**УДК [616.98:579.842.15:579.835.12] — 053.2 — 078:577.118**

**Microelement disturbances in children suffering from shigellosis and infected with Helicobacter pylori**

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In spite of progress achieved in treatment of infectious diseases, acute enteric infections (AEI) are still considered to be a significant problem in countries all over the world, as in the same way as before, they rank second in the structure of morbidity and mortality [1]. In this regard, 60-70% of all cases of enteric infections, where shigellosis rate is quite high [2], occur in children [3]. Dynamic monitoring of children suffering from shigellosis over the last few years is indicative of significant changes of its clinical presentation, increased number of lingering forms, and aggravated course of the disease. Probably, the apparent peculiarities do not result only from changes of the immune reactivity [3], impaired premorbidity background of children as well as the social level [4], but also from combined character of shigellosis with other diseases including infectious ones. The latter group of diseases is predominantly represented by Helicobacter infection. The infection rate of Helicobacter pylori (H. pylori) is quite high and it reaches 30-60% even in children, especially from social risk groups [5].

Over the last years, the issue of macro- and microelements in different pathologic conditions is rather challenging. It is due to the fact that most macro- and microelements are included as compounds of biologically active substances. They have direct or indirect influence on most metabolic and immune processes, determine functional condition of many organs and systems [6].

Microelements form a part of many enzyme body systems, including those ones, which take place in providing inflammatory and anti-inflammatory reactions. An example of such activity can be the system of antioxidants. The key chain of this system is superoxide dismutase enzyme catalyzing dismutation reaction, i.e. neutralization of superoxide radicals. One of the variants of superoxide dismutase is Cu/Zn-dependent one, which is found in the cytoplasm of cells [7].

The balance between activity of pro- and antioxidant enzymes is considered as an essential condition for optimal state and continuity of cellular membranes. Deficiency of microelements can lead to inadequate capacity of natural antioxidants [8, 9]. Another application point of microelements is matrix Zn-dependent metalloproteinases, the synthesis of which is stimulated by TNFα, IL-1β and some other factors [10, 11].

According to the current classification, based on influence on the immune system and performing immunophysiologic functions, Cu and Zn minerals belong to those ones, which are characterized by immunomodulatory impact. For example, zinc is a compound of more than 300 enzymes and it is of great importance in metabolism of nucleic acid, replication and regeneration of cells [12]. Zinc deficiency results in significant changes of functions of the thymus and, consequently, in loss of T-cell responses with increased susceptibility to infectious diseases [13]. Zn stimulates secretion of thymalin, which activates maturation of T-lymphocytes and regulates protective functions of mature T-cells. The zinc-containing enzyme Nucleoside Phosporylase, which participates in catabolism of purines, is of great importance in providing adequate functioning of T- and B-lymphocytes. Zn deficiency leads to impairment of phagocytosis, T-mediated cellular responses, proper antibody formation [14]. Many papers provide the facts that early phase of acute inflammatory response is accompanied by decreased concentration of Zn in blood serum [10, 9]. It has been revealed, that Zn deficiency in children aged up to 5 significantly increases risk of GI tract disorders. On the average, Zn deficit is observed in 31% of population (from 4 to 73% depending on the region). Considering these data and assessing the risk of mortality caused by the factors, one way or another associated with Zn deficit, the scientists consider it as the cause of 176000 diarrhea-related deaths per year [15].

One of the most important biometals, involved into immune reactions, is also copper. It is of great concern in antioxidant protection of the body, as in combination with Zn they are compounds of superoxide dismutase (Cu, Zn-superoxide dismutase), which is present in cytosol of erythrocytes and is considered as an important acceptor of O2, which is a free radical causing damage of membranes and other biological structures. Besides, copper deficiency leads to reduced antimicrobial activity of macrophages, as well it can be associated with humoral immunodeficiency [16]. Copper takes part in processes of biosynthesis of heme and, consequently, of hemoglobin. It is characterized by anti-inflammatory and antiseptic features (indirectly due to antioxidant activity) [17]. Increased content of copper in the body is observed in acute and chronic inflammatory diseases [11]. Some papers provide data concerning increased content of copper in blood plasma in case of recrudescence of chronic gastritis. Many researchers provided evidence that mucosal healing process in GI (gastrointestinal) tract diseases was characterized by an increase in copper [11]. Alongside this, irritation by external factors, stressful situations and infectious diseases generally cause increased content of copper and ceruloplasmin in blood, influencing the exchange of this metal partially through the neurohumoral system [13].

 The findings obtained due to nutritiological and immunologic studies made it possible to define general conditions of interrelation between adequate number of microelements and level of anti-infective protection of the human body. It is reliably known that inadequate number of macro- and microelements results in suppression of functional activity of many cells of the body, predominantly those ones, which are involved into providing of specific and non-specific mechanisms of anti-infective protection [10]. Consequently, adequate number of micro- and microelements is the essential condition of proper functioning of separate immunocompetent cells as well as the immune system in general.

The level of microelements of blood serum depends directly on gastric mucosa condition. Persistence of H. Pylori and inflammatory processes, caused by it in the stomach lining, can change its physiology and, consequently, influence homeostasis of vitamins and minerals [16]. This bacterium changes proper secretion of hydrochloric acid and precipitates development of achlorhydria in people who are infected. Due to such intervention into secretion of gastric acid H. Pylori can result in impaired assimilation of many nutrients and lead to different clinical manifestations of chronic disorders of nutrition. Some studies provide the evidence of interaction of H. Pylori with different extra GI tract disorders including deficiency of vitamin В12, folic acid, iron and some other micronutrients [18]. There are data concerning reduced Zn content in blood of patients suffering from peptic ulcer, gastritis and other GI tract diseases such as chronic gastroduodenitis, celiac disease, chronic enteritis. At the same time, increased amount of this bioelement was revealed in the gastric mucosa [9]. Differently directed character of change of Zn content in blood and stomach lining at the stage of recrudescence of gastroduodenal diseases is explained by researchers through the ability of metal to be redistributed from blood to tissues associated with increased demand for Zn in order to provide conditions for healing of injured stomach or intestinal lining. Some authors showed distinct interrelation between frequency of diarrheal disease episodes and low plasmatic level of coper and zinc. Besides, there are data proving the fact that copper deficiency is more widespread than zinc deficiency [19]. Zinc tends to be involved into competitive relations with some other metals. There is evidence concerning physiological antagonism of zinc and copper, which is explained by stimulation of synthesis of metallothioneins in the liver, where copper forms stronger compounds with protein [11, 18].

However, unfortunately there are no corresponding research data proving interrelation between H. Pylori infection and content of zinc and copper of serum in children. These minerals are properly assimilated in case they are located in the intestinal lumen in a soluble form. Taking into account the fact, that solubility of these cations is carried out in acid environment, sufficient secretion of hydrochloric acid and corresponding stomach acidity are principal factors for absorption of these minerals. Therefore, interrelation of Helicobacter infection and deficiency of such important micronutrients as zinc and copper seems to be possible.

**Purpose of the study** is assessment of serumlevels of zinc and copper in children suffering from shigellosis, infected with H. pylori.

**Materials and methods.** The study enrolled 89 children aged from 1 to 3, who were hospitalized in Regional children’s infectious diseases hospital (Kharkiv) and diagnosed with shigellosis. Diagnosis of shigellosis was established on the basis of clinical and epidemiological data and confirmed by bacteriological and serological methods. All children underwent additional examination aimed to detect presence of Helicobacter infection. Presence of H. pylori in the stool was detected by means of PCR technique and immunoenzymometric CITO TEST H.Pylori Ag. Besides, zinc and copper content in blood serum of all children was studied in acute period of the disease and in period of early convalescence. The study was carried out by means of the semiautomatic photometer BTS-330 Biosystems S. A. (Іспанія): copper (Lachema), zinc (Olvex). All children received medical treatment according to the currently approved clinical protocols.

The children were divided into two groups: Group 1 (21 children) represented by patients with shigellosis with confirmed infection with H. pylori and Group 2 (68 children) made up by patients with shigellosis infection without laboratory markers of Helicobacter infection. The children of both groups were compared according to age (3.15±1.15 and 3.04±1.27, p>0.05), sex and comorbidity. In order to compare levels of microelements of blood serum of patients, the diagnostic findings of 30 apparently healthy children (control group), obtained due to the thesis for a Candidate Degree of Medical Sciences defended by the post-graduate student of Infectious Diseases Department of KNMU T.S. Zharkova in 2010, were used. Statistical processing of the obtained data was carried out by means of Excel and Statistica 6.0 applications.

**Findings.** The study, which was carried out, made it possible to reveal differences in the content of microelements in blood plasma of children suffering from shigellosis with background infection and without it in different phases of the diseases. The level of microelements of blood serum in patients of both groups in acute phase of shigellosis is represented in Table 1.

Table 1

Content of microelements of blood serum in patients in acute phase of shigellosis (М±m, mcmol/l)

|  |  |  |  |
| --- | --- | --- | --- |
| Microelements | Group 1 (n=21) | Group 2 (n=68) | Control group (n=30) |
| Zinc | 9.5±0.561,2 | 11.07±0.361,2 | 12.32±0.45 |
| Copper | 17.28±0.462 | 14.24±0.471,2 | 18.35±0,28 |

Note: 1 — significance of evidence in control group, р˂0.05;

 2 — significance of evidence between groups, р˂0.05.

It was revealed that the levels of Zn in blood serum of children suffering from shigellosis in acute phase of the disease without regard to presence of background Helicobacter infection are significantly lower than similar indices of apparently healthy children: 9.5±0.56 and 11.27±0.36 mcmol/l in Group 1 and Group 2 respectively in comparison with 12.32±0.35 mcmol/l of the control group, p<0.05. Such changes can be explained due to the factor of early phase of acute inflammatory response which is accompanied by decreased concentration of serum Zn.

Rather different tendencies were detected in analysis of study of copper levels. The blood serum copper level in children with shigellosis without background infection was significantly lower than that one in apparently healthy children, i.e. 14.24±0.21 in comparison with 18.35±0.28 mcmol/l, p<0.05. In patients with Helicobacter infection this index was lower than the normal range (17.28±0.46 in comparison with 18.35±0.28 mcmol/l of the control group), but the difference turned out to be unreliable, p>0.05.

In addition, significant difference of levels of both microelements of blood plasma between the groups of patients under the study was assessed. Zinc content of blood serum of the patients infected with H. pylori was significantly lower than that one in the patients of Group 2 (9.5±0.56 in comparison with 11.27±0.36 mcmol/l, p<0.05). However, copper content in blood serum of children with background infection with H. pylori in acute phase of shigellosis was significantly higher and reached 17.28±0.46 mcmol/l in comparison with 14.24±0.21 mcmol/l in patients, who were not infected, p<0.05.

 The level of microelements of blood serum in patients in the period of early convalescence of shigellosis is represented in Table 2.

 Table 2

Content of microelements in blood serum of children with shigellosis in the period of convalescence, (М±m, mcmol/l)

|  |  |  |  |
| --- | --- | --- | --- |
| Microelements  | Group 1 (n=21) | Group 2 (n=68) | Control group (n=30) |
| Zinc | 9.8±0.491,2 | 12.16±0.332 | 12.32±0.45 |
| Copper | 17.89±0.39 | 17.32±0.59 | 18.35±0.28 |

Note: 1 — significance of evidence in control group, р˂0.05;

 2 — significance of evidence between groups, р˂0.05.

Comparing Zn content in children without background infection in the period of reconvalescence with similar data of the control group (12.16±0.33 and 12.32±0.45 mcmol/l), significant difference of indices was not revealed, p>0.05. The substantial difference of zinc levels between patients with Helicobacter infection and control group (9.8±0.49 in comparison with 12.32±0.45 mcmol/l, p˂0.05) as well between the patients of experimental groups (9.8±0.49 in comparison with 12.16±0.33 mcmol/l, p˂0.05) was observed.

Copper level in children infected with H. pylori in the period of early convalescence of shigellosis was not significantly different from indices of healthy children (17.89±0.39 in comparison with 18.35±0.28 mcmol/l, p>0.05) and reliably did not differ from the corresponding data of patients suffering from shigellosis without background infection (17.89±0.39 in comparison with 17.32±0.59 mcmol/l, p>0.05). In the period of convalescence substantial difference of copper content in the patients of Group 2 and the control group (17.32±0.59 in comparison with 18.35±0.28 mcmol/l, p>0.05) was not detected.

Analyzing dynamics of content of microelements of blood serum in children suffering from shigellosis, in the process of infectious course the following data were revealed. In spite of slightly increased concentration of zinc in patients with Helicobacter infection in the period of convalescence of shigellosis (9.8±0.49 in comparison with 9.5±0.56 mcmol/l), it did not reach the values in healthy children (9.8±0.49 in comparison with 12.32±0.45 mcmol/l, p<0.05). In the patients of Group 2 zinc content in the period of convalescence tended to be increased in comparison with indices of acute phase (12.16±0.33 in comparison with 11.07±0.36 mcmol/l, p<0.05) and was not significantly different from the indices of the control group (12.16±0.33 in comparison with 12.32±0.45 mcmol/l, p>0.05). Decreased indices of zinc content in the period of convalescence (especially in the patients of Group 1) can be indicative of impaired absorption as well as redistribution of Zn between plasma and mucosa, which is typical for Helicobacter infection.

Serum level of copper in the children of Group 1 in the period of convalescence was increased in comparison with acute phase, but without significant difference (17.89±0.39 in comparison with 17.28±0.46 mcmol/l, p˃0.05) and remained comparative with control group (17.89±0.39 in comparison with 18.35±0.28 mcmol/l, p˃0.05). In Group 2 significantly increased content of copper in blood serum of children in the period of convalescence in comparison with the control group (17.32±0.59 in comparison with 14.24±0.47 mcmol/l, p<0.05) as well as reaching indices of the control group (17.32±0.59 in comparison with 18.35±0.28 mcmol/л, p˃0.05) were recorded. The level of copper in plasma of the children with background infection was relatively high (in comparison with copper level of children who were not infected), that, on the one hand is typical for long-term inflammatory processes in the stomach, but, on the other hand, can be explained through competitive interrelations between the two microelements under study.

Therefore, assessment of zinc and copper content of serum in the children suffering from shigellosis is indicative of significant disorders in the system of microelement homeostasis in acute phase. Prior to the period of early convalescence, substantial increase of content of microelements occurs, but in children, infected with H. pylori, complete restoring of Zinc level is not observed. The data, which have been obtained, represent complicated character of restoring processes with absence of full recovery of functional features of GI tract in children infected with H. pylori, despite regression of clinical manifestations of shigellosis, and can be used for further improvement of treatment provided for such category of patients. These data are the ground for reasonable application of Zn-containing medical agents in combined therapy of shigellosis in children with Helicobacter infection.

Conclusions:

1. The course of shigellosis in children is accompanied by disturbance of content of basic blood serum microelements, i.e. copper and zinc.
2. Zinc deficiency of blood serum was observed in acute phase of shigellosis in children; in children infected with H. pylori this deficit was more pronounced in comparison with children who were not infected.
3. The course of acute phase of shigellosis in children without background infection is accompanied by significantly decreased levels of copper, while in children infected with H.pylori these levels are inconsiderable.
4. In children suffering from shigellosis, infected with H. pylori, decreased levels of Zn content are preserved in phase of early convalescence, which is considered as a ground for application of medical agents containing zinc not only within acute phase, but also at the stage of recovery.

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**Мікроелементні порушення у хворих** **на шигельоз та інфікованих Helicobacter pylori дітей**

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Інфікування Helicobacter pylori може змінювати клінічну картину, тривалість та перебіг інших інфекційних хвороб у дітей, в т.ч. шигельозу, а також впливати на рівень біометалів сироватки крові, які беруть участь в імунних реакціях. З метою дослідження сироваткових рівнів цинку і міді у хворих на шигельоз та інфікованих H. pylori дітей на базі Обласної дитячої інфекційної клінічної лікарні м. Харкова нами було обстежено 89 хворих на шигельоз дітей, з яких 21 дитина інфікована H. pylori. Виявлені значні порушення в системі мікроелементного гомеостазу в гострому періоді шигельозу. Визначено дефіцит вмісту цинку сироватки крові, який є більш вираженим у дітей, інфікованих H. рylori. Достовірне зниження рівнів міді спостерігали у дітей без фонового інфікування H. рylori. До періоду ранньої реконвалесценції відбувалось підвищення вмісту мікроелементів, але у дітей, інфікованих H. рylori, повного відновлення рівню цинку не відмічено. Отримані дані відображають складну динаміку відновних процесів при шигельозі з відсутністю повного відновлення функціональних характеристик шлунково-кишкового тракту у інфікованих H. рylori дітей, незважаючи на регресію клінічних проявів, і можуть бути використані для подальшого вдосконалення терапії хворих на шигельоз.

**Ключові слова:** шигельоз, Нelicobacter рylori, мікроелементи, цинк, мідь, діти.

**Microelement disturbances in children suffering from shigellosis and infected with Helicobacter pylori**

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Helicobacter pylori infection can change clinical presentation, duration and course of other infectious diseases in children including shigellosis as well as affect the level of biometals of blood serum involved in immune responses. In order to make an assessment of serum levels of zinc and copper in children with shigellosis and infected with H. pylori, the study enrolled 89 children suffering from shigellosis, where 21 children were infected with H. pylori. Significant changes in the system of microelement homeostasis in acute period of shigellosis were revealed. The deficiency of blood serum zinc content, more pronounced in children, was estimated. Significant reduction of levels of copper was observed in children without background H. pylori infection. Increase of content of microelements occurred prior to convalescence period. However, in children infected with H. pylori, complete recovery of zinc level was not noted. The data, which have been obtained, represent complicated character of restoring processes in shigellosis along with absence of complete restoring of functional features of GI tract in H. pylori children despite regression of clinical manifestations as well as they can be used for further improvement of treatment of patients suffering from shigellosis.

Key words: shigellosis, Helicobacter pylori, microelements, zinc, copper, children.

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