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Biochemical markers of the severity and occurrence of non-smooth course of pseudotuberculosis in children

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Abstract

Background. Currently, pseudotuberculosis is characterized by a high incidence in children and it is the second acute intestinal infection by frequency after shigellosis. The search for relationships between early changes in cell metabolism, dysfunctions of some organs and body systems under the pathogenic effect of an infectious agent and the severity, the occurrence of a non-smooth course of pseudotuberculosis in children is relevant.

Aim. Search for biochemical markers to predict the severity and occurrence of a non-smooth variant of the course of pseudotuberculosis in children.

Material and methods. A prospective study was performed in 125 patients with pseudotuberculosis during periods of the disease height, early convalescence and recovery: 17 patients with mild severity and a smooth course of the disease; 64 — with moderate severity and smooth course of the disease; 28 — with moderate severity and non-smooth course, 16 — with high severity and non-smooth course of the disease. The comparison group consisted of 45 children from the IIA health group. The distribution of the examined children by sex was equal, the age ranged from 9 to 13 years. The spectrophotometric method determined the following: in blood plasma — the content of malondialdehyde; in erythrocytes — the concentration of reduced glutathione, the activity of glutathione reductase, glutathione peroxidase, glutathione-S-transferase and catalase. The following ratios were calculated: reduced glutathione/malondialdehyde, reduced glutathione/glutathione reductase, reduced glutathione/glutathione peroxidase, reduced glutathione/glutathione S-transferase, glutathione peroxidase/catalase. Normality of the obtained data distribution was checked using the Shapiro–Wilk test, and the statistical hypotheses about the difference between the studied groups were checked using the nonparametric Mann–Whitney test at a significance level of $p < 0.05$.

Results. In the acute period of the disease, based on the analysis, a statistically significant decrease in the ratios of reduced glutathione/malonic dialdehyde, reduced glutathione/glutathione reductase, reduced glutathione/glutathione peroxidase by 3.0, 3.5 and 3.1 times, respectively, was established ($p < 0.05$) in patients with mild severity and smooth course; 5.3, 5.1 and 3.8 times ($p < 0.05$) — with moderate severity and smooth course; 5.8, 4.0 and 3.0 times ($p < 0.05$) — with moderate severity and non-smooth course; by 8.1 and 6.1 times ($p < 0.05$) — with heavy severity and non-smooth course relative to control values. However, during the period of early convalescence of a non-smooth course, these indicators significantly decreased by 2.2, 4.4 and 1.8 times ($p < 0.05$) in patients with moderate severity and by 3.4, 6.8 and 2.2 times ($p < 0.05$) — with heavy severity relative to control values.

Conclusion. Prognostically significant criteria for an increase in the severity and occurrence of a non-smooth course of pseudotuberculosis in children in the acute period of the disease are a decrease in the ratios of reduced glutathione/malonic dialdehyde less than 17.0, reduced glutathione/glutathione reductase below 38.0 and reduced glutathione/glutathione peroxidase less than 12.0.

Keywords: children, pseudotuberculosis, oxidative stress, lipid peroxidation, antioxidant system, glutathione system.

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Background

At present, studies that enable the identification of early changes in cell metabolism and dysfunctions of individual organs and body systems under the pathogenic effect of an infectious agent are particularly relevant. It is known that the development of an infectious process is associated with the activation of free-radical processes and the formation of oxidative stress, the manifestations of which depend largely on the power of the body's antioxidant system [1–3].

Pseudotuberculosis (PT) is an acute intestinal infectious saprozoonotic disease. The causative agent, *Yersinia pseudotuberculosis*, has a number of toxins, namely thermostable and thermolabile enterotoxins, cytotoxins, and factors that impair the permeability of skin vessels (early and late), as well as a lethal toxin, which contribute upon introduction of bacteria not only to increased generation of reactive oxygen species by effector cells of the macroorganism but also to impaired vascular permeability and organ microcirculation [4, 5].

A special aspect of the course of PT in pediatric patients is the development of a uneven course (up to 55% of cases), even with timely antibiotic therapy [6]. The study of the effect of free-radical oxidation products during the introduction of *Yersinia pseudotuberculosis* on the components of the cell's antioxidant defense will reveal the factors that contribute to the occurrence of a more severe and uneven course of inflammation. Under modern conditions, in clinical laboratory diagnostics, the search for biochemical markers and patterns of their changes is relevant, as it enables the prediction of a more pronounced disease severity in the acute disease phase and, with the elimination of clinical symptoms, the possibility of recurrence of the inflammatory process.

Aim

The study aimed to search for biochemical markers to predict the increase in severity and uneven course of PT in pediatric patients.

Materials and methods

The prospective study included 125 PT patients hospitalized in the G.E. Sibirtsev Children's Infectious Diseases Hospital of Tomsk. All patients were distributed into four groups:

– group 1 included 17 patients (mean age 9.96 ± 0.56 years) with mild severity and a smooth disease course;

– group 2 included 64 patients (mean age 10.39 ± 0.43 years) with moderate severity and smooth disease course;

– group 3 included 28 patients (mean age 11.00 ± 0.71 years) with moderate severity and an uneven disease course;

– group 4 included 16 patients (mean age 12.00 ± 0.55 years) with a severe and uneven disease course.

The diagnosis of PT was performed according to modern diagnostic standards [7]. The distribution of children by gender was equal. In 77.4% of pediatric patients, the disease was regarded as sporadic. The patients were hospitalized throughout the year, but 78.0% of them were admitted between February and June. The patients received conventional therapy.

The comparison group (control) included 45 children (mean age 9.98 ± 0.44 years) in health group IIA.

The study was performed in accordance with the ethical principles of medical research of the Declaration of Helsinki of the World Health Organization and approved by the ethical committee at the Siberian State Medical University (protocol No. 4267 dated September 21, 2015).

Blood was drawn by puncture of the cubital vein, in the morning, on an empty stomach, in an amount of 5 ml, with the addition of sodium heparin (25 IU/ml). Further, blood plasma and erythrocytes were obtained by centrifugation at 3000 rpm (CM-6 ELMI, Latvia) for 15 min. Blood plasma was used to determine the level of malondialdehyde (MDA). The sedimented erythrocytes were washed three times with cold 0.9% sodium chloride solution, and then lysates were prepared using cold distilled water at a 1:10 ratio to determine the level of reduced glutathione (RG) and the activity of glutathione reductase (GR), glutathione-S-transferase (GST); and at 1:200 to determine the activity of catalase (CAT) and glutathione peroxidase (GPO).

The intensity of lipid peroxidation processes was assessed by determination of the concentration of MDA, which forms a trimethine complex with thiobarbituric acid with an absorption maximum at 532 nm [8].

The non-enzymatic link to antioxidant defense was studied by determining the RG level by the reaction of the RG groups of the tripeptide with 5,5'-dithio-bis-(2-nitrobenzoic acid) and the formation of a product with an absorption maximum at 412 nm after preliminary precipitation of proteins with a 5% solution of sulfosalicylic acid; the enzymatic link was analyzed by determining the activity of GR (enzyme classification 1.8.1.7) by NADPH-dependent[1] conversion of the oxidized form of glutathione to the reduced one; GPO (en-

[1] NADPH — nicotinamide adenine dinucleotide phosphate (reduced form).

zyme classification 1.11.1.9) was determined by the ability of the enzyme to catalyze the reaction of interaction of RG with t-butyl hydroperoxide; GST (enzyme classification 2.5.1.18) was determined by the method based on the rate of formation of glutathione-S-2,4-dinitrobenzene in the reaction between RG and 1-chloro-2,4-dinitrobenzene; and CAT (enzyme classification 1.11.1.6) was determined according to the rate of hydrogen peroxide utilization in the reaction mixture [9].

The protein concentration in the samples was determined by the biuret method according to the manufacturer's protocol (Protein-Novo, Vector-Best, Novosibirsk). The optical density of the samples was recorded on an SF-2000-02 spectrophotometer (Spektr, Russia). Then, based on the results obtained, the values of integral indicators (RG/MDA, RG/GR, RG/GPO, RG/GST, and GPO/CAT) were calculated.

Statistical processing of the data obtained was performed using the Statistica 6.0 program. Results were presented as medians (Me), with upper and lower quartiles ($Q_{25\%}$–$Q_{75\%}$). The data obtained were tested for the normal distribution using the Shapiro–Wilk test. To test the statistical hypotheses about the difference between the studied groups, the nonparametric Mann–Whitney test was used due to the non-normal distribution of the samples. Differences were considered statistically significant at $p < 0.05$.

Results

The acute period of infectious inflammation was accompanied by the active production of peroxidation products and the consumption of RG to reduce their damaging effects. Along with this, glutathione-dependent enzymes and CAT were activated. We revealed a significant increase in the blood plasma concentration of MDA ($p < 0.05$) and a decrease in the level of RG ($p < 0.05$) in erythrocytes in proportion to the severity of PT in pediatric patients relative to the results obtained in the control group (Tables 1, 2).

The response to a decrease in RG concentration was a significant increase in GR activity ($p < 0.05$) in erythrocytes relative to the control value. At the same time, with an increase in the inflammatory process severity, an imbalance arose in the work of enzymes using RG as one of the reaction substrates (GPO and GST), most likely due to its deficiency and/or the damaging effect of active oxygen metabolites on enzyme molecules (Tables 1 and 2). During the period of early convalescence, which corresponded to the attenuation of clinical symptoms, in pediatric patients with moderate and severe PT, a significant increase in the blood plasma MDA was noted, a significant decrease in the concentration of RG was recorded in erythrocytes, and an increase in the activity of CAT and GR with the comparable values of GPO and GST was observed, relative to the results obtained in the control group (Tables 1, 2).

Thus, the results obtained indicate that with an increase in the severity of the infectious process, more pronounced oxidative stress was noted in the child's body, and at the same time, the prognosis and outcome of the disease depended on the adequacy of functioning of intracellular antioxidant systems.

At the next stage of the work, we calculated the ratios of the obtained indicators in order to detect patterns of their change in accordance with the severity of the infectious process. At the height of mild clinical symptoms of PT, we recorded the most minimal changes in the calculated values of the ratios, compared to similar values in the control group of children, namely a significant decrease in the value of the ratio of RG/MDA by 3.0 times ($p < 0.05$), that of RG/GR by 3.5 times ($p < 0.05$), that of RG/GPO by 3.1 times ($p < 0.05$), and that of RG/GST by 4.4 times ($p < 0.05$). The convalescence phase in patients with mild PT was characterized by the absence of significant differences in all calculated values, compared to those in healthy children (Tables 3, 4).

The acute period of a smooth course of moderate PT was characterized by a more significant decrease in the values of integral indicators, namely RG/MDA by 5.3 times ($p < 0.05$), RG/GR by 5.1 times ($p < 0.05$), RG/GPO by 3.8 times ($p < 0.05$), and RG/GST by 7.0 times ($p < 0.05$), relative to similar values in pediatric patients of the control group. During the convalescence period, in patients with a smooth course of moderate PT, all the studied integral indicators increased; however, they did not reach the values of the control group, and at the same time, the ratios of RG/MDA, RG/GR, and RG/GPO in the presence of comparable indicators of RG/GST and GPO/CAT were significantly decreased, relative to similar values registered in healthy children ($p < 0.05$) (Tables 3, 4).

In the case of an uneven course of moderate PT in the acute period, there was a significant decrease in the value of the RG/MDA ratio by 5.8 times ($p < 0.05$), of RG/GR by 4.0 times ($p < 0.05$), of RG/GPO by 3.0 times ($p < 0.05$), and of RG/GST by 6.1 times ($p < 0.05$), relative to the control group. With the attenuation of clinical symptoms during the period of early convalescence, the values of the calculated indicators increased, but they were also significantly lower than the corresponding values in the control group children,

Table 1. Level of malondialdehyde in blood plasma, reduced glutathione, and catalase activity in erythrocytes in pediatric patients with pseudotuberculosis with varying severity and a smooth and uneven disease course, Me ($Q_{25\%}$–$Q_{75\%}$).

Groups of children	Disease period	Malondialdehyde, μmol/l	Reduced glutathione, μmol/l	Catalase, mmol/min × g protein
Control, n = 45		2.58 (2.32–2.73)	120.03 (115.02–146.00)	1.56 (1.35–1.82)
Group 1, n = 17	AP	5.45 (4.78–5.90)*	86.00 (78.12–88.09)*	2.34 (2.12–2.37)*
	C	2.87 (2.39–3.05)	95.33 (93.01–99.20)*	1.48 (1.35–1.80)
Group 2, n = 64	AP	6.07 (5.30–7.05)*	58.50 (55.10–66.00)*,♦	2.73 (2.48–3.03)*
	C	3.00 (2.64–3.23)	89.00 (84.00–94.00)*	1.33 (0.96–1.56)
Group 3, n = 28	AP	6.50 (6.12–6.90)*	59.00 (55.00–61.01)*,♦	2.85 (2.70–3.57)*,♦
	EC	3.68 (3.45–3.90)*	81.50 (74.00–89.00)*	2.60 (2.47–2.73)*
	C	3.64 (2.96–3.90)*	90.50 (89.10–98.00)*	1.77 (1.53–1.78)
Group 4, n = 16	AP	8.59 (7.05–8.97)*,♦	55.00 (54.05–56.00)*,♦	2.64 (2.60–2.73)*
	EC	4.20 (4.01–4.50)*	62.00 (60.00–65.00)*	2.73 (2.70–2.77)*
	C	2.90 (2.83–3.02)*	79.00 (77.50–80.00)*	1.56 (1.35–1.78)

Note: AP — acute period; C — convalescence; EC —early convalescence; $p < 0.05$ — *compared to the control, ♦compared to group 1.

Table 2. Activity of glutathione-dependent enzymes in erythrocytes in pediatric patients with pseudotuberculosis with varying severity and a smooth and uneven course, Me ($Q_{25\%}$–$Q_{75\%}$)

Groups of children	Disease period	Glutathione reductase, μmol/min × g protein	Glutathione peroxidase, mol/min × g protein	Glutathione-S-transferase, μmol/min × g protein
Control, n = 45		0.90 (0.42–1.23)	4.20 (3.32–4.94)	0.92 (0.38–1.39)
Group 1, n = 17	AP	2.18 (2.12–2.33)*	6.89 (6.68–7.83)*	2.76 (2.54–2.78)*
	C	1.13 (0.90–1.30)	4.01 (3.32–4.95)	1.19 (0.87–1.77)
Group 2, n = 64	AP	2.45 (2.30–2.70)*	6.50 (6.01–8.11)*	3.01 (2.78–3.70)*,♦
	C	1.40 (1.12–1.56)	3.99 (3.52–4.20)	1.07 (0.87–1.39)
Group 3, n = 28	AP	1.93 (1.67–2.16)*,▪	5.90 (4.70–6.29)♦	2.42 (2.10–2.78)*
	EC	3.32 (2.03–4.62)*	4.30 (4.10–4.50)	2.75 (2.60–2.90)*
	C	1.01 (0.98–1.23)	3.13 (2.27–4.01)	1.27 (1.23–1.77)
Group 4, n = 16	AP	2.56 (2.47–2.60)*	1.98 (1.80–2.54)*,♦	1.23 (1.09–1.34)
	EC	3.10 (3.09–3.12)*	3.98 (3.83–4.01)	1.34 (1.23–1.45)
	C	3.91 (3.09–3.99)*	2.10 (1.46–2.13)*	2.16 (2.01–2.73)*

Note: AP — acute period; C — convalescence; EC — early convalescence; $p < 0.05$ — *compared to the control, ♦compared to group 1, ▪compared to group 2.

namely by 2.2 times for RG/MDA ($p < 0.05$), by 4.4 times for RG/GR ($p < 0.05$), by 1.8 times for RG/GPO ($p < 0.05$), and by 4.6 times for RG/GST ($p < 0.05$). The convalescence phase in these patients was characterized by comparable values of the integral indicators of RG/GR and RG/GPO, and the values of RG/MDA (by 1.8 times; $p < 0.05$) and RG/GST (by 1.9 times; $p < 0.05$) were lower than those registered in healthy children (Tables 3, 4).

In examined patients of all the groups described (1, 2, and 3), in the acute period, the period of early convalescence, and the convalescence phase, the value of the GPO/CAT ratio was comparable to that in control group children (Tables 3 and 4).

The lowest values of the RG/MDA and RG/GR ratios were registered in the acute period of uneven course of severe PT; it was significantly lower than the corresponding parameters in healthy children, namely by 8.1 times ($p < 0.05$) and 6.1 times ($p < 0.05$), respectively. The studied parameters in the patients remained significantly lower in the period of early convalescence, namely by 3.4 times ($p < 0.05$) and 6.8 times ($p < 0.05$), as well as in the convalescence phase, namely by 1.8 times ($p < 0.05$) and 6.5 times ($p < 0.05$), respectively, relative to the values registered in the control group of children (Table 3).

The integral indicator of RG/GPO in the acute period and in the convalescence phase of an uneven

Table 3. Integral indicators in pediatric patients with pseudotuberculosis with varying severity and a smooth and uneven course, Me ($Q_{25\%}$–$Q_{75\%}$)

Groups of children	Disease period	RG/MDA	RG/GR	RG/GPO
Control group, n = 45		49.68 (42.40–55.74)	131.58 (95.24–170.08)	34.53 (25.25–42.22)
Group 1, n = 17	AP	16.32 (13.64–17.49)*	37.61 (36.45–40.18)*	11.32 (10.35–12.69)*
	C	33.28 (31.15–40.34)	86.38 (73.85–105.03)	23.50 (19.45–28.65)
Group 2, n = 64	AP	9.31 (8.08–11.86)*	25.88 (22.92–29.18)*	9.13 (7.52–10.56)*
	C	29.96 (25.83–34.57)*	63.57 (56.54–83.81)*	23.66 (19.46–27.03)*
Group 3, n = 28	AP	8.60 (8.36–9.52)*	32.94 (25.21–34.38)*	11.47 (9.54–11.70)*
	EC	22.39 (18.97–25.80)*	29.94 (16.03–43.84)*	18.91 (18.05–19.78)*
	C	27.57 (23.64–32.13)*	84.73 (79.67–89.45)	30.75 (24.16–43.27)
Group 4, n = 16	AP	6.13 (5.82–7.66)*	21.48 (19.23–21.86)*	27.27 (21.65–27.78)
	EC	14.44 (14.29–14.96)*	19.35 (19.23–21.04)*	15.79 (14.96–16.33)*
	C	27.59 (26.16–28.21)*	20.20 (19.80–25.89)*	38.28 (37.09–54.48)

Note: RG — reduced glutathione; MDA — malondialdehyde; GR — glutathione reductase; GPO — glutathione peroxidase; AP — acute period; C — convalescence; EC — early convalescence; *$p < 0.05$ compared to the control.

Table 4. Integral indicators in pediatric patients with pseudotuberculosis with varying severity and a smooth and uneven course, Me ($Q_{25\%}$–$Q_{75\%}$)

Groups of children	Disease period	RG/GST	GPO/CAT
Control group, n = 45		138.73 (84.99–202.47)	2.57 (1.89–3.27)
Group 1, n = 17	AP	31.30 (25.91–34.65)*	3.14 (2.82–3.36)
	C	88.45 (53.82–109.83)	2.81 (2.31–3.27)
Group 2, n = 64	AP	19.93 (17.70–22.08)*	2.45 (2.08–3.22)
	C	77.88 (65.71–112.14)	2.97 (2.44–4.26)
Group 3, n = 28	AP	22.92 (21.58–26.32)*	1.52 (1.32–2.62)
	EC	29.87 (25.52–34.23)*	1.66 (1.50–1.82)
	C	71.53 (60.47–73.25)*	1.80 (1.32–2.27)
Group 4, n = 16	AP	43.90 (41.04–45.87)*	0.73 (0.68–0.96)*
	EC	44.78 (41.38–52.85)*	1.47 (1.35–1.52)*
	C	37.77 (28.94–40.98)*	1.36 (0.81–1.55)*

Note: RG — reduced glutathione; GST — glutathione-S-transferase; GPO — glutathione peroxidase; CAT — catalase; AP — acute period; C — convalescence; EC — early convalescence; *$p < 0.05$ compared to the control.

course of severe PT had comparable values, and in the period of early convalescence, it was significantly lower, namely by 2.2 times ($p < 0.05$), compared with the same indicator in healthy children (Table 3).

The dynamics of changes in the value of the RG/GST ratio in children of this group was different than in patients with mild and moderate disease. Thus, the value of the RG/GST ratio was significantly lower, namely by 3.2 times at the height of clinical symptoms ($p < 0.05$), by 3.1 times in the early convalescence phase ($p < 0.05$), and by 3.7 times during convalescence, relative to the control result (Table 4).

In addition, in the uneven course of severe PT, we revealed the lowest values of the GPO/CAT ratio relative to the control values, namely a decrease of 3.5 times in the acute period ($p < 0.05$), of 1.7 times in the period of early convalescence ($p < 0.05$), and of 1.9 times in the convalescence phase ($p < 0.05$), compared with the same indicator in healthy children (Table 4).

Discussion

A wide variety of prooxidants and antioxidants exist, and the specificity of the latter with respect to certain reactive oxygen species makes it difficult to determine them in both *in vitro* and *in vivo* sys-

tems. Because the results obtained have a functional relationship, we decided to calculate the values of the integral ratios and compare the results obtained with the degree and course of PT in pediatric patients in order to substantiate their prognostic significance. These calculated values enable characterization of the activity of antioxidant systems at different degrees of severity and course of the inflammatory process in pediatric patients.

The plasma membrane performs the main barrier function [10] when protecting the cell from the action of prooxidants and toxins of the infectious agent. Structural and functional rearrangements of the membrane due to increased activity of lipid peroxidation processes contribute to changes in its barrier properties [11, 12]. Considering that the probability of diffusion from the MDA cell into the extracellular fluid (including blood plasma) is the same [13] and that RG is the main component of erythrocyte antioxidant defense [14, 15], an integral indicator of the ratio of the RG concentration in erythrocytes to the blood plasma MDA level was proposed.

The enzymatic link of the glutathione system is represented by the main enzymes GPO, GST, and GR [3, 16–19]. The calculated indicator "erythrocyte RG level/erythrocyte GR activity" enabled assessment of the adequacy of regeneration of the oxidized form of glutathione to the reduced form, and the ratios "erythrocyte RG level/erythrocyte GPO activity" and "erythrocyte RG level/erythrocyte GST activity" indicated not only the sufficiency of RG intake as one of the substrates of reactions but also, in the case of GST, the power of the body's detoxification function. The ratio of the erythrocyte activity of GPO and CAT showed the contribution of one of the enzymes to the neutralization of peroxides in the cell.

An analysis of the data obtained in the dynamics of PT at various degrees of severity and course in pediatric patients clearly established inhibition of the functional activity of the glutathione system as a whole, both in the acute period and in the period of early convalescence, as well as the lack of normalization of indicators in the convalescence phase with the disappearance of clinical symptoms of the disease.

Based on the data obtained and the constancy of dynamics of the changes identified, integral relationships can be distinguished to predict the severity and nature of the disease course in pediatric patients. The most significant integral indicators for predicting the development of a uneven course of PT are RG/MDA, GPO/CAT, and RG/GR. The more severe the degree of PT, the lower the values of these ratios registered by us. In the period of early convalescence of the disease in patients with attenuation of clinical symptoms, the formation of a uneven course of moderate and severe PT was evidenced by the low values of the studied ratios, compared to the same values in healthy children.

Based on the analysis of the ratio of GPO and CAT activities, the contribution of each of these enzymes to the utilization of hydrogen peroxide in erythrocytes during acute inflammation can be determined. Comparable values of the GPO/CAT index at the initial stage of the disease compared with the control value indicate a greater contribution of GPO to the utilization of hydrogen peroxide than that of CAT. A decrease in the ratio indicates, by contrast, the predominance of CAT activity over GPO, which manifests itself with an increase in the severity of clinical manifestations of PT.

For this reason, when studying the change in the GPO/CAT value, attention should be paid to the fact that the absence of an increase in the index during the period of early convalescence can be a prognostically unfavorable sign of the onset of an uneven course of PT, and a decrease in this indicator during the most intense clinical symptoms indicates the formation of the most severe degree of PT.

Thus, with a uneven course of severe PT, a low value of the GPO/CAT ratio indicated not only a predominant increase in CAT activity but also a high level of H_2O_2 in the cell and the possibility of a damaging effect of this reactive oxygen species on intracellular organelles and the cell plasma membrane.

The revealed dynamics of changes in the RG/GPO ratio in an uneven course of PT is probably associated with manifestation of greater oxidative stress, resulting in GPO inhibition by active oxygen metabolites and acidification of the intracellular environment in the acute period of the disease. Adequate function of GPO in the glutathione system can be implemented only with the timely active regeneration of oxidized glutathione in the GR reaction [16, 17, 19].

It follows from the above that not only the accumulation of lipid peroxidation products but also the level of RG in the cells significantly affects the severity of PT in pediatric patients, and the indicator of its level in erythrocytes in patients is highly informative in predicting the uneven course of moderate and severe forms of the disease already in the initial period.

Patients with moderate PT accounted for the major share of sick children, so it is most important to identify criteria that contribute to predicting a uneven disease course. Among the described

integral indicators, the best predictive criterion was the value of the RG/MDA ratio, since in the case of an uneven course of moderate or severe disease, with the disappearance of clinical symptoms during the period of early convalescence, a significant decrease was only recorded in the value of this index. In the convalescence phase, this indicator increased in patients with moderate and severe PT but did not reach control values.

The regularities revealed enable use of the calculation of the RG/MDA index to predict the dynamics and assess the severity of PT in pediatric patients in the initial period of the disease. The blood plasma concentration of MDA was determined; therefore, the state of lipid peroxidation processes in the body as a whole was assessed. The interpretation of the results obtained in blood erythrocytes reflects the intracellular processes of maintaining antioxidant balance.

The level of RG and MDA is technically simple and cost effective to determine, does not require the use of expensive equipment and reagents, and can be performed in conventional clinical diagnostic laboratories. The analysis requires no additional blood sampling, since blood plasma and erythrocyte mass can be used according to standard methods (coagulogram) using the patient's heparinized blood. The use of erythrocytes enables a sufficient amount of material to be obtained for research. All this makes the determination of the RG and MDA ratio very promising for widespread implementation in clinical practice.

Thus, by evaluating the integral indicators of the ratios of RG and MDA, RG and GPO, RG and GR, and GPO and CAT at the initial stage of the disease and during early convalescence, the nature of the course of PT can be predicted, and, if necessary, effective additional corrective antioxidant therapy should be prescribed.

Based on the data obtained, prognostically valuable criteria for an increase in the severity and the occurrence of a uneven disease course are a decrease in the acute period of PT in the absolute values of the ratios of RG/MDA below 17.0, of RG/GR below 38.0, and of RG/GPO below 12.0, as well as the lack of recovery of these indices to control values during the period of early convalescence.

When detecting a decrease in the level of RG in erythrocytes in the acute period of PT, it is necessary to determine further, with the attenuation of clinical symptoms, the full range of indicators of the glutathione system (RG level, and GR and GPO activity) and CAT activity to identify the degree of imbalance of the enzymatic link of antioxidant protection in pediatric patients with moderate and severe PT in order to predict the disease course.

Conclusion

Prognostically significant criteria for an increase in the severity and occurrence of a uneven course of pseudotuberculosis in pediatric patients included a decrease in the absolute values of the reduced glutathione/malondialdehyde ratios to less than 17.0 in the acute period of the disease, reduced glutathione/GR below 38.0, and reduced glutathione/glutathione peroxidase to less than 12.0, as well as the absence of an increase in these indices in the period of early convalescence.

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