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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

EVALUATION OF SEVERAL IMMUNE PARAMETERS IN CHILDREN WITH IRON DEFICIENCY ANAEMIA

Specialty: 3220.01 – Pediatrics

Field of science: Medicine

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GENERAL CHARACTERISTIC OF THE RESEARCH

Relevance of the problem. Iron deficiency is still one of the main causes of anemia worldwide[1].

Iron deficiency anemia (IDA) is a common hematological problem among children, with a prevalence of 20.1% in developed countries between the ages of 0-4 years and 5.9% between the ages of 5-14 years, and 39,0% and 48.1% in developing countries, respectively[2].

It is known that iron is required for T-cell proliferation and effector functions, metabolic and reduction reactions, while heme and iron sulfate (Fe-S)-containing enzymes are essential for cell division and cytokine production[3].

Iron is considered an essential element not only for DNA synthesis and red blood cell formation but also for the integrity of the immune system[3]. However, a complex relationship between iron status and immune function has not been quite clarified [4] .

Iron deficiency affects the body's ability to adequately respond to infections, as iron is essential for the proliferation of immune cells and the specific response to infection. Despite evidence that functional

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[1] Moscheo, C. New Insights into Iron Deficiency Anemia in Children: A Practical Review / C. Moscheo, M. Licciardello, P. Samperi // Metabolites, – 2022. 12(4), – p. 1-33

[2] Wang, M. Global burden and inequality of iron deficiency: findings from the Global Burden of Disease datasets 1990-2017 / M. Wang, H. Gao, J. Wang [et al] // Nutrition Journal, –2022. 21, – p. 1-10.

[3] Cronin, S.J.F. The Role of Iron Regulation in Immunometabolism and Immune-Related Disease / S.J.F. Cronin, C.J. Woolf, G. Weiss [et al.] // Frontiers in molecular biosciences, – 2019. 6 (116), – p. 1-19.

[4] AlRajeh L. Effects of Iron Deficiency and Its Indicators on Lymphocyte Subsets: A Study at King Fahd Hospital of the University, Saudi Arabia / L. AlRajeh, A. Zaher, A. Alghamdi // Journal of blood medicine, – 2022. 13, – p. 61-67.

immunological defects can be corrected, the relationship between iron deficiency and susceptibility to infection remains controversial[5].

Despite numerous studies on IDA, timely detection of iron deficiency is considered one of the urgent problems facing medicine. Studies conducted in Azerbaijan also confirm the existence of the problem of anemia in the country.

The Ministry of Health of the Republic of Azerbaijan, in the final report of the Demographic and Health Survey for 2011, revealed the prevalence rate of anemia among 2,107 children aged 6-59 months[6]. Thus, 44.6% of children aged 6-59 months in Azerbaijan were shown to have varying degrees of anemia. Anemia was more prevalent among children living in rural areas (47.8%) than among children living in urban areas (41.5%).

In one of the studies, it is shown that IDA remains a public health problem among both children and women in Azerbaijan. When children aged 6-59 months were examined, the prevalence of anemia was 24.2%, the prevalence of iron deficiency in this age group was 15.0%, and the prevalence of iron deficiency anemia was 6.5%[7]. The relevance of iron deficiency anemia is due not only to its high prevalence but also to changes in the immune status of the child's body. Low iron levels in children lead to impaired immune function and recurrent infectious diseases of viral and bacterial origin. In most cases, long-term iron deficiency anemia leads to recurrent infections, especially acute respiratory infections[8].

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[5] Das, I. Impact of iron deficiency anemia on cell-mediated and humoral immunity in children: A case control study / I. Das, K.Saha, D. Mukhopadhyay [et al.] // Journal of natural science, biology, and medicine, – 2014. 5 (1), – p.158-63.

[6] Azərbaycan Respublikası Səhiyyə Nazirliyi Demoqrafiya və sağlamlıq sorğusu. Azərbaycan, 2011,Yekun hesabat 2013. – 302 s. (s. 151)

[7] Wirth, J.P. Micronutrient Deficiencies, Over and Undernutrition, and Their Contribution to Anemia in Azerbaijani Preschool Children and Non-Pregnant Women of Reproductive Age / J.P. Wirth, T. Rajabov, N. Petry [et al.] // Nutrients, –2018. 10(10), – p. 1-37. (p.1, p. 14)

[8] Jayaweera, J. Childhood iron deficiency anemia leads to recurrent respiratory tract infections and gastroenteritis / J. Jayaweera, M. Reyes, A. Joseph // Scientific reports, – 2019. 9 (1), – p. 1-8.

Thus, iron deficiency anemia affects humoral, cellular and nonspecific immunity and the activity of cytokines that play an important role in various stages of immune mechanisms[9].

Iron homeostasis plays a major role in the functioning of the immune system, including innate and adaptive immunity[10].

Recent advances in the study of lymphocyte subpopulation indicators and cytokine fractions have paved the way for the elucidation of new aspects affecting the pathogenesis of iron deficiency, as in many other diseases. Despite the fact that a number of studies were conducted on changes in the level of lymphocyte subpopulation indicators and cytokine fractions in blood serum during iron deficiency, the contradictory results obtained indicate the relevance of this issue. Although studies on IDA in children have been conducted in Azerbaijan, its relationship with the immune system has not been clarified. In this regard, the study of lymphocyte subpopulation indicators and cytokine fractions in children with IDA remains relevant.

Object and subject of the research. The object of the study was 95 children aged 6 months to 5 years with varying degrees of IDA, and the subject of the study was to evaluate the relationship between iron metabolism parameters, and immune parameters in children with IDA.

The purpose of the research. To study the effect of IDA on lymphocyte subpopulation indicators and some cytokine fractions in children.

Tasks of the research:

1. Study of risk factors - nutrition and family socioeconomic status- for the development of IDA in children aged from 6 months to 5 years;

[9] Шахвердиева, И.Д. Взаимосвязь между цитокинами и антимикробными пептидами у беременных женщин с анемией / И.Д. Шахвердиева, А.М. Эфендиев., И.А. Керимова [и др.] // Современные проблемы науки и образования. – 2019. № 3, – с. 1-10.

[10] Ni, S. Iron Metabolism and Immune Regulation / S. Ni, Y, Yuan, Y. Kuang [et al.] // Frontiers in immunology, – 2022. 23 March (13), – p. 1-11.

2. Evaluation of lymphocyte subpopulation indicators (CD3+, CD4+, CD8+, CD4/CD8, CD19+, CD56+) in the serum of children in the early childhood period with IDA according to the severity of the disease and determination of the correlation between immune indicators and iron metabolism indicators;
3. Identification of changes in cytokine fractions (INF-γ, TNF-α, IL-2) according to the severity of the disease in children with IDA and determination of the sensitivity and specificity of these indicators;
4. Conducting a comparative analysis of iron metabolism, and immunological parameters before and after 8-16 weeks of replacement therapy with iron supplements (iron (II) sulfate, iron (II) gluconate, iron (III) hydroxide polymaltose), as well as evaluating the effectiveness of the treatment.
5. Study of the incidence of respiratory tract infections in children with IDA before and after treatment.

Research methods: The examination methods included anamnestic (conducting a questionnaire survey with the mothers of 123 children), and clinical data. Besides, laboratory tests were performed – the evaluation of iron metabolism indicators (CBC and iron status parameters), total protein levels, C-reactive protein (CRP) levels, cytokine fractions in blood serum (IL-2, TNF-α, INF-γ), and markers of specific cellular components of the immune system, such as lymphocyte subpopulations (CD3+, CD4+, CD8+, CD4+/CD8+, CD19+, CD56+). Mathematical and statistical analysis of the results was performed.

The main provisions of the dissertation presented for defense:

- IDA negatively affects the functioning of immune indicators in early childhood;
- For the diagnosis of immune imbalance during IDA in young children, it is advisable to determine some CD+ markers and cytokine fractions in blood serum;
- For children with IDA, an increase in hematological indicators, positive dynamics of iron status indicators, and activation of cellular immunity indicators following treatment can be considered a satisfactory outcome;

– Evaluation of immune markers during IDA can be used to monitor the effectiveness of treatment measures in children.

Scientific novelty of the research:

– A negative impact of IDA on the activity of lymphocyte subpopulation parameters ($CD3^+$, $CD4^+$, $CD8^+$, $CD4^+/CD8^+$, $CD56^+$) and cytokine fractions (INF-γ, TNF-α, IL-2) in children was determined.

– Significant correlations were found between iron metabolism (HgB, serum iron, serum ferritin) and immunological parameters in children with IDA.

– The sensitivity, specificity, and cutoff points of cytokine fractions (INF-γ, TNF-α, IL-2) were determined to identify immune imbalance in children with IDA.

– A positive effect of replacement therapy with iron supplements on the function of immune parameters in children with IDA was observed.

Practical significance of the research

The elimination of risk factors, detection of latent iron deficiency, and implementation of preventive measures can contribute to the regulation of the immune system in children and help prevent potential complications.

Changes in cellular immunity (CD3+, CD4+, CD4+/CD8+, CD56+) leading to an increased frequency of respiratory infections highlight the necessity of timely diagnosis and treatment of IDA.

In cases of IDA in children, determining indicators such as INF-γ and IL-2, which exhibit high sensitivity and specificity, can serve as valuable parameters in pediatricians' clinical practice to identify the imbalance in cellular immunity.

Approbation: The main provisions of the dissertation were reported at scientific and practical conferences:

– Scientific and practical conference dedicated to the birthday of the outstanding statesman and scientist of Azerbaijan, Professor Aziz Mammadkarim Aliyev (Azerbaijan, Baku, 2022); Scientific and practical conference dedicated to the 100th anniversary of the national leader Heydar Alirza Aliyev – The XXXVII International Scientific Symposium- Haydar Aliyev and development strategy of Azerbaijan:

New Trends of Modernization (Eskishehir / Türkiye -Sheki / Azerbaijan 2023).

The discussion of the dissertation work was held at a joint meeting with the participation of the employees of the Department of Pediatrics of the Azerbaijan State Advanced Training Institute For Doctors named after A. Aliyev (protocol No. 9). The results of the study were also reported and discussed at the Scientific Seminar on the specialty 3220.01 - "Pediatrics" (protocol No. 7) operating under the ED 2.27 Dissertation Council of the Azerbaijan Medical University.

Publications on the topic of the research. Seven articles covering the results of the dissertation have been published (2 articles in foreign press, 1 article in SCOPUS indexed journal) in scientific-practical journals on the relevant list of the Higher Attestation Commission, and 6 theses (4 local, 2 foreign) in proceedings of scientific conferences in the last five years.

The organization where the research was performed: The Department of Pediatrics of the Azerbaijan State Advanced Training Institute For Doctors named after A. Aliyev.

Application of research results: The results of the research are applied in the practice of the pediatric department of the National Hematology and Transfusiology Center and the pediatric polyclinic department of the Lachin District Hospital. Besides, individual provisions of the dissertation have been included in the curriculum of the Pediatrics Department of the ASATID.

Structure and volume of the dissertation:

The dissertation contains 150 typed pages with the following sections: Introduction – 6 pages (10,175 characters), Literature Review – 28 pages (54,491 characters), Materials and Methods – 6 pages (10,671 characters), Chapter III – 912 pages (16,372 characters), Chapter IV – 23 pages (30856 characters), Chapter V – 8 pages (11,202 characters), Chapter VI – 23 pages (31,169 characters), Discussion and Conclusion – 14 pages (26,494 characters), Results- 2 pages (2147 characters), Practical recommendations – 1 page (999 characters). The Bibliography includes 201 sources. Of them, 19 are

local, 2 are Turkish, 6 are Russian, and 174 are other foreign sources. The dissertation also includes 24 figures and 22 tables.

The total volume of the dissertation (excluding spaces, title page, tables of contents, graphs, bibliography, and abbreviated terms) is 194,576 characters.

MATERIALS AND METHODS

Data collection was carried out at the National Center for Hematology and Transfusion during 2019-2020. The study included 123 children aged from 6 months to 5 years. A questionnaire survey was conducted with the mothers of 123 children and an initial examination of the children was performed. General examination and clinical examinations of the children were carried out in the pediatric outpatient department of the National Center for Hematology and Transfusion.

The main group (total IDA group) consisted of 95 children (58 boys [61.1%] and 37 girls [38.9%]) with varying degrees of IDA, who were born at term and had not received iron-containing supplements in the past year. The control group included 28 practically healthy children (16 boys [57.1%] and 12 girls [42.9%]).The mean age (in months) of the main group was 29.39±17.8, the mean age (in months) of the control group was 26.04±16.58. Statistical processing was performed using the Student's T-test, and no statistical difference was recorded between the main and control groups in terms of mean age (in months) ($t=0.140$, $p=0.891$, $p>0.050$).

The main group was divided according to the severity of IDA:

– mild IDA – 32 (33.68%) patients;

– moderate IDA – 37 (38.95%) patients;

– severe IDA – 26 (27.37%) patients.

During the analysis of nutrition, children included in the main and control groups were divided into groups of 6 months - 2 years (main group - 55 children, control group - 18 children), and 2-5 years (main group - 40 children, control group - 10 children).

During the analysis of height, weight, hematological and immunological indicators, children were divided into groups of 6

months - 1 year old (main group - 24 children, control group - 9 children), 1-2 years old (main group - 31 children, control group - 9 children), and 2-5 years old (main group - 40 children, control group - 10 children).

The main group of patients were divided into two groups based on the occurrence of respiratory system diseases: the group with respiratory system diseases (58 patients) and the group without respiratory system diseases (37 patients).

During the study, patients with premature birth (gestational age >36 weeks), anemia of chronic inflammatory diseases, acute inflammatory diseases, parasitic infections, hereditary diseases, genetic disorders, B12 and folic acid deficiency anemia were excluded.

Laboratory examinations used during the study were conducted in the clinical diagnostic laboratory of the National Center for Hematology and Transfusiology and the Central Scientific Research Laboratory of the Azerbaijan State Advanced Training Institute for doctors named after A. Aliyev.

To assess specific cellular components of the immune system, cell markers of T-lymphocytes (CD3+, CD4+, CD8+), B-lymphocytes (CD19+), NK-lymphocytes (CD56+) were determined using immunofluorescence-labeled commercial monoclonal antibody FITC reagents (Sorbent Company, Russia), and microscopy was performed on a Lyuma PI microscope.

The BioScreen MS-500 semi-automatic device (USA) and Vector Best (Russia) reagent were used to determine the number of cytokine fractions (IL-2, TNF-α, INF-γ) in blood serum by the Enzyme immunoassays (EIA) method.

In the main group, after 8-16 weeks of replacement therapy with iron supplements CBC, iron metabolism, and immune parameters were re-evaluated in 35 children.

The “EXCEL-2016” spreadsheet and the “SPSS Statistics” package program were used to analyze the obtained indicators. In the processing of quantitative indicators in the groups, non-parametric Mann-Whitney U), Pearson's goodness-of-fit criterion – $\chi 2$ (Chi-square Pearson), Fisher (F), Student's t criteria, Kruskal Wallis H,

ROC-analysis (Receiver Operating Characteristic), Spearman's correlation coefficient and 95% confidence interval (CI) were applied. The difference between the compared indicators was considered statistically significant if $p<0.050$.

RESULTS AND DISCUSSION

In the course of the research, we analyzed the features of the nutrition of children aged from 6 months to 2 years and 2-5 years old, included in the main and control groups.

Despite the low concentration of iron in breast milk (0.4 mg/l), it is considered an important nutrient for the developing infant's organism. According to anamnestic data obtained from mothers, 25.5% of children in the main group (6 months to 2 years) and 72.2% in the control group were exclusively breastfed during the first 6 months of their life ($p_{\chi^2}<0.001$, $p_F<0.050$).

The intake of non-adapted foods in addition to breast milk was 12.7% in the main group contrary to the control group ($p_{\chi^2}=0.112$, $p_F>0.050$).

The study revealed that 76.2% of breastfed children aged from 6 months to 2 years in the main group and 18.8% of children in the control group did not receive IDA prophylaxis during the first 6 months of life ($p_{\chi^2}<0.001$, $p_F <0.050$).

It was clear that among the children in the study group, cow's milk was preferred for complementary feeding and cow's milk in drinking form was started earlier than 12 months, which was 47.3% and 11.1% in the main and control groups, respectively ($p_{\chi^2}=0.007$, $p_F<0.050$).

Children who consumed meat products daily according to age were 58.2% and 88.9% in the main and control groups, respectively ($p_{\chi^2}=0.018$, $p_F<0.050$).

According to the anamnestic data, a lack of iron-rich products was observed in the diet of children aged 2-5. Thus, the intake of red meat in the main and control groups was 42.5% and 100.0%, respectively ($p_{\chi^2}=0.002$, $p_F<0.050$).

The analysis of the families' socio-economic status revealed that among the mothers of children aged 6 months to 5 years in the main and control groups, respectively, 70.5% and 35.7% had completed primary or secondary education ($p_{\chi 2}<0.001$, $p_F<0.050$). It was found that 76.8% of children in the main group and 28.6% in the control group belonged to low-income families ($p_{\chi 2}<0.001$, $p_F<0.050$).

Characteristics of hematological and immunological indicators of children based on the severity of IDA. The study üas investigated changes in hematological parameters in children with IDA aged 6 months to 5 years. According to the results, in the total IDA group, compared to the control group, the level of Hgb concentration decreased by 1.4 times, the erythrocyte count decreased by 1.3 times, MCH and MCV decreased by 1.2 times, and the hematocrit (Hct) level decreased by 1.5 times, with all differences being statistically significant ($p_u<0.001$).

The erythrocyte sedimentation rate (ESR) and platelet (PLT) indicators were 1.4 times higher in the main group compared to the control group ($p_u<0.001$, $p_H<0.001$). The total protein level was statistically significantly lower in the main group, 1.1 times lower than in the control group ($p_u<0.001$, $p_H<0.001$). The lower level of this indicator in the main group may be associated with the lower consumption of protein-based products, especially meat products, during the children's nutrition. No difference was observed in the results for CRP in the main group compared to the control group ($p_u=0.108$).

The serum iron, transferrin saturation, and serum ferritin levels in the total IDA group were statistically significantly lower compared to the control group, by 1.9 times, 2.6 times, and 6.4 times, respectively ($p_u<0.001$), while total iron binding capacity (TIBC) and latent iron binding capacity (LIBC) were statistically significantly higher, by 1.4 times and 1.7 times, respectively ($p_u<0.001$). Significant correlations were detected between HgB and serum iron, transferrin saturation coefficient, and serum ferritin ($\rho=0.771$, $p<0.001$; $\rho=0.832$, $p<0.001$; $\rho=0.710$, $p<0.001$, respectively). The obtained results confirm the diagnosis of IDA in the main group.

We analyzed the changes in immune parameters in the IDA groups (Table 1), and in the main and control groups overall (Table 2) based on severity.

Table 1. Immune indicators in the main group according to the severity of IDA

Indicators	Mild degree of IDA (n=32)	Moderate dgree of IDA (n=37)	Severe degree of IDA (n=26)	p_{u1}	p_{u2}	p_H
CD3+%	54.7±2.4	49.3±1.7	45.4±3.7	<0.001	<0.001	<0.001
CD4+%	31.5±1.3	27.4±1.4	25.0±2.3	<0.001	<0.001	<0.001
CD8+%	23.2±1.8	21.5±1.7	20.5±2.3	<0.001	0.142	<0.001
CD4/CD8	1.37±0.11	1.28±0.12	1.23±0.16	0.002	0.042	0.011
CD19+%	28.5±2.5	29.6±3.8	31.1±1.4	0.006	0.053	<0.001
CD56+%	12.5±0.7	12.0±1.1	11.1±1.2	0.013	0.002	<0.001
INF-γ, pg/ml	2.47±0.48	1.60±0.31	0.81±0.16	<0.001	<0.001	<0.001
TNF-α, g/ml	2.72±0.92	3.32±1.00	4.00±0.94	<0.001	<0.001	<0.001
IL-2, pg/ml	2.11±0.63	1.55±0.35	0.49±0.23	<0.001	<0.001	<0.001

Note: statistical integrity $p_u<0.050$ (According to the Mann-Whitney U test) p_{u1}- between mild and moderate IDA groups; p_{u2}- between moderate and severe IDA groups; p_H - The level of significance of the Kruskal-Wallis H test among groups ($p<0.050$)

The relative number (%) of CD3+ (all T-lymphocytes) a factor of adaptive cellular immunity, was 54.7±2.4% in the mild IDA group, 49.3±1.7% in the moderate IDA group, 45.4±3.7% in the severe IDA group, 50.1±4.5% in the total IDA group, and 64.8±3.3% in the control group. Based on the evaluation, the relative number of CD3+ cells in the moderate IDA group was statistically significantly lower than in the mild IDA group ($p_{u1}<0.001$) and in the severe IDA group was statistically significantly lower than in the moderate IDA group ($p_{u2}<0.001$, $p_H<0.001$), the Kruskal-Wallis-H test among the groups was statistically significant ($p_H<0.001$), (Figure 1). A statistically significant decrease of 1.3 times was observed in the total IDA group compared to the control group ($p_u<0.001$).

In the main group, a significant correlation was established between the relative number of CD3+ cells and iron metabolism

parameters (HgB, serum iron, and serum ferritin) respectively; ρ=0.894, p<0.001 and ρ=0.785, p<0.00, ρ=0.736, p<0.001). The results obtained suggest a deficiency of T-cell defense mechanisms that carry out immunological control of antigen homeostasis in the body.

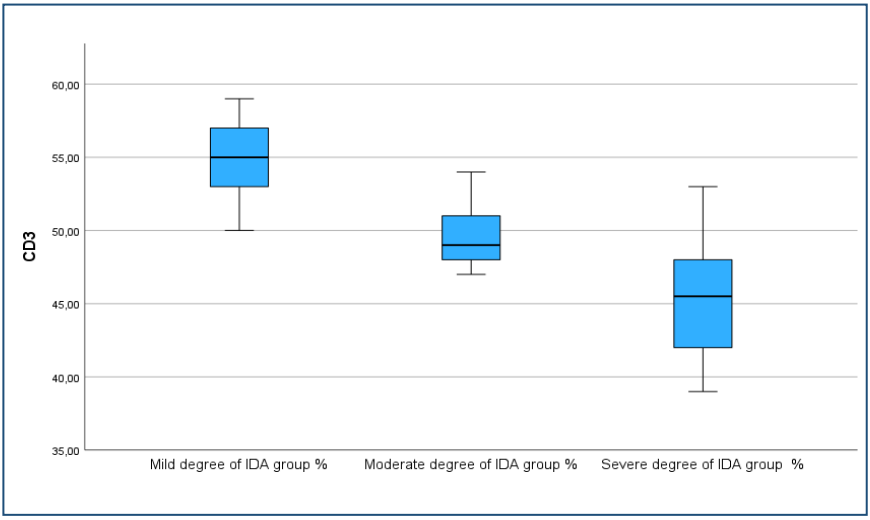


Figure 1. Comparative analysis of CD3+ indicators between IDA groups

The relative number of T-helpers (CD4+) was 31.5±1.3%, 27.4±1.4%, 25.0±2.3%, 28.1±3.1%, and 39.2±4.1% in the mild, moderate, severe, total, and control groups, respectively. A statistically significant decrease was also observed in the relative number of CD4+ as the severity of IDA increased (p_{u1}<0.001, p_{u2}<0.001, p_H<0.001). A statistically significant 1.4-fold decrease was observed in the total IDA group compared to the control group (p_u<0.001). There were also significant correlations between the relative number of CD4+ cells and HgB, serum iron, and serum ferritin. (ρ=0.881, p<0.001, ρ=0.820, p<0.001 ρ=0.698, p<0.001). T-helpers are cells that regulate the strength of the body's immunological response to foreign antigens, ensure the stability of the body's internal environment, and increase antibody production. A decline in the

relative number of CD4+ cells increases the likelihood of impaired cellular immunity in children with IDA.

Table 2. Evaluation of immune indicators in the main and control groups

Indicators	Control group (n=28)	Main group (total IDA) (n=95)	p_u
CD3+%	64.8±3.3	50.1±4.5	<0.001
CD4+%	39.2±4.1	28.1±3.1	<0.001
CD8+%	25.3±2.4	21.8±2.2	<0.001
CD4+/CD8+	1.56±0.22	1.30±0.14	<0.001
CD19+%	19.0±1.8	29.6±3.0	<0.001
CD56+%	14.9±1.4	11.9±1.1	<0.001
INF- γ pg/ml	3.04±0.22	1.74±0.67	<0.001
TNF- α pg/ml	1.74±0.55	3.30±1.07	0.003
IL-2 pg/ml	2.86±0.22	1.45±0.70	<0.001

Note:p_u- statistical significance of the difference between groups ($p<0.05$) (According to the Mann-Whitney U test)

The relative number of T-cytotoxic suppressors with the CD8+ surface marker decreased statistically significantly ($p_{u1}<0.001$) in the moderate IDA group compared to the mild IDA group, and statistically insignificantly ($p_{u2}>0.142$) in the severe IDA group compared to the moderate IDA group. The Kruskal-Wallis-H test was statistically significant among the groups ($p_H<0.001$). The latter indicator decreased statistically significantly by 1.2 times in the total IDA group compared to the control group ($p_u<0.001$). In the total IDA group, moderate positive correlations were formed between CD8+ cells and HgB, serum iron, and serum ferritin (respectively, $\rho=0.586$, $p<0.001$, $\rho=0.521$, $p<0.001$, $\rho=0.506$, $p<0.001$). The results obtained suggest that the cytotoxic activity of T-lymphocytes is weakened during IDA.

Changes in the level of CD4+ and CD8+ indicators caused an inversion of the CD4+/CD8+ immunoregulatory ratio and it was statistically significantly reduced by 1.2 times in the total IDA group compared to the control group ($p_u<0.001$). The correlations of the

CD4+/CD8+ immunoregulatory ratio with HgB, serum iron, and serum ferritin were: ρ=0.326, p=0.001, ρ=0.278, p=0.006, and ρ=0.167, p=0.107, respectively.

CD19+ B lymphocytes are indicators of humoral immunity and in our study, their levels increased by 1.6 times compared to the control group, reaching 29.6±3.0% in the total IDA group and 19.0±1.8% in the control group (p<0.001). This increase can be viewed as a compensatory increase in response to the decrease in the relative number of cellular immunity indicators and can be associated with the T-cell deficit identified during the study. In the total IDA group, a negative correlation of moderate strength was obtained between CD19+ and HgB, serum iron and serum ferritin (respectively, ρ=-0.536, p<0.001; ρ=-0.530, p<0.001; ρ=-0.482, p<0.001).

A statistically significant decrease in the relative number of NK-natural killers with the CD56+ surface marker was observed among the IDA groups (p_{u1}<0.001, p_{u2}<0.001, p_H<0.001). The level of CD56+ cells decreased by 1.3 times compared to the control group, reaching 11.9±1.1% in the general DDA group and 14.9±1.4% in the control group (p_u<0.001). In the main group, moderate positive correlations were formed between CD56+ cells and HgB, serum iron, and serum ferritin (respectively, ρ=0.547, p<0.001, ρ=0.517, p<0.001, ρ=0.460, p<0.001). Based on the results obtained, it can be assumed that there is a weakening in the cytolytic activity of CD56+ cells.

The results suggest that the functional activity of both innate (CD56+) and adaptive (CD3+, CD4+, CD8+, CD4+/CD8+) components of cellular immunity is likely weakened due to the impact of İDA.

According to clinical anamnestic data, the risk of upper and lower respiratory tract infections in children with IDA was higher than in the control group. The annual incidence (more than 4 times a year) of acute respiratory viral infections (ARVI) in the mild, moderate, severe, total IDA, and control groups was 34.4%, 64.9%, 88.5%, 61.1%, and 17.9%, respectively. A statistical difference was recorded between the mild and moderate ($p_{\chi 2}$=0.012 p_F<0.050), moderate and severe ($p_{\chi 2}$=0.035, p_F<0.050), total IDA and control groups ($p_{\chi 2}$=0.010, p_F<0.050).

The incidence of lower respiratory tract infections (pneumonia) was not observed in the mild IDA and control groups, while it was 16.2% in the moderate IDA group, 42.3% in the severe IDA group, and 17.9% in the total IDA group. A statistical difference was recorded between the mild and moderate ($p_{\chi 2}$=0.018, p_F<0.050), moderate and severe ($p_{\chi 2}$=0.022, p_F<0.050), and total IDA and control groups ($p_{\chi 2}$=0.016, p_F<0.050).

Thus, the high incidence of upper and lower respiratory tract infections in children with IDA may be related to a deficiency in cellular immunity.

Some cytokine fractions (INF γ, TNF- α, IL-2) were also analyzed in the study groups. INF-γ in the mild, moderate, severe, total IDA, and control groups was 2.47±0.48 pg/ml, 1.60±0.31 pg/ml, 0.81±0.16 pg/ml, 1.74±0.67 pg/ml, and 3.04±0.22 pg/m, respectively. The level of INF-γ in the moderate IDA group decreased by 1.5 times (p_u <0.001) compared to the mild IDA group, in the severe IDA group by 2.0 times (p_u<0.001) compared to the moderate IDA group, and in the total IDA group by 1.7 times (p_u<0.001) compared to the control group. The Kruskal-Wallis H test was statistically significant between the IDA groups (p_H<0.001). INF-γ is considered an important factor in innate and adaptive cellular immunity, as it is synthesized by natural killers – CD56+, CD4+ Th1 cells. In our study, the low level of INF-γ may be associated with a decrease in the relative number of CD4+ and CD56+ cells during IDA. The correlations between INF-γ HgB, serum iron, and serum ferritin were ρ=0.890, p<0.001; ρ=0.682, p<0.001; ρ=0.604, p<0001, respectively (Figure 2).

TNF-α levels were also analyzed in the study groups. It was found to be 2.72±0.92 pg/ml in the mild DDA group, 3.32±1.00 pg/ml in the moderate DDA group, and 4.00±0.94 pg/ml in the severe DDA group. A statistically significant 1.2-fold increase was observed in the moderate DDA group compared to the mild DDA group and in the severe DDA group compared to the moderate DDA group (p_{u1}<0,001, p_{u2}<0,001, p_H<0,001). The TNF-α indicator increased 1.9 times compared to the control group, reaching 3.30±1.07 pg/ml in the total DDA group and 1.74±0.55 pg/ml in the control group (p_u=0.003).

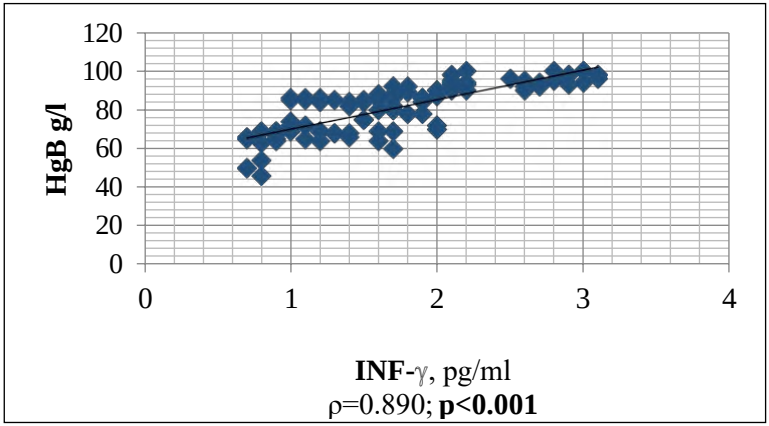


Figure 2. Correlation between IFN-γ and HgB indicators

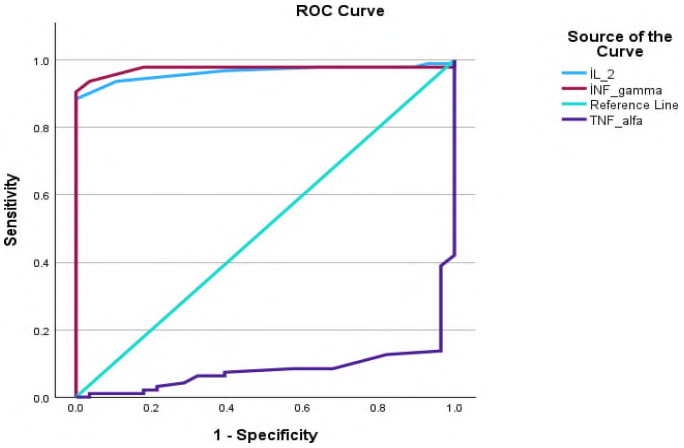
TNF-α showed a weak negative correlation with HgB, serum iron, and serum ferritin, respectively; ρ=-0.454, p=0.001; ρ=-0.323; p=0.001, ρ=-0.310; p=0.002. Since TNF-α is a pro-inflammatory cytokine, its elevated levels can be associated with inflammatory processes that may occur during IDA.

Considering that IL-2, as an immunomodulatory cytokine, controls intracellular signaling pathways in T cells, IL-2 levels were also evaluated during the study. These levels were 2.11±0.63 pg/ml, 1.55±0.35 pg/ml, 0.49±0.23 pg/ml, 1.45±0.70 pg/ml, and 2.86±0.22 pg/ml in the mild, moderate, severe, total IDA and control groups, respectively. The level of IL-2 indicator decreased statistically significantly by 1.4 times in the moderate IDA group compared to the mild IDA group (p_u<0.001), by 3.0 times in the severe IDA group compared to the moderate IDA group (p_u <0.001), and by 2.0 times in the total IDA group compared to the control group (p_u <0.001). The Kruskal-Wallis H test was statistically significant among IDA groups (p_H<0.001). IL-2 showed a positive correlation with HgB, serum iron, and serum ferritin, respectively; ρ=0.810, p<0.001; ρ=0.712, p<0.001; ρ=0.711, p<0.001.

Since INF-γ and IL-2 are cytokines involved in the cellular immune response, a decrease in their levels suggests a weakening in the cellular immune response during IDA.

In the study, the level of INF-γ in the group of patients with respiratory infections was 1.6 times lower (pu > 0.103) compared to the group without infections (main group of 58 and 37 patients, respectively) and 2.2 times lower (pu < 0.006) compared to the control group. The level of TNF-α was 1.3 times higher and 2.0 times higher, respectively (pu = 0.001, pu < 0.001). The level of IL-2 was 1.6 times lower and 2.4 times lower, respectively (pu = 0.039, pu < 0.001).

ROC statistical analysis was used to determine the specificity and sensitivity of INF-γ, IL-2, and TNF-α indicators during IDA in children (Figure 3).



Parameters	Area	Standard error	Stat. sig. (p)	95% Confidence Interval	
				Lower bound	Upper bound
INF- γ	0.974	0.015	<0.001	0.944	1.003
TNF-α	0.083	0.026	<0.001	0.033	0.133
IL-2	0.983	0.016	<0.001	0.932	0.996

Figure 3. AUC-ROC indicators of immune parameters

Based on the ROC curve, the area of INF-γ was 0.910±0.026 pg/ml (95% CI: 0.859-0.961, p<0.001), the area of TNF-α was 0.784±0.041 pg/ml (95% CI: 0.703-0.866, p<0.001), and the area of

IL-2 was 0.984±0.09 pg/ml (95% CI: 0.968-1.001, p<0.001). The cut-off points for INF-γ, TNF-α, and IL-2 during IDA in children were <2.35 pg/ml, >3.25 pg/ml, and <1.85 pg/ml, respectively. INF-γ, TNF-α, and IL-2 showed 82,1%, 42,1%, and 71,6% sensitivity, 100.0%, 100%, and 100.0% specificity, respectively. Based on the data in the ROC curve, the high sensitivity and specificity of INF-γ and IL-2 during IDA in children can be considered a useful parameter for determining the imbalance in cellular immunity. Although the sensitivity of the TNF-α indicator is low, a comprehensive analysis of the results may be important.

Comparative analysis of hematological, iron status and immune parameters in children with IDA before and after treatment.

Hematology, iron status, and immune parameters were re-evaluated in 35 children with IDA after oral administration of 3-6 mg/kg (elemental iron) of iron-containing (iron (II) sulfate, iron (II) gluconate, iron (III) hydroxide polymaltose) preparations (aktiferrin, totema, ferrum lek) for 8-16 weeks.

Hematological parameters – HgB concentration and hematocrit increased 1.5 times statistically significantly after treatment compared to the pre-treatment group; erythrocytes, Hct and MCH increased 1.2 times after treatment compared to the pre-treatment group; MCV increased 1.1 times statistically significantly after treatment compared to the pre-treatment group, the difference between the groups for all parameters was statistically significant (p_w<0.001), The ESR and PLT indicators have decreased by 1.4 times, respectively (pw < 0.001), and all indicators have approached the control group indicators. The total protein level increased by 1.1 times after treatment compared to the pre-treatment group (p_w<0.001), while no statistically significant difference was observed in the results for CRP levels (p_w = 0.215).

After treatment, the iron metabolism indicators - serum iron increased by 1.7 times compared to the pre-treatment group (p_w<0,001); TIBC decreased by 1.5 times and LIBC decreased by 1.7 times compared to the pre-treatment group (p_w<0,001); transferrin saturation coefficient increased by 2.4 times (p_w<0,001)and serum

ferritin increased by 4.8 times compared to the pre-treatment group (p_w<0,001), and all indicators approached the control group indicators.

Lymphocyte subpopulation parameters were evaluated in the main group (35 children) before and after treatment (Figure 4).

In the group of children with IDA, the relative number of CD3+ (all T-lymphocytes) lymphocytes was 49.4±4.2% before treatment and 60.0±3.5% after treatment. The relative number of T-helpers (CD4+) was 27.5±2.6% and 35.7±3.2%, respectively. In 32 patients, the relative number of CD3+ and CD4+ cells increased compared to the pre-treatment group. In 1 patient, it decreased, and no change was detected in 2 patients. The results were statistically significant (p_w<0.001). The relative number of T-cytotoxic suppressors with the CD8+ surface marker was 21.4±2.1% before treatment and 24.2±1.8% after treatment (p_w<0.001).

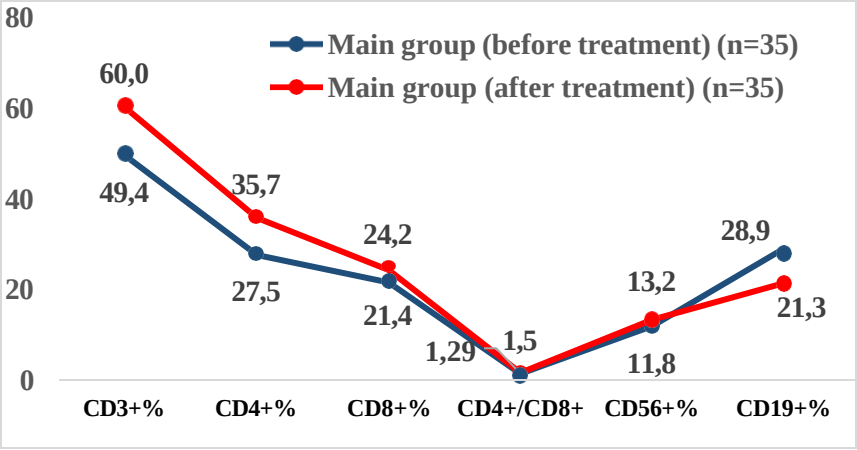


Figure 4. Evaluation of lymphocyte subpopulation indicators in the dynamics of treatment in the main group

The relative number of CD8+ lymphocytes increased in 28 patients after treatment compared to the pre-treatment group, decreased in 4 patients, and remained unchanged in 3 patients. The difference between the groups was statistically significant (p_w<0.001). The ratio of CD4+/CD8+ immunoregulatory factor was 1.29±0.11

before treatment and 1.50±0.11 after treatment. The immunoregulatory factor increased in 32 patients after treatment and decreased in 3 patients. The difference between the groups was statistically significant (p_w<0.001).

The relative number of CD19+ B lymphocyte cells, an indicator of humoral immunity, was 28.9±4.3% before treatment and 21.3±2.5% after treatment (p_w<0,001). The latter indicator increased in 2 patients and decreased in 33 patients, approaching the control group indicators.

The relative number of CD56+ cells before and after treatment was 11.8±1.2% and 13.2±1.4%, respectively. In 7 patients, the relative number of CD56+ cells did not change, and in 28 patients, an increase in the relative number of natural killer cells was observed after treatment. The difference between the groups was statistically significant (p_w<0.001). This suggests that CD56+ cells have an active cytolytic activity.

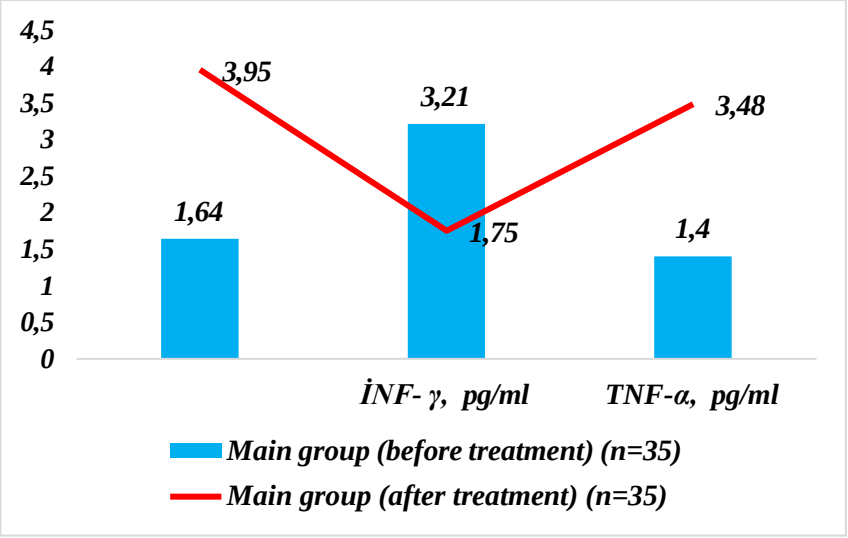


Figure 5. Cytokine fractions in the treatment dynamics in the main group

The INF-γ indicator before and after treatment was 1.64±0.84 pg/ml and 3.95±0.48 pg/ml (pw<0.001), respectively, increasing in all

patients and approaching the control group indicators. The positive dynamics of the INF-γ indicator can be evaluated as an effective result of the treatment. The values of the TNF-α indicator before and after treatment were 3.21±1.13 pg/ml and 1.75±0.41 pg/ml, respectively. A decrease in TNF-α was observed in 32 patients included in the group, and there was no change in 3 patients (p_w<0.001). Since TNF-α is an inflammatory cytokine, the change can be attributed to the inflammatory process that may occur during IDA. The decrease in the amount of the indicator after treatment can be attributed to the positive effect of the treatment. IL-2 was 1.40±0.86 pg/ml before treatment and 3.48±0.49 pg/ml after treatment. IL-2 indicator increased statistically significantly in all patients included in the group after treatment (p_w<0.001).

The incidence of upper and lower respiratory tract infections was assessed in the main group before and after treatment. The annual incidence of ARVI (more than 4 times a year) was 17 patients (48.6%) in the pre-treatment group and 2 patients (5.7%) in the control group. A statistical difference was recorded between the groups ($\chi 2$=16,254, $p_{\chi 2}$<0.001, F<0.001, p_F<0.050).

The incidence of lower respiratory tract infections (pneumonia) was 8 patients (22.3%) in the main group before treatment but none in the post-treatment group. A statistical difference was recorded between the groups ($\chi 2$=9.032, $p_{\chi 2}$=0.003, F=0.002, p_F<0.050).

A questionnaire survey was conducted for all children included in the study group and an initial examination of the children was performed. The results of subjective and objective examinations of patients included in the main group before and after treatment were examined.

In the main group, the complaints of weakness and loss of appetite decreased after treatment compared to before treatment by 3.3 times (57.1% to 17.1%) and 4.3 times (85.7% to 20.0%), respectively ($p_{\chi 2}$<0.001, p_F<0.050).

Complaints of hair loss and sleep disturbances decreased after treatment by 7.5 times (42.9% to 5.7%) and 4.3 times (37.1% to 8.6%), respectively ($p_{\chi 2}$<0.001, p_F<0.050).

During the objective examination, dry skin was observed 5.5 times less frequently (62.9% to 11.4%), and pale skin tone was observed 6.8 times less frequently (77.1% to 11.4%) after treatment compared to before treatment ($p_{\chi 2}<0.001$, $p_F<0.050$).

Thus, a statistically significant increase in the relative number of cellular immunity indicators (CD3+, CD4+, CD4+/CD8+, CD56+) was observed ($p_w<0.001$).

After taking the prescribed iron-containing preparations, the levels of INF-γ and IL-2 in the main group increased statistically significantly by 2.6 and 2.8 times, respectively, after treatment compared to the pre-treatment period ($p_w<0.001$).

The level of TNF-α indicators decreased by 1.9 times statistically significantly after treatment compared to the pre-treatment period ($p_w<0.001$).

Thus, the positive outcome of replacement therapy conducted with iron supplements, may prevent future complications of the disease from an immunopathological point of view. After treatment, an increase in hematological indicators, positive dynamics of iron metabolism indicators, and activation of cellular immunity can be considered as a satisfactory outcome of treatment.

The decrease in the frequency of complaints and respiratory diseases of patients in the main group after treatment with iron preparations may be attributed to the positive effect of the treatment on the activity of immune indicators.

CONCLUSIONS

1. A lack of iron-rich foods was observed in the diet of children with IDA included in the main group of children aged from 6 months to 5 years ($p_{\chi 2}<0.050$; $p_F<0.050$). In the main group, 76.2% of breastfed children aged 6 months - 2 years did not receive IDA prophylaxis during the first 6 months. Analysis of socio-economic status revealed that 76.8% of children with IDA were from low-income families ($p_{\chi 2}<0.001$; $p_F<0.050$) [2].

2. With the increasing severity of IDA in children, the relative number of lymphocyte subpopulation indicators - components of cellular immunity (CD3+, CD4+, CD8+, CD4+/ CD8+, CD56+) statistically significantly decreased compared to the control group (p_u<0.050); positive correlations were found between the relative number of CD3+ and CD4+ cells with HgB, serum iron and serum ferritin (respectively, ρ=0.894 p<0.001; ρ=0.785, p<0.001; ρ=0.736, p<0.001 and ρ=0.881, p<0.001; ρ=0.820, p<0.001; ρ=0.698, p<0.001) [6; 8; 13].
3. The levels of INF-γ and IL-2 in the main group were 1.4 times and 2.0 times statistically significantly lower than in the control group, respectively (p_u<0.050). INF-γ and IL-2 positively correlated with HgB, serum iron and serum ferritin (respectively, ρ=0.890, p<0.001; ρ=0.682, p<0.001; ρ=0.604, p<0.001 and ρ=0.810, p<0.001; ρ=0.712, p<0.001; ρ=0.711, p<0.001). According to the ROC curve, the cut-off points for INF-γ and IL-2 indicators were found to be 2.35 ng/ml and 1.85 ng/ml, the sensitivity – 82,1% and 71,6%, and the specificity -100.0% and 100.0%, respectively [3;13].
4. After taking iron supplements for 8-16 weeks, cellular immunity indicators (CD3+, CD4+, CD4+/CD8+, CD56+, INF-γ, IL-2) in the main group showed a statistically significant increase (p_w<0.050) compared to the pre-treatment period and approached the levels of the control group [4; 5; 11; 13].
5. The high incidence of respiratory tract infections in children with IDA is associated with a deficiency in cellular immunity. Among children in the main and control groups, acute respiratory viral infections (more than 4 times a year) were observed in 61.1% and 17.9%, respectively ($p_{\chi 2}$<0,001; p_F<0,050), pneumonia was not observed in the control group, while in the total IDA group, it was 17.9% ($p_{\chi 2}$=0,016; p_F<0,050). The decrease in the incidence of respiratory tract diseases after treatment with iron preparations (p_F<0.050) is attributed to the positive effect of treatment on the activity of immune indicators [7; 9].

PRACTICAL RECOMMENDATIONS

1. The increase in respiratory infections associated with changes in the immune system (CD3+, CD4+, CD8+, CD4+/CD8+, CD56+) in children with iron-deficiency anemia necessitates the timely diagnosis and treatment of iron deficiency.
2. The high sensitivity and specificity of IL-2 and IFN-γ indicators can be used in pediatric practice as diagnostic and prognostic parameters for respiratory complications caused by immune system deficiencies in children with IDA.
3. Children from socio-economically disadvantaged families, those with a diet deficient in iron-rich products, and children with iron-deficiency anemia who experience respiratory infections more than four times a year should be included in the risk group and monitored under special supervision during dispensary observation.
4. To prevent the long-term consequences of IDA, screening measures aimed at early detection of iron deficiency should be conducted among children in the risk group. Pediatricians should strengthen awareness efforts with parents and implement preventive strategies to prevent IDA.

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ABBREVIATIONS

CBC - complete blood cell
CRP - c-reactive protein
ESR - erythrocyte sedimentation rate
Hct - hematocrit
HgB - hemoglobin
IDA - iron deficiency anemia
IL- interleukin
INF - γ-interferon gamma
LIBC - latent iron binding capacity
MCH - mean corpuscular hemoglobin
MCHC - mean corpuscular hemoglobin concentration
MCV - mean corpuscular volume
TIBC - total iron binding capacity
TNF-α - Tumor necrosis factor-alpha
WHO - World Health Organization
ROC - (Receiver Operating Characteristic)

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