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Integrative Analysis of Oxidative Stress Biomarkers And Their ” Clinical correlates in Pediatric Type 1 Diabetes A Multi Parametric Approach

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Abstract

In this study, we used the neutrophil granulocytes functional activity model to examine oxidative stress indicators, antioxidant protection, lipid peroxidation, and T1D in children. According to research, children with type 1 diabetes show heightened lipoperoxidation and antioxidant defense system activation in response to reactive oxygen species (ROS) damage, which includes bidirectional alterations in non-enzymatic pathways. The development of tolerance to oxidative stress in children with compensated type 1 diabetes is indicated by the coordinated synthesis of reactive oxygen species and oxygen-dependent blood cell metabolism. In children with decompensated type 1 diabetes, it is indicated that the third stage of oxidative stress, which involves the depletion of insulin-producing β-cells, begins with a decrease in reactive oxygen species production, a decrease in the activation of the phagocyte oxygen-dependent metabolism, and incomplete phagocytosis mechanisms. The severity of metabolic problems in children with type 1 diabetes is influenced by the level of the antioxidant defense system's "respiratory explosion" of neutrophilic granulocytes.

Keywords: type 1 diabetes mellitus, child population, chemiluminescence, neutrophil granulocytes, lipid peroxidation, antioxidant protection

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INTRODUCTION

The prevalence rate of diabetes mellitus (DM) approaches epidemic proportions, and it is a non-communicable illness (UN and WHO definition, 2006). Experts from the World Health Organization and the United Nations Commission on illnesses estimate that type 2 diabetes ranks fourth among all illnesses and poses a major medical and social problem; autoimmune type 2 diabetes affects one out of every 500 children and one out of every 200 teenagers. The medical and social importance of type 1 diabetes in children stems from the following factors: the disease's high prevalence (peak incidence between the ages of 7 and 11), its chronic course, the development of complications, early disability while patients are still socially active, a decrease in total life expectancy, and premature mortality. It is important to remember that type 1 diabetes in children requires a lot of mental and physical energy, influences the child's future, and impacts the whole family's life [1-7]. Looking at the kid's body as a whole makes it easy to develop diagnostic and treatment procedures for endocrine disease [8-22].

There has been a steady rise in the global illness incidence rate despite improvements in understanding endocrine pathophysiology, new

research methods, diagnostic tools, and regulatory frameworks. Its total number of T1D Approximately 40% of the 218,000 cases reported each year are in children less than 14 years old, according to statistics from the International Diabetes Federation (2013). By 2017, 22,969 children and 8,758 adolescents were found to have type 1 diabetes in Russia, as reported in the National Register. Mortality and morbidity indices were 0.05 and 0.10, respectively, while the prevalence of type 1 diabetes was 203.29 for "adolescents" and 86.73 for "child" categories, according to studies [23–28]. In humans, lipoperoxidative processes are crucial. Evidence from studies shows that even weakly functioning free radical reactions may alter the shape and function of cell membranes and play a direct role in their repair and regeneration. Proof of a function for free-radical oxidation processes in phagocytosis and microbial lysis has been found. Additionally, it has been shown that lipoperoxidative processes have a role in cell division and nerve impulse transmission [29-32].

Atoms, molecular building blocks, or whole molecules with unpaired electrons in their outer orbitals are free radicals. Since free radicals are so reactive, they may interact with and destroy a wide variety of substances. Free radical derivatives and exo- and endocellular superoxidant oxygen metabolites, which are directly harmful, spike sharply (abruptly) when lipid peroxidation is amplified. Internal and external factors influencing the human body have been found to have varying degrees of functional activity in free-radical and peroxide reactions. Some of these factors include low antioxidant levels, stress, the effects of synthetic drugs and xenobiotics, hypodynamia, aging, and an excessive consumption of lipids and carbohydrates. Diabetes, bronchopulmonary pathology, adaptive overstrain syndrome, peptic ulcer disease, coronary heart disease, communicable illnesses, peroxidation syndrome, heart attacks, strokes, and coronary heart conditions are all significantly impacted by peroxidation syndrome [33–37].

The pathogenetic processes of type 1 diabetes have been proved to include oxidative stress and activation of lipid peroxidation, according to studies collected by researchers from Russia and other countries. Free radicals form and accumulate excessively due to glycation, glucose auto-oxidation, and polyol pathway intracellular activation, all of which significantly raise blood plasma glucose levels. This imbalance in the NADH/NAD+ ratio is amplified. Mechanisms that activate lipid inflammation mediators control lipid peroxidation and antioxidant protection in the injured area; these changes in metabolism (hyperglycemia, dyslipidemia, insulin secretion change, and reduced antioxidant reserve) in type 1 diabetes also activate mechanisms that activate cell membrane functional status. The formation of reactive oxygen species is a crucial prerequisite for lipoperoxidation. Due to neutrophils' oxygen consumption and the subsequent development of an oxygen-dependent bactericidal activity necessary for agent elimination, a thorough investigation into the severity of the homeostatic imbalance is necessary for an objective evaluation of the oxidative stress intensity in type 1 diabetes [38–43].

We can claim, based on a systematic review of the literature, that determining the characteristics of the antioxidant mechanisms and the patterns of free-radical processes safety components, and the metabolism of oxygen-dependent neutrophils in children with type 1 diabetes will help define early diagnostic criteria for endocrine diseases, while also enhancing our knowledge of the pattern of changes in lipoperoxidation intensity throughout the disease's progression. Additionally, the results will validate the approach of seeing the body as an integrated system, which will aid in the search for integrated solutions in arranging treatment and rehabilitation for endocrine diseases, and improve the predictive and diagnostic value in pediatric practice.

Aim of study

To assess the status of lipid peroxidation, antioxidant protection and oxidative stress indicators based on the functional activity model of neutrophilic blood granulocytes in children with T1D depending on the endocrinopathy compensation stage.

MATERIALS AND METHODS

Research involving minors could not proceed without first obtaining a finding from the Committee on Bioethics and obtaining the parents' or guardians' explicit voluntary agreement. The research protocols were found to comply with national and international regulations, according to the results of the ethical review. These regulations include the following: the World Medical Association Declaration of Helsinki, 1964 ETHICAL PRINCIPLES FOR MEDICAL RESEARCH INVOLVING HUMAN SUBJECTS, as amended by the WMA LXIV General Assembly (2013); Cl. 24 of the Russian Federation Constitution; Rules of Clinical Practice in the Russian Federation (Decree 266 issued by the Ministry of Healthcare of the Russian Federation on June 19, 2003); ethical standards by the Committee on Experiments, Standards for Clinical Trials (GOST R 52379-2005); Federal Law of the Russian Federation 323-FL ON THE PRINCIPLES OF THE PROTECTION OF CITIZENS' HEALTH IN THE RUSSIAN FEDERATION (of 11/21/2011).

After getting their parents' or guardians' free assent, researchers conducted clinical and laboratory diagnostic examinations on 121 youngsters in the second childhood stage (boys aged 8-12, females 8-11). Two groups were formed from all of the patients. The 32 kids that made up the comparison group were from Veltischev's 1994 Health Groups I and II. Assuming the pediatrician comes to the same judgment, the diagnosis of healthy was issued. From 2010 to 2017, 89 individuals were a part of the study. The majority of these individuals were children with type 1 diabetes who were receiving treatment at the endocrinology departments of two Russian hospitals: G. K. Filippsky Child Clinical Hospital in the city of Stavropol and Child Regional Clinical Hospital of Krasnodar. Subgroup 1 consisted

of 46 children (52.9%) with compensated type 1 diabetes, whereas Subgroup 2 consisted of 43 children (47.1%) with decompensated type 1 diabetes; the two subgroups were formed from the main group of patients based on the degree of endocrinopathy compensation. Children with type 1 diabetes had a long history of the condition; 27 patients (30.3%) reported having it for less than a year, 43 patients (48.3%) for one to five years, while For the remaining 19 patients (21.4%), the illness had persisted for more than five years.

Twenty children (74.1% of the total) had decompensated type 1 diabetes, whereas only seven children (25.9% of the total) had compensated type 1 diabetes in the group whose endocrinopathy lasted up to a year. Endocrinopathy in children diagnosed with type 1 diabetes was classified according to the degree of compensation using the parameters of carbohydrate metabolism (Dedov, 2007). The child's clinical history was updated with the glycemia level markers.
Results from laboratory tests (including a general blood test, a urine test, and a biochemical blood test that assesses blood glucose levels) and information gleaned from clinical examinations conducted by hospital endocrinologists led to the diagnosis of type 1 diabetes in the groups under observation. Blood serum and hemolysate generated from erythrocytes were used to evaluate lipid peroxidation and antioxidant defense markers. Using a vacuum apparatus (venipuncture), blood was drawn from the ulnar vein in the morning while the patient was fasting, in accordance with the standard procedure for vein blood sample. This study used the following metrics to assess the antioxidant protection status:

Absolute antioxidant capacity (AOA). In order to explore the ability of blood serum to slow down the accumulation of active products by thiobarbituric acid (TBA) in a solution of chicken egg yolk lipoproteins, a model system was used (Klebanova, 1988). Ability to neutralize superoxide (SOD). The spectrofluorophotometer was used to measure the SOD activity, at a wavelength of 320 nm, by following a curve that represented the enzymatic inhibition of adrenaline auto-oxidation. According to research by H.P. Misra and I. Fridovich (1972), the quantity of superoxide dismutase (SOD) needed to suppress adrenaline auto-oxidation into adrenochrome by 50% was considered the unit of enzymatic activity.
The fluorometric approach was used to identify retinol and α-tocopherol (R.Ch. Chernyauskene, 1984). Serva and All-trans-retinol (Sigma) were used as the external standards.

• Under identical circumstances for fluorescence recording, the fluorometric approach was used to identify the amount of reduced (GSH) and oxidized (GSSG) glutathione (P.J. Hissin and R. Hilf 1976). Both the 350 nm and 420 nm wavelengths were measured using a spectrofluorophotometer.

Investigating the extent of lipid peroxidation was done by measuring the concentration of substrates that had conjugated double bonds (DB). An optical region-based spectrophotometric approach was used to identify diene conjugates (DC), ketodienes (KD), and conjugated trienes (CT) by measuring the absorbance of electromagnetic radiation of conjugated lipids.

Here are the diene structures of hydroperoxides: DB (λ = 220 nm), DC (λ = 232 nm), KD, and CT (λ = 278 nm) according to J. Stocks's technique (1974), with some modifications made by I. Volchegorsky (1989, 2000). The theory behind the technique for analyzing malondialdehyde (MDA), a byproduct of free-radical polyunsaturated higher fatty acid oxidation, is that NH2 protein groups interacting with MDA aldehyde groups cause irreversible protein denaturation. A trimethyl complex (the stained molecule) was formed when MDA and TBA reacted. V.B. Gavrilova (1987) described a method for determining the MDA concentration using the molar extinction coefficient of the trimethyl (stained) complex and fluorimetric assessment of the optical density of TBA-active lipoperoxidation products at λ = 532 nm.

A semi-automatic biochemical analyzer called BioChem SA (High Technology Inc., USA) was used in conjunction with a reagent kit from Spinreact, Spain, to study the total lipids (TL) level by the spectrophotometric technique. A CM2203 spectrofluorometer (Solar, Belarus) and an RF-5301PC spectrofluorophotometer (SHIMADZU, Japan) were used for the measurements.

Objective evaluation of antioxidant protection and lipid peroxidation was achieved by calculating the oxidative stress coefficient (OSC). Children with compensated type 1 diabetes (Subgroup 1) and decompensated type 2 diabetes (Subgroup 2), relative to the average values in healthy children (comparison group), have antioxidant protection and lipid peroxidation levels that make up this coefficient. It was determined that oxidative stress had occurred when the OSC value above 1.

The functional activity of neutrophilic granulocytes was examined using the chemiluminescent (CL) technique (De Sole, 1983) for a more in-depth in vivo examination of the free-radical oxidation state in the groups that were tested. When a material goes from an electrically excited state to its ground state, a quantum flux appears, and this flux may be measured using the chemoluminescent analysis technique. For 90 minutes, a 36-channel analyzer CL3604 (Russia) was used to measure luminol-dependent spontaneous chemiluminescence (LDCL) and zimosan-induced chemiluminescence (ZICL). According to Figure 1, the analyzer's fluorescence intensity of 5.1×105 quanta per second was considered as 1 cu.

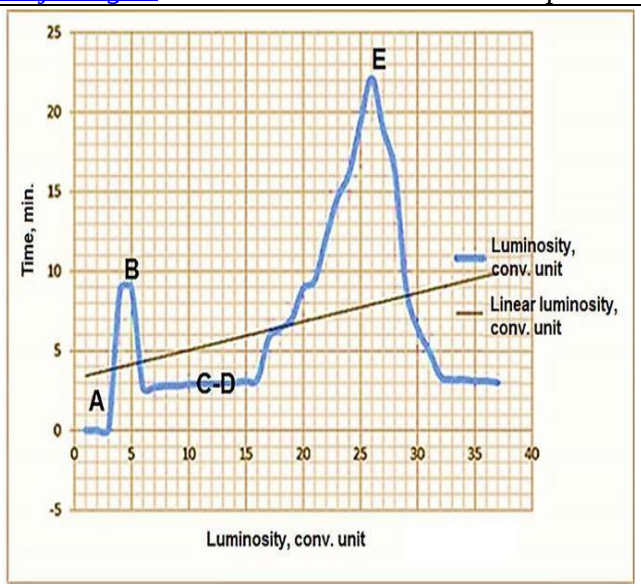

Figure 1. Vein blood spontaneous LDCL curve: A – spontaneous fluorescence; B – fast flash; C, D – latent period; E – slow fluorescence phase.

The following indicators laid the basis for the outcomes: T*max* – time to reach the maximum; I*max* – the maximum intensity level; S – the area under the CL curve; the activation index (AI) – the ratio of the ZICL area to the area of LDCL, which determines the CL enhancement induced by zymosan. The analyzer control along with the result record was performed through a PC. Statistical processing, including the data systematization, and construction of the graphic images tables were carried out following variation statistics methods. The results can be seen as the arithmetic mean and its standard error. The differences significance between the groups (p) was assessed subject to Student's t-test. The differences in indicators were considered significant at $p<0.05$. The calculations were performed employing the following software: STATISTICA 10.0, DBASE, STATGRAF, STAT4 (Stat Soft Inc., USA), as well as Med Calc (version 9.3.5.0), SPSS (version 7.5).

Results and Discussion

Both direct and indirect components make up the antioxidant defense system. Indirectly, it enhances basic metabolism, which does not need producing reactive oxygen species and lipid peroxidation products in excess. Consequently, altering the mitochondrial base—which encompasses activity and quantitative and qualitative mitochondrial components—is an ideal approach to controlling the oxygen-peroxide situation and the dependent signaling pathways that dictate the trajectory of all essential cellular activities. Antioxidant defense relies on a group of naturally occurring micro- and macromolecular molecules. Enzymes that catalyze the reduction of free radicals and lipoperoxidation products—among them, the glutathione complex—play an essential role in the antioxidant defense system.

superoxide dismutase, aconitase, catalase, myeloperoxidase, paraoxonase, system (GR, GPO, GST), and peroxidase. Patients in the aforementioned categories display antioxidant defense signs in Table 1.

Table 1- Indicators of antioxidant protection in patients of the studied groups, (M±m), (p≤0,05).

Indicators, units of measurements	Research groups		
	Comparison group	First group	Second group
Total antioxidant activity, c.u.	15,07 ± 1,61	16,84 ± 1,19	21,16 ± 1,33
Retinol, mkmol/l	1,87 ± 0,19	1,76 ± 0,16	1,39 ± 0,28
α-tocopherol, mkmol/l	6,69 ± 0,58	6,28 ± 0,43	4,51 ± 0,67

Recycled glutathione, mkmol/l	2,72 ± 0,13	2,66 ± 0,11	2,57 ± 0,16
Oxidized glutathione, mkmol/l	1,78 ± 0,19	1,84 ± 0,22	2,17 ± 0,14
Superoxide dismutase, c.u.	1,52 ± 0,08	1,46 ± 0,06	1,27 ± 0,11

In a healthy state, the body's antioxidant defense activity status and the amount of oxidants (free radicals) are in perfect harmony with one another. An increase in free radicals, which may damage cellular structures, is a response to oxidative stress. When free radicals build up in bodily tissues, it throws the system out of whack. With its many enzymatic and non-enzymatic linkages, total AOA is a key parameter controlling the antioxidant defense system's buffer capacity. Superoxide dismutase, retinol, and α-tocopherol, which are fat-soluble vitamins, are the fundamental enzymes. Scientific studies have shown that α-tocopherol, a necessary component of every plasma membrane, encourages the formation of inactive radicals that cannot sustain lipid peroxidation cascades. Furthermore, by increasing the packing density of membrane phospholipids, this fat-soluble vitamin makes them less accessible for peroxidation. Table 2 shows that when comparing healthy children with those who have type 1 diabetes, the antioxidant defense system is activated in the former group. Based on our analysis, the main group of children showed an increase in total AOA (11.7 ± 0.7% in the compensation stage and 40.4 ± 2.3% in the decompensation stage), a decrease in retinol concentration (5.6 ± 0.3% and 25.7 ± 1.4% in the decompensation stage, respectively) and α-tocopherol concentration (6.1 ± 0.2% and 32.6 ± 1.7% in the compensation stage), which can be seen as a defense mechanism against the increasing production of reactive oxygen species (oxidative stress).

pressure) with the goal of alleviating the severity of endocrinopathology. Research has shown that glutathione is only protective in its reduced (GSH) form, and that changes in glutathione status negatively impact the development of comorbidities and the outcome of the illness. In children with type 1 diabetes, there is an increase in the oxidized glutathione form (GSSG) and a decrease in superoxide dismutase (SOD) activity (3.9 ± 0.4% and 16.4 ± 0.9%, respectively), as well as a decrease in the reduced glutamine content (GSH) and an increase in the SSG form (2.2 ± 0.2% and 5.5 ± 0.3%, respectively). This suggests that the antioxidant defense system is activated, that there is stress in the glutathione redox system, and that lipid chain oxidation is being slowed down, since the primary action of SOD is to remove superoxide radicals. Research on the glutathione system in red blood cells from children with type 1 diabetes shows that GSH activity (the primary antioxidant system component) is reduced, which, in our opinion, is caused by reactive oxygen species' harmful effects. Lipid peroxidation products are more able to damage membranes when GSH levels are low. The oxidative stress is worsened when the cellular antioxidant defense is compromised, leading to an increase in GSSG concentration. This, in turn, promotes the inactivation and oxidation of the thiol protein groups.

Scientific evidence suggests that elevated levels of lipid peroxides are associated with insulin insufficiency (absolute or relative) in type 1 diabetes. Insulin is a hormone that inhibits lipid peroxidation. Its effects include bidirectional changes in the non-enzymatic component, protein glycosylation, as well as the use of peroxide molecules and an increase in the mobility (lability) of membrane lipids. Lipid peroxidation activity advances, leading to a lethal impact, just as endocrinopathy severity increases. Caused by harm to erythrocyte membranes and lysosomes, this pathophysiological process leads to the inactivation (inhibition) of cytochrome oxidase activity, an enzyme attached to the cell membrane. Diabetic angiopathies and vascular problems are facilitated by the emergence of morphological, structural, and functional alterations in the smooth muscle components of the vascular wall and endothelial cells. These changes often lead to rupture. The lipoperoxidation markers in the relevant groups' patients are shown in Table 2.

Table 2- Indicators of lipoperoxidation in patients of the studied groups, (M±m), (p≤0,05).

Indicators, units of measurements	Research groups		
	Comparison group	First group	Second group

Conjugated double bond substrates, c.u.	1,41 ± 0,14	1,53 ± 0,11	1,92 ± 0,19
Diene conjugates, mkmol/l	0,48 ± 0,04	0,57 ± 0,03	0,86 ± 0,09
Ketodienes and conjugated trienes, c.u.	0,16 ± 0,02	0,19 ± 0,03	0,26 ± 0,05
Malonic dialdehyde, mkmol/l	1,46 ± 0,13	1,59 ± 0,12	2,29 ± 0,17
Total lipids, g/l	4,09 ± 0,27	4,37 ± 0,38	5,96 ± 0,59

Children with T1D, compared with healthy children, had all the respective indicators showing lipid peroxidation intensification, including an increase in the substrates with conjugated double bonds (compensation stage, 8.5 ± 0.5 %; decompensation stage, 36.2 ± 2.1%), DC accumulation (lipid peroxidation primary products; 18.8 ± 1.2% and 79.2 ± 3.7%, respectively), accumulation of KD and CT (the intermediate products of lipoperoxidation; 18.7 ± 1.4% and 62.5 ± 3.1%, respectively), an increase in the MDA level (the final product of lipid peroxidation; 8.9 ± 0.7% and 56.8 ± 2.7%, respectively), and total lipids (6.8 ± 0.4% and 45.7 ± 2.3%, respectively). It should be noted that children with decompensated T1D have a significant accumulation of malonic dialdehyde (the most toxic product). From our point of view, in children with decompensated T1D, the most prominent increase in the lipid peroxidation initiation at the stage of primary, intermediate and final products, in comparison with the antioxidant defense system activation parameters, contribute to thickening of the blood vessel walls basement membrane, a higher blood viscosity, and a slower blood flow, thus increasing the probability of intravascular coagulopathy (aggregation of blood cells) and disturbance through various hemostasis stages.

Table 3 shows the range of oxidative stress coefficient fluctuations in the studied groups.

Table 3. The range of fluctuation of the coefficient of oxidative stress in patients of the studied groups (c.u.), (M±m), (p≤0,05).

	Research groups	
Comparison group	First group	The second group
Less than 1	1,03 −1,18	1,08 −1,79

A multi-tiered mechanism known as the antioxidant defense prevents normal lipid peroxidation from becoming pathological (oxidative stress). Classical stress is accompanied by oxidative stress of varied degrees, which develops as a result of impaired antioxidant defense systems and might be a critical component of the disease.
Due to disrupted component interaction in a single stress mechanism of all physiological systems, the chance of oxidative stress occurring (OSC ≥ 1) in children with long-term decompensated type 1 diabetes is greatly increased.

Damage to biological membranes, both structural and functional, is associated with an upsurge in free-radical oxidation activity, a good measure of the health of macroorganisms. At the molecular level, the pathogenesis of many illnesses is based on this collection of events, which includes a decrease in the stability of the lipid layers of membranes, increased peroxidation of proteins and lipids, and increasing ion permeability. By studying photochemical reactions, electronically excited molecule states, the structure and properties of biological systems, and the dynamics of molecular transitions, CL analysis—which is highly informative, sensitive, and dependable—enables the objective and dependable identification of possible morphological and metabolic disorders in the development and progression of endocrine pathology at the molecular level.

Evidence from CL analysis of biological structures points to a link between free radical oxidation in macroorganisms and the reduction of molecular oxygen to active species like hydroxyl and superoxide anion radicals as well as singlet oxygen. When lipids undergo auto-oxidation, they create free radicals, which are the primary source of reactive oxygen species. Emission of light quanta happens when

peroxides recombination develops as a result of free radicals interacting with oxygen. Additionally, photon emission may be seen when oxygen dimer molecules, biogenic amines, aldehydes, oxalates, cyclic hydroperoxides, and intermediate products of reactions with molecular oxygen (peroxides) are excited. Natural antioxidants of hydrophobic (flavonoids, tocopherols, steroids, and carotenoids) and hydrophilic (ascorbic acid and sulfhydryl compounds of the SH-group of proteins) phases inhibit free-radical oxidation in the body.

In this light, CL activity shows that antioxidants are ineffective and that free radical oxidation is rampant. Examining the correlation between the rate of free radical recombination and the intensity of spontaneous emission does not provide a solid picture of the causes of the change in the rate of free radical oxidation. Here, the luminol-based approach to modulating free-radical reactions followed by an examination of induced CL stands out. Oxidation of luminol to produce carbonyl chromophores excited by electrons occurs in the presence of reactive oxygen species. The generation of reactive oxygen species causes the indicated functional groups with a high quantum yield to considerably boost the light intensity. Researchers have found success in using this phenomena to explore phagocytic immunity at a functional level. The activation rate of oxygen-dependent phagocytic metabolism is lowered, and phagocytosis is partial, due to an inadequate formation of reactive oxygen species that aims to inactivate antigens.

The first steps in the immune response to antigens (foreign agents) are the processes of "non-specific immunity," according to scientific evidence. The kind of inflammation that develops is dictated by the responsiveness of neutrophil granulocytes, which, in reaction to several signals indicating an internal environment disturbance, may execute functional reconfiguration quickly. A rapid increase in oxygen consumption due to phagocytes converting it into active species triggers what is known as the "respiratory (oxygen) explosion," which in turn controls the pace of neutrophil mobilization and strengthens the body's defensive mechanisms. Neutrophil blood granulocytes' reactive oxygen species production capacity is an indicator of the kind of inflammation, whereas stimulation response provides an objective measure of the body's defensive activity. Since free-radical oxidation is considered a factor that indicates the health status through different phases of type 1 diabetes in children, studying oxidative stress using a model of neutrophilic granulocytes with high diagnostic significance allows a substantial expansion of the information pool to be obtained. For each of these patient categories, Table 4 displays the markers of luminol-dependent chemiluminescence of neutrophilic blood granulocytes.

Table 4- Indicators of luminol-dependent chemiluminescence of neutrophilic blood granulocytes in patients the studied groups (M±m).

Indicators	Research groups					
	Comparison group		First group		Second group	
	Value range	Average value	Value range	Average value	Value range	Average value
Spontaneous chemiluminescence						
Tmax., сек	573,4-1478,7	905,3±34,1	417,6-1084,9*	667,3±23,8*	614,6-1583,5*	968,9±31,7*
Imax, o.e.×103	3,03-12,96	9,93±0,67	28,87-80,33**	51,46±3,78**	16,23-43,91**	27,68±2,14**
S, o.e.×105	2,82-6,74	3,92±0,31	17,58-49,22**	31,64±2,06**	9,84-28,21**	18,37±1,95**
Zymosan-induced chemiluminescence						
Tmax., сек	772,8-1297,4	1035,1±38,6	593,9-1311,3*	717,2±26,3*	472,2-1559,2*	1087,0±43,1*
Imax, o.e.×103	9,72-29,06	19,34±1,26	36,63-135,16**	98,53±5,34**	34,18-99,57**	65,39±4,02**
S, o.e.×106	3,19-10,32	7,13±0,58	30,46-82,03**	51,57±3,29**	16,44-51,16**	34,72±2,19**
Activation index		1,82		1,63		1,89

Note: statistically significant differences with the children of the comparison group (* - p <0.05; ** - p <0.01).

The results of studying the luminol-dependent chemiluminescence parameters in neutrophilic blood granulocytes in the main group

indicated that children with T1D in the compensation stage have bidirectional change dynamics in the spontaneous LDCL if compared with similar factors in healthy children, including an increase of Imax (5.2 times) and S (8.1 times), and a decrease in Tmax values (1.4 times). This type of change in the values indicates a proper generation of reactive oxygen species and blood cells' oxygen-dependent metabolism processes in response to the early phases of selective organ-specific destruction of insulin- producing β-cells in the Langerhans pancreatic islets. A decrease in the maximum intensity growth rate value (Imax) during zymosan-induced LDCL in this subgroup, in comparison with the change in dynamics of spontaneous LDCL, confirms that the development of the destruction initial stage in the islet cells correlates with a decrease in the reserve capacity of neutrophil granulocytes antimicrobial protection.

In case of an increasing severity of endocrinopathy in children with T1D, if compared with the main group (Subgroup 1), there were also bidirectional changes registered in the spontaneous LDCL values (a 1.9 decrease in Imax and a 1.7 times decrease in S; Tmax parameters growth by 1.5 times) and zymosan-induced LDCL (decrease in Imax (1.5 times) and S (1.4 times), and increase in Tmax parameters (1.6 times)). The reduction of nonspecific antimicrobial defense in children with decompensated T1D, occurring along with an increasing pancreas lesion area (destruction of insulin-producing β-cells of the Langerhans islets), is an effect of the following pathophysiological mechanisms:

- reduced rate of metabolic processes, which is accompanied by a respiratory burst developing;
- reduced production of reactive oxygen species;
- depleted phagocytic activity of macrophages.

From our point of view, this condition points to a long chronic inflammatory process, which is combined with the depletion

of defense & compensation mechanisms aimed at improving the body's vital processes under inflammation.

CONCLUSIONS

1. During the whole course of type 1 diabetes in children, the antioxidant defense system is always engaged to counteract the harmful effects of reactive oxygen species, which may cause alterations in non-enzymatic processes that go in both directions. The overall antioxidant activity increases (11.7 ± 0.7% in the compensation stage and 40.4 ± 2.3% in the decompensation stage), while the levels of retinol and α-tocopherol decrease (5.6 ± 0.3% and 25.7 ± 1.4%, respectively) when compared to healthy children. This protective response is meant to reduce the severity of endocrinopathy by reducing the generation of reactive oxygen species.
2. In contrast to healthy children, children with type 1 diabetes show a marked rise in the amount of oxidized glutathione (GSSG) in their venous blood (3.4 ± 0.2% in the compensation stage and 21.9 ± 1.3% in the decompensation stage). This is accompanied by a decline in superoxide dismutase activity (3.9 ± 0.4% and 16.4 ± 0.9%, respectively) and a level of reduced glutathione (GSH) (2.2 ± 0.2% and 5.5 ± 0.3%, respectively). These findings point to a glutathione redox system stress and a slow chain oxidation of lipids. Contributing to the stability of the antiperoxide and antiradical cell potential, redox system stress inhibits anti-peroxide enzymes and decreases the quantity of antioxidants.

3. At every stage of disease, children with type 1 diabetes show increased lipid peroxidation. In comparison to children in good health, there is a noticeable rise in the following levels: substrates with conjugated double bonds (8.5 ± 0.5% in the compensation stage and 36.2 ± 2.1% in the decompensation stage), diene conjugate accumulation (18.8 ± 1.2% and 79.2 ± 3.7%, respectively), ketodien accumulation (18.7 ± 1.4% and 62.5 ± 3.1%, respectively), malondialdehyde level (8.9 ± 0.7% and 56.8 ± 2.7%, respectively), and total lipids (6.8 ± 0.4% and 45.7 ± 2.3%, respectively).

4. Intravascular problems (coagulopathy) are more likely to occur in children with decompensated type 1 diabetes due to evident changes in lipid metabolism and indications of oxidative stress, which worsen the endocrine pathology.
5. The total antioxidant activity level, diene conjugate content, and total lipids in the venous blood should be considered highly reliable and diagnostically important risk factors for the early development of microvascular angiopathies.
6. Results from luminol-dependent chemiluminescence studies of functional activity of neutrophilic blood granulocytes in children with type 1 diabetes show that, when compared to healthy children, children with T1D develop reactive oxygen species more intensely during both spontaneous and zymosan-induced (stressed) chemiluminescent reactions.
7. The study of oxidative stress in children with type 1 diabetes used induced luminol-dependent chemiluminescence, which is a highly sensitive, cost-effective, and dependable express method for evaluating the functional status of phagocytic immunity. It also allows for the registration of the kinetic component of the phagocytosis process.

Changes in the Lipid peroxidation and antioxidant defense system are observed in children with decompensated type 1 diabetes. This is because activated antioxidant defense mechanisms cause lipid peroxidation to intensify, which in turn corresponds to the systemic inflammatory response syndrome that occurs when the body's protective-compensatory mechanisms are under the greatest stress.

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