

ORIGINAL ARTICLE

CONTENTS

Violation of vitamin and mineral homeostasis in children with recurrent respiratory diseases

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ABSTRACT
Aim: To examine and analyze the state of vitamin and mineral homeostasis in children with recurrent respiratory diseases.
Materials and Methods: To achieve the goal, 62 children of primary school age with a diagnosis of Recurrent Respiratory diseases were examined in comparison with data of healthy children of the control group (n=26). The study included a clinical examination of children, determination of serum vitamin and mineral levels.
Results: A dinamic decreasing in the level of vitamins beyond the reference range was found: vitamin D (in 1.4 times) vitamin C (in 1.4 times lower than the data of the control group, and Cyanocobalamin (in 1.9 times) in relation to the data of the control group. Calcium levels were below the limit of reference values, both total (2.14 ± 0.04 mmol/l vs. 2.53 ± 0.07 mmol/l, $p<0.01$) and ionized (1.15 ± 0.01 mmol/l compared 1.26 ± 0.05 mmol/l, $p<0.01$). At the lower limit of the reference, there were significant differences in the levels of Zinc ($p<0.01$, 1.6 times), Copper ($p<0.01$, 1.4 times) and unreliable - Magnesium ($p<0.12$), sodium ($p<0.62$).
Conclusions: Positive correlations were observed with vitamins - Cyanocobalamin ($r=0.40$, $p<0.01$), vitamin C ($r=0.43$, $p<0.01$), Ferritin ($r=0.35$, $p<0.01$), Magnesium ($r=0.27$, $p<0.003$). Negative correlations of vitamins, in particular, Folic acid ($r=-0.50$, $p<0.01$), vitamin A ($r=-0.40$, $p<0.01$) and Phosphorus minerals ($r=-0.75$, $p<0.01$), Sodium ($r=-0.51$, $p<0.01$), Potassium ($r=-0.24$, $p=0.008$), Chlorine ($r=-0.38$, $p<0$,01).

KEY WORDS: acute respiratory infections, Recurrent Respiratory infections, vitamins, minerals, homeostasis disorders, children

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INTRODUCTION

Recurrent respiratory infections (RRIs) are a very common clinical condition in childhood with an important social and economic impact. According to estimates, about 25% of children under the age of 1 year and 6% of children in the first 6 years of life have PRIs, which makes them one of the most common reasons for visiting a pediatrician in the first years of life [1–3]. Although it is a benign condition that is likely to gradually improve by age 12, it significantly interferes with the child's well-being and causes significant medical and social costs. Within RRIs, a specific definition of relapse has yet to find consensus in the literature, on the contrary, the relapse of some specific respiratory diseases is well expressed. These include infectious rhinitis [4], which is defined as recurrent if it occurs more than 5 times per year, or acute otitis media, which is classified as recurrent with 3 episodes in 6 months or 4 episodes in 12 months [5].

In the past, a clinical score for the assessment of PRIs was proposed for children, and it was based on the type of infectious episode, its duration, visits to the pediatrician, therapy, and absence from the community [1]. Cases that scored more than 30 points in 6 months were classified as PRIs. Alternatively, recently, a definition has been introduced that takes into account different trends in the development of respiratory infections depending on age: to define them as PRIs, 8 or more infections per year are required in subjects under 3 years of age, 6 or more infections in children older than 3 years [6].

To guide physicians in the treatment and prevention of children with PRIs, based on the analysis, an inter-societal consensus document was developed, which includes an updated definition of PRIs, a practical diagnostic algorithm, and recommendations for the use of possible measures to prevent PRIs in children. Available international scientific literature, developed according to the GRADE (Grading of Recommendations Assessment, Development and Evaluation) method. Regarding the treatment of individual infectious episodes, the commission recommends that each individual infection be controlled according to national and international guidelines published for each respiratory disease (eg, tonsillitis, rhinitis, otitis, etc.) [7].

AIM

The aim was to examine and analyze the state of vitamin and mineral homeostasis in children with recurrent respiratory diseases.

MATERIALS AND METHODS

To achieve the goal, 62 children of primary school age with a diagnosis of recurrent respiratory diseases were examined in comparison with the data of healthy children of the control group (n=26) identical in age and anthropometric data. The study included a clinical examination of children, determination of serum vitamin and mineral levels.

RESULTS

Recurrent respiratory infections (RRIs) are characterized by impaired immune function and deficiency of micronutrient reserves, particularly vitamins, including vitamins A, B9, B12, C, D3, and E [8]. Some nutritional components are reported to be actively involved in the proper functioning and strengthening of the immune system, including proteins, omega-3 fatty acids, vitamins A, D3, E, B1, B6, B12, and C [8].

Based on studies that show that vitamins are effective in the prevention of respiratory pathology, we will consider the role of vitamins and their effect on PRIs (Table 1).

According to Table 1, there is a decrease in the level of vitamins beyond the reference limits: vitamin D3 (26.82 ± 2.90 ng/ml and in comparison with the data of the control group 36.05 ± 2.58 ng/ml, $p<0.01$), vitamin C (4.78 ± 2.41 mg/l vs. 9.17 ± 2.63 mg/l, $p<0.01$) and cyanocobalamin (206.39 ± 24.91 pg/ml vs. 385.57 ± 54 .26 pg/ml, $p<0.01$).

In infectious diseases, vitamin D3 plays a role in maintaining the physical barrier of the immune system by maintaining the integrity of the mucous membrane, which is usually disrupted by viruses and other microorganisms, enhances innate and cellular immunity, partly through the induction of antimicrobial peptides, directly exhibits antimicrobial, antiviral, antifungal effects, reduces the replication of the influenza A virus. In the adaptive immune response, vitamin D3 suppresses pro-inflammatory cytokines mediated by T-helper cells type 1 (Th1), and enhances the production of anti-inflammatory cytokines by T-helper cells 2 (Th2), promotes the induction of T-regulatory cells, thereby suppressing inflammatory processes [9].

Despite strong evidence for the important role of vitamin D3 in the immune system, clinical trials have shown conflicting findings regarding the effects of vitamin D3 supplementation on respiratory infections. A systematic review that included 39 studies showed a significant association between low vitamin D3 and an increased risk of upper and lower respiratory tract infections [10,11].

According to our research, the values of vitamin D3 were lower than the reference and 1.4 times lower than the data of the control group. In the context of respiratory infections, there is increasing scientific evidence that vitamin D3 can have a protective effect against various infections and reduce the severity of the disease. These findings have increased scientific interest in the relationship between vitamin D3 and infections, particularly the molecular mechanisms involved in the effect of vitamin D3 status on respiratory tract infections. Although between-study heterogeneity is high for most outcomes, and publication bias may have led to an overestimation of the effect size of the incidence data, vitamin D3 supplementation may be beneficial in increasing resistance to common respiratory infections, especially when used daily [12].

From a functional point of view, vitamin C is involved in many biological processes: it prevents oxidative damage to biomolecules by directly absorbing free radicals, by donating electrons to these radicals, and indirectly absorbing free radicals by reactivating other free radical scavengers, such as alpha-tocopherol (vitamin E) and glutathione (GSH), also acts as a cofactor for more than sixty enzymes that catalyze important reactions, including the synthesis of collagen, carnitine, serotonin, and norepinephrine (Ang et al. 2018) [13].

In addition, suppressive effects on the level of inflammatory mediators are observed by inhibiting the activity of nuclear factor kappa B (NF-κB) and the penetration of immune cells into the microcirculation by inhibiting the expression of intracellular adhesion molecules (Du et al. 2022) [14]. In line with this extensive biological activity, beneficial effects of vitamin C have also been reported in the context of various diseases. In addition, numerous findings have been published on the effects of vitamin C on respiratory, allergic, and immunological diseases. In this regard, treatment with vitamin C has been shown to improve

Table 1. Level of vitamins and proliferative activity of lymphocytes in children with PRIs

Laboratory parameters	Control group (n=26)	1st group – OT (n=62)	Statistical significance of differences
Vitamin D3 (30-50, ng/ml)	36,05±2,58	26,82±2,90	p<0,01
Vitamin A (0.26-0.49 ng/ml)	0,39±0,07	0,38±0,05	p<0,045
Vitamin E (3.0-9.0, ng/ml)	7,28±1,47	10,56±2,67	p<0,01
Vitamin C (5.00-15.00, ng/ml)	9,17±2,63	4,78±2,41	p<0,01
Folic acid (B9)(3.00-17.00, ng/ml)	8,26±2,61	3,75±0,54	p<0,01
Cyanocobalamin (211.0-911.0, pg/ml)	385,57±54,26	206,39±24,91	p<0,01
Proliferative activity of lymphocytes with mitogen (1.2-1.68, opt.unit)	1,45±0,07	1,54±0,08	p<0,01

Notes: p – the statistical significance of the differences between the indicators of the 1st and control group

Table 2. Levels of mineral metabolism in children with RRI

Laboratory parameters	Control group	1st group (n=62)	Statistical significance of differences
Za (46-150, μ/dL)	75,35±18,05	47,27±3,46	p<0,01
Cu (10.4-21.4, μmol/l)	15,89±1,32	11,05±1,53	p<0,01
Se (0.05-0.28, μg/l)	0,17±0.05	0,11±0,02	p<0,01
Mg (0.7-0.86, mmol/l)	0,80±0,04	0,79±0,02	p<0,12
Total Ca (2.2-2.7, μmol/l)	2,53±0,07	2,14±0,04	p<0,01
Ionazed Ca (1.16-1.32, mmol/l)	1,26±0,05	1,15±0,01	p<0,01
P (phosphorus)(0.95-1.75, mmo;/l)	1,64±0,18	1,69±0,19	p<0,26
Na (136.0-145.0, mmol/l)	139,66±2,07	139,89±1,94	p<0,62
K (3.5-5.1, mmol/l)	4,43±0,29	4,42±0,34	P=0,89
Cl (98.0-107.0, mmol/l)	103,07±2,64	102,35±2,15	P=0,18

Notes. P2 – statistical significance of differences between indicators of the 1st and control groups

the course of influenza and upper respiratory tract infections (Colunga Biancatelli et al. 2020; van Driel et al. 2019) [15].

In studies on vitamin C and Colds, 3 out of 5 studies showed an effect of reducing the frequency of cold diagnoses and the duration of cold symptoms with the consumption of 500–6000 mg of vitamin C [16-19].

Considering that vitamin B12 plays a crucial role in metabolic processes, cardiovascular and circulatory systems, as well as controls the immune system and antiviral activity, participates in the recovery of damaged tissues and compensates for the reduction of reserves in the liver during viral hepatitis [20], it is possible to think about the reduction of positive effects in our studied contingent on the above biological characteristics. The values of the level of vitamin B12 show a decrease in the level relative to the reference values and a significant decrease of 1.9 times in relation to the data of the control group (206.39 ± 24.91 pg/ml vs. 385.57 ± 54.26 pg/ml, p<0.01).

According to our research, there was a slight increase in vitamin E beyond the reference values (10.56 ± 2.67 mg/l vs. 7.28 ± 1.47 mg/l, p<0.01). According to the researchers, compared with the pneumonia group, the vitamin E level increased in the recurrent respiratory infection group, and the difference was statistically significant (P<0.05), which is consistent with our research. Vitamin E levels in the asthma with cough group were decreased compared to the recurrent respiratory infection group, asthma and pneumonia group (P<0.05) [21].

According to our data, the level of folic acid is within the reference range (3.75 ± 0.54 ng/ml vs. 8.26 ± 2.61 ng/ml data of the control group), that is, it is 2 times lower than in the children of the control group. Given the properties of the functioning of the body system, folic acid is necessary for the creation and maintenance of new cells in a healthy state, therefore its presence is especially necessary for the development of the body, especially in childhood. One can think about the pre-pathological level of folic acid in our patients, which needs correction. [22]. Two studies related to folic acid have shown that folic acid has a positive effect on the prevention of viral infections.

Table 3. Correlation relationships of lymphocytes proliferative activity with mitogen and vitamin-mineral components

Parameter	Parameter	r	p
Lymphocytes proliferative activity with mitogen	Focil acid	-0,50	<0,01
	Cyanocobalamin	0,40	<0,01
	Ferritin	0,35	<0,01
	Vitamin A	-0,40	<0,01
	Vitamib C	0,43	<0,01
	Mg	0,27	<0,003
	Phoshorus	-0,75	<0,01
	Na	-0,51	<0,01
	K	-0,24	<0,008
	CL	-0,38	<0,01

A study related to folic acid and respiratory infections indicated that deficiency of micronutrients such as folic acid was strongly associated with the occurrence of pneumonia or colds ($p < 0.001$) [23].

Considering the level of vitamin A in our patients, no increase was found, and no significant differences were identified between the studied parameters (p=0.45). From numerous scientific studies, it has been proven that children with low serum vitamin A levels were significantly associated with respiratory diseases in children and adolescents, contrary to our results. [24]

The study of indicators of the proliferative activity of lymphocytes with mitogen in patients with recurrent respiratory diseases (RRD) showed that its level is significantly different from the values of the control group of children and varies within the reference values of anti-infective protection of patients (1.54 ± 0.08 opt. units vs. 1.45 ± 0.07 opt. units, p<0.01).

Saturation of the children's body was considerated with micro and macroelements in the RRI childs (table 2).

According to the data of table II, Calcium levels were observed below the limit of reference values, both total (2.14 ± 0.04 µmol/l vs. 2.53 ± 0.07 µmol/l, p<0.01) and ionized (1.15 ± 0.01 mmol/l vs. 1.26 ± 0.05 mmol/l, p<0.01). At the lower reference limit, there were significant differences in the levels of Zinc (47.27 ± 3.46 µg/dL vs. 75.35 ± 18.05 µg/dL, p<0.01, 1.6 times), Copper (11.05 ± 1.53 µmol/l versus 15.89 ± 1.32 µmol/l, p<0.01 in 1.4 times) and unreliable Magnesium (p<0.12), Sodium (p<0.62). The value of the Selenium level varied within the reference range, but with a significant decrease of 1.5 times compared to the data of the control group (0.11 ± 0.02 µg/l vs. 0.17 ± 0.05 µg/l, p<0.01). The values of Potassium (p=0.89), Chlorine (p=0.18), Phosphorus (p=0.26) were also presented as unreliable differences.

Calcium has many important functions, including intracellular signaling, muscle function, nerve transmission, and mediating vasoconstriction and dilation. Total serum calcium concentration varies significantly with serum albumin concentration and hydration status, without any change in ionized calcium concentration. The gold standard for assessing calcium status is measurement of ionized calcium. Although Hypocalcemia can lead to acute respiratory failure due to muscle weakness, tetany, laryngospasm, and bronchospasm, patients with severe Hypercalcemia may present with lethargy, confusion, and coma. Low serum ionized calcium can also cause cardiac dysfunction, including QTc prolongation and reduced left ventricular systolic function, leading to acute pulmonary edema. Low serum ionized Calcium may also reflect the severity of respiratory Alkalosis, which eventually leads to fatigue and diaphragmatic weakness due to hypocalcemia, as well as heavy diaphragmatic stress due to the underlying disease itself [25]. Zinc, Copper and Iron are trace elements that are involved in the development of the immune response. Regarding the effectiveness of micronutrients in the prevention of RRIs, there are few low-quality studies available, burdened by insufficient reproducibility, methodological imprecision, small population size, and heterogeneity of the study population and the results obtained, so it is not possible to recommend the use of micronutrients for the prevention of RRIs [26].

Zinc is involved in the body's cell-mediated and humoral immune responses, each of which is generated for a specific pathogen. Its low level can affect the ability of the immune system to fight infections [27]. Copper is an important trace element for various physiological processes in almost all types of human cells. Dysfunctional Cu metabolism or regulatory pathways can lead to an imbalance

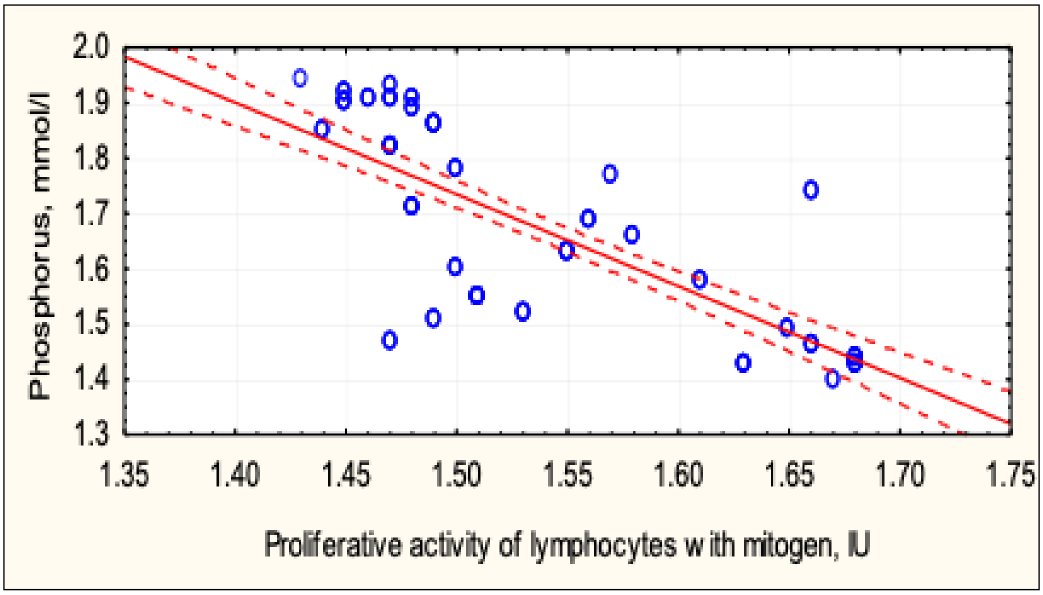

Fig.1. Correlation between Phosphorus concentration and Lymphocytes Proliferative activity with mitogen in the blood ($r=-0.75$; $p<0.01$).

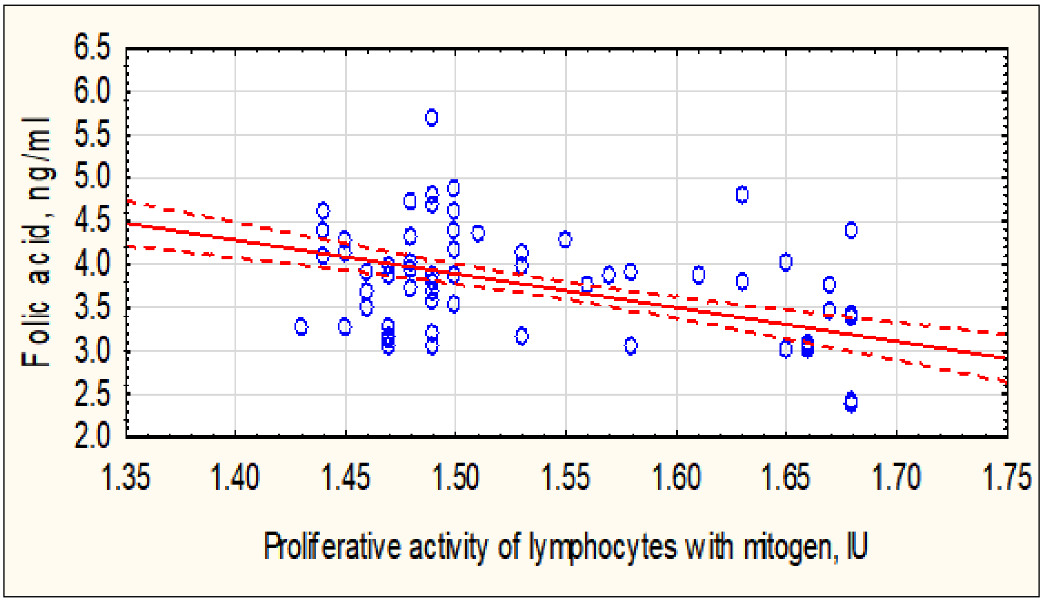

Fig. 2 Correlation between the Lymphocytes Proliferative activity with mitogen and the level of Folic acid in the blood ($r=-0.50$; $p<0.01$).

of Cu homeostasis in the lung, affecting both acute and chronic pathological processes. Recent studies have identified a new form of Cu-dependent cell death called cuproptosis, which has sparked renewed interest in the role of Cu homeostasis in disease. Cuproptosis differs from other known pathways of cell death. This occurs through direct binding of Cu ions to lipoylated components of the tricarboxylic acid cycle during mitochondrial respiration, which causes toxic stress to proteins and ultimately leads to cell death. When considering the homeostasis of Cu on the pathogenesis of various respiratory diseases, it is necessary to take into account the influence of the presence of unregulated metabolism. The therapeutic potential of copper interaction mechanisms with infections and immune inflammation as a component of cellular processes involved in airway homeostasis in the context of respiratory diseases and studying the potential of therapeutic strategies is also possible [28].

In the group with a low content of Selenium in blood serum, serum levels of CRP were increased, which served as an indicator of systemic inflammatory reactions. Consistent with this, multivariate analysis also showed that low serum Selenium levels were also significantly correlated with higher serum CRP levels. Se deficiency affects immune function, Selenoprotein expression, alters the antioxidant response, contributing to greater susceptibility to severe viral and bacterial infections [29].

Lymphocyte proliferation tests are based on the ability of Lymphocytes to proliferate when cultured. Proliferation can be enhanced by mitogens that are polyclonal activators of T-lymphocytes (eg, concanavalin A) or B-lymphocytes (eg, LPS). Activation-induced proliferation and clonal expansion of antigen-specific Lymphocytes is a hallmark of the adaptive Immune response to pathogens. This proliferative burst is autonomously synchronized, ensuring an appropriate magnitude of response while preventing uncontrolled expansion. Steady clonal expansion of a small number of antigen-specific T cells, as well as the simultaneous emergence of a large cellular diversity creates immunity to a large number of different pathogens. Thus, a precise description of the regulatory principles governing Lymphocyte proliferation, differentiation, and survival is essential for a unified understanding of the Immune system [30].

We also analyzed the proliferative capacity of Lymphocytes with mitogen in the studied children. The correlations of vitamin-mineral components and Lymphocytes Proliferative activity with mitogen are considerated (Table 3).

According to the obtained data, positive correlations are observed with vitamins Cyanocobalamin ($r=0.40$, $p<0.01$), vitamin C ($r=0.43$, $p<0.01$) and Ferritin, as an intracellular depot of Iron ($r=0.35$, $p<0.01$), the mineral Magnesium ($r=0.27$, $p<0.003$), which testifies to the protective role of the components of the metabolic direction at the stage of development of the cellular immune response. Negative correlations of vitamins, in particular, Folic acid ($r=-0.50$, $p<0.01$), vitamin A ($r=-0.40$, $p<0.01$) and minerals Phosphorus ($r=-0.75$,$p<0.01$) and Potassium ($r=-0.24$, $p=0.008$),Sodium ($r=-0.51$, $p<0.01$), Chlorine ($r=-0.38$, $p<0$,01). Negative correlations may indicate the exclusion of components from possible preventive factors to optimize prevention [7].

We presented some correlograms of the relationship between the Lymphocytes Proliferative activity with mitogen in the blood and the level of Phosphorus and the values of Folic acid (Fig. 1-2).

The negative direction of relationships (Fig.1) shows that the body has a balanced saturation of the body with phosphorus and does not need to add this mineral to the adaptive nutritional balance. Phosphorus, like Phosphates, are negatively charged ions that are highly reactive with oxygen to form phosphate ions that form complexes with several ions such as calcium and form ester bonds with other molecules such as peptides and proteins. Phosphorus is needed for bone growth, cell metabolism, energy and signaling through phosphorylation reactions, structures (Phospholipid membranes and skeletal tissue), protein and nucleic acid synthesis (DNA and RNA), and oxygen delivery. In children, serum Phosphorus levels are higher, vary throughout the day, and depend on age, genetics, sex, chemical factors in environmental pollution, food intake, use of certain medications, serum pH, disease, and levels of other nutrients and hormones involved in the Phosphorus balance. As for excessive Phosphorus intake at a higher level than the norm, it is associated with an increased risk of chronic diseases [22].

The relationship between Folic acid and the Lymphocytes proliferative activity with mitogen also has a negative direction of relationship (Fig. 2), which indicates the actual balancing of the vitamin with an adequate adaptive immune response. Folic acid plays a role in mitigating inflammatory reactions in the human body. High serum Folate was positively associated with measures of Lung function in patients with COPD. Further longitudinal studies are needed to elucidate the underlying mechanisms [22].

DISCUSSION

Vitamins are multifunctional compounds that carry out biological activity necessary for the completion of enzymatic processes in the child's organism and the possibility of modulating the functions of the Immune system. Trace elements, which are present in the body in very small quantities, play a basic role in metabolic processes and adequate functioning of the Immune system. The increased risk of infection in deficient states has led to the hypothesis that optimization by micronutrient supplementation may improve the efficiency of the Immune response [23].

The results of numerous scientific studies are contradictory and present significantly lower serum levels of vitamin D3 in children with RRIs, while other studies do not indicate a significant difference in serum levels of vitamin D, which are low in both children with RRIs and controls [12].

The effects of vitamin D3 administration in the prevention of RRIs have also been the subject of systematic reviews and meta-analyses. When conducting a study in children under 5 years of age, the effect of vitamin D3 on the prevention of infections in general, including gastrointestinal ones, was considered [7].

The authors concluded that vitamin D3 was effective in preventing infections (adjusted Odds ratio), and that the effect was stronger in subjects with vitamin D3 < 25 nmol/L. A modest statistically significant protective effect of vitamin D3 supplementation compared with placebo was found (OR 0.91, 95% CI 0.84–0.99), in contrast to the results of a previous meta-analysis [11]. Vitamin D has attracted the most attention during the COVID-19 pandemic due to its purported protective effects, among of all the metabolic components. Several systematic reviews and meta-analyses have been published about vitamin

D3 in the prevention of ARI, of which the most recent and large-scale study was by Joliffe et al. in 2020 [12,22]. Recent evidence has shown no positive effects of vitamin D3 and vitamin C together with zinc in the treatment of moderate and mild cases of COVID-19, respectively [9].

Currently, there are no studies in the literature which indicate that low levels of vitamins A and E is leaded to the respiratory infections predispose in children, so their use cannot be recommended for the prevention of RRIs [21,24].

Zinc is a micronutrient whose role in supporting the immune response against viral infections has been extensively studied, especially in children. A systematic review and meta-analysis of the preventive effect of Zinc supplements in children was conducted. Despite many published studies on Zinc supplementation for the prevention of viral respiratory diseases, some studies have reported only modest effects in children, which warrants further research [26,27].

Prevention of RRD in healthy children has recently become the subject of several clinical studies. There is no consensus as to which children should be considered susceptible to RRD (i.e., those who suffer from recurrent ARIs), as different countries use different criteria. The specific sites of respiratory tract infection, the total number of ARIs, and the period of life during which infection should occur vary between countries [27]. On the basis of the conducted research, numerous factors influencing the implementation of RRD in children were identified, which have a significant impact on the condition of sick children, their families, society and the health care system. It is possible to draw conclusions about the importance of metabolic and pro-inflammatory disorders in the prevention of this pathology. It is possible to draw conclusions about the importance of metabolic and pro-inflammatory disorders in the prevention of this pathology.

CONCLUSIONS

1. A decrease in the level of vitamins beyond the reference range: vitamin D3 (26.82 ± 2.90 ng/ml and in comparison with the data of the control group 36.05 ± 2.58 ng/ml, $p<0.01$) and in 1, 4 times lower than the data of the control group; vitamin C (4.78 ± 2.41 mg/l vs. 9.17 ± 2.63 mg/l, $p<0.01$) and 1.4 times lower than the data of the control group and Cyanocobalamin (206.39 ± 24.91 pg/ml versus 385.57 ± 54.26 pg/ml, $p<0.01$), the level of which had a significant decrease of 1.9 times in relation to the data of the control group.
2. The values of Folic acid were within the reference range (3.75 ± 0.54 ng/ml vs. 8.26 ± 2.61 ng/ml, $p<0.01$), i.e. in 2 times lower than in children of the control group. Considering the properties of the functioning of the body system, one can think about the pre-pathological level of folic acid in our patients.
3. According to our research, there was a slight increase in vitamin E beyond the reference values (10.56 ± 2.67 mg/l vs. 7.28 ± 1.47 mg/l, $p<0.01$).
4. Considering the level of vitamin A in our patients, no increase was found, and no significant differences were identified between the studied parameters ($p=0.45$).
5. The study of indicators of the proliferative activity of lymphocytes with mitogen in patients with RRD showed that its level is significantly different from the values of the control group of children and varies within the reference values of anti-infective protection of patients (1.54 ± 0.08 opt. units vs. 1.45 ± 0. 07 opt. units, $p<0.01$).
6. Calcium levels were observed below the limit of reference values, both total (2.14 ± 0.04 μmol/l vs. 2.53 ± 0.07 μmol/l, $p<0.01$), and ionized (1.15 ± 0.01 mmol/l versus 1.26 ± 0.05 mmol/l, $p<0.01$). At the lower limit of the reference, there were significant differences in the levels of Zinc (47.27 ± 3.46 μg/dL vs. 75.35 ± 18.05 μg/dL, $p<0.01$, 1.6 times), Copper (11.05 ± 1.53 μmol/l versus 15.89 ± 1.32 μmol/l, $p<0.01$, 1.4 times) and unreliable - Magnesium ($p<0.12$), Sodium ($p<0.62$). The value of the Selenium level varied within the reference range, but with a significant decrease of 1.5 times compared to the data of the control group (0.11 ± 0.02 μg/l vs. 0.17 ± 0.05 μg/l, p<0, 01). The values of Potassium ($p=0.89$), Chlorine ($p=0.18$), Phosphorus ($p=0.26$) were also presented as unreliable differences.
7. Positive correlations of Lymphocytes Proliferative activity with mitogen are observed with vitamins – Cyanocobalamin ($r=0.40$, $p<0.01$), vitamin C ($r=0.43$, $p<0.01$) and Ferritin, as an intracellular Iron depot (r= 0.35 $p<0.01$). Corelationship with mineral Magnesium ($r=0.27$, $p<0.003$) testifies about the protective role of the metabolic direction components at the stage of the Cellular Immune response development. Negative correlations of vitamins, in particular, Folic acid ($r=-0.50$, $p<0.01$), vitamin A ($r=-0.40$, $p<0.01$) and Phosphorus minerals (r=-0.75 , $p<0.01$), Sodium ($r=-0.51$, $p<0.01$), Potassium ($r=-0.24$, $p=0.008$), Chlorine (r=-0.38, p<0 ,01) were identified. Negative correlations may indicate the exclusion of components from possible preventive factors to optimize prophylactic measure.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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