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Author contribution

Proskurina M.V. – conception and design (collection of clinical and laboratory data, measurement of skin autofluorescence level, database creation), justification of the manuscript or critical revision for important intellectual content. Kiseleva N.G. – conception and design. Taranushenko T.E. – conception and design, final approval of the manuscript for publication. Salmin V.V. – conception and design, analysis and interpretation of data, final approval of the manuscript for publication.

Author information

Proskurina Margarita V. – Postgraduate Student, Department of Pediatrics IPE, Prof. V.F. Voino-Yasenetsky Krasnoyarsk State Medical University, Krasnoyarsk, pmargov@rambler.ru, <http://orcid.org/0000-0002-7360-6121>

Kiseleva Natalya G. – Cand. Sc. (Medicine), Associate Professor, Department of Pediatrics IPE, Prof. V.F. Voino-Yasenetsky Krasnoyarsk State Medical University, Krasnoyarsk, kinatta@rambler.ru; <http://orcid.org/0000-0001-6425-5086>

Salmin Vladimir V. – Dr. Sc. (Physical and Mathematical Sciences), Professor, Department of General Physics, Moscow Institute of Physics and Technology (National Research University), Moscow; Professor, Department of Fundamental Sciences-4, Bauman Moscow State Technical University (National Research University), Moscow, vsalmin@gmail.com, <http://orcid.org/0000-0003-4441-9025>

Taranushenko Tatyana E. – Dr. Sc. (Medicine), Professor, Head of the Department of Pediatrics IPE, Prof. V.F. Voino-Yasenetsky Krasnoyarsk State Medical University, Krasnoyarsk, tetar@rambler.ru, <http://orcid.org/0000-0003-2500-8001>

(✉) **Proskurina Margarita V.**, prmargov@rambler.ru

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