

Peculiarities of Morphodensitometric Indices of Epitheliocytes of the Oral Cavity Mucous Membrane in Children with Latent Iron Deficiency and Mild Iodine Deficiency

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Abstract: The article represents the results of the study of the micronutrient deficiency effect (iron and iodine) on the morphometric and optical indices of the oral cavity mucous membrane (OCMM) in school children. In order to achieve this goal, 99 school children aged 6-18 years with adequate iodine and iron supply (control group), latent iron deficiency, and mild iodine deficiency were examined. The morphodensitometric study included the determination of the perimeter and area of cells and nuclei, the nuclear-cytoplasmic ratio, the state of chromatin condensation of the epitheliocyte OCMM. The study material was: epitheliocytes of the buccal scraping. It was determined that the area of epitheliocytes in girls aged 6-11 has significantly increased in mild iodine deficiency and in latent iron deficiency. Instead, the decrease of nuclei area was more noticeable in boys of this age in mild iodine deficiency. During the analysis of the general level of chromatin condensation of epitheliocyte nuclei in children, a gender peculiarity has been proved, which was manifested by a greater sensitivity of girls to microelementosis (increase of the integrative optical density of nuclei in the latent iron deficiency at 97.9%, $p_{1-3} < 0.05$, and cell area at 45.8%, $p_{1-4} < 0.01$ relative to control). Among children aged 12-18 years, young men were more sensitive to the deficiency of the studied trace elements. Significant growth of the cell area at 38.7% ($p_{1-2} < 0.05$) in adolescents with mild iodine deficiency and total optical density of nuclei at 63.6% ($p_{1-2} < 0.05$) was substantiated with respect to control in the decrease of karyoplasmic area.

Key words: Mild iodine deficiency, latent iron deficiency, morphodensitometry of epitheliocytes, school-aged children.

1. Introduction

The most common microelementoses are iodine and iron deficiency states. The infant body is particularly sensitive to the micronutrient deficiency; it, due to its intense growth, requires more trace elements (in particular, iron and iodine) in its diet [1, 2]. There is a close connection between all trace elements and the lack or excess of one trace element can lead to the impaired absorption or biological action of another [1]. Iron is known to be involved in metabolism, oxygen transport, tissue respiration, and important physiological processes that take place at the cellular and molecular levels, in particular DNA biosynthesis,

cellular immunity [3, 4]. Iron is the active center of peroxidase involved in the conversion of iodine into the organic form and tyrosine binding to thyroglobulin [4, 5]. In iron deficiency intracellular hypoxia occurs, to which the surface epitheliocytes of oral cavity mucous membrane (OCMM) are particularly sensitive [6]. Iodine is an essential element, because it is necessary for the synthesis of thyroid hormones, and is involved in the activation of enhancers of genes that initiate the activity of their expression.

To study the mechanisms of disorders of cell metabolic activity in iodine and iron deficiency, a complex of morphometric and optical characteristics of OCMM epitheliocytes was studied. At the same time, an important component of the study was the analysis of the morphometric indices of the nuclei, since their

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perimeter and area are related to the state of the chromatin. It is well known that nuclear chromatin is a necessary substrate for the metabolic functions of cells. Structural-functional transformations of chromatin cause the modification of DNA and histone proteins that form chromosomes and depend on the volume of nuclei [7, 8].

The objective of the study is to characterize the morphometric and optical changes of the epitheliocytes of the oral cavity mucous membrane in school-aged children with latent iron deficiency, mild iodine deficiency and in the conditions of their combination.

2. Materials and Methods of the Study

There were examined 99 practically healthy children (50 boys and 49 girls) aged 6-18 years. All pupils were divided into three groups: group I ($n = 33$)—pupils with adequate iodine and iron supply (control group), group II ($n = 34$)—children with limited iodine supply, group III ($n = 32$)—school-children with latent iron deficiency. The analysis of indices in each group was performed taking into account age (6-11 and 12-18 years) and gender peculiarities.

The condition of iodine supply of the organism was studied according to the level of iodine excretion with urine in single portions of urine and with the subsequent calculation of the ioduria median. According to these indices, the severity of iodine deficiency was judged [9]. The functional state of the thyroid gland was studied by determination of the content of thyroid hormones in blood serum: free triiodothyronine (fT_3) and thyroxine (fT_4), thyrotropic hormone of adenohypophysis (TSH) by the method of enzyme immunoassay using test-kits “DRG” (Germany). The state of iron supply was estimated by hemoglobin (Hb) contents in capillary blood, serum iron (SI) level (colorimetric method using “Cormay” test-kit, Poland), total blood serum iron binding ability (BSIBA) using photometric method (test-kit “Cormay”, Poland) and the coefficient of iron transferrin saturation (CITS) was calculated. The state of iron

depot was estimated by the level of serum ferritin (SF) using chemiluminescent method (test-kit “DRG”, Germany) [10]. Morphodensitometric study of OCMM epitheliocytes included determination of the perimeter and area of cells and nuclei, nuclear-cytoplasmic ratio, and chromatin condensation state. The material for the study was: epitheliocytes of the buccal scraping as the most functionally active site. In every child, 2-3 cytological preparations were analyzed; they were stained with aceto-orcein. There were studied 100-200 cells in each drug with an increase of 600- and 900-fold. Nuclei chromatin and its changes were evaluated using a semi-automatic image analyzer based on Image Tool for Windows software (v 3.0).

In order to exclude any pathology, a thorough clinical-endoscopic examination of the oral cavity was performed for every examined patient. All clinical and functional indices of the oral cavity in children were within the physiological norm.

Statistical analysis of the data was performed using the statistical software package Microsoft Office Excel 2016 and STATISTICA 10.

3. Results of the Study

As a result of the study, children with urinary iodine concentration greater than 100 $\mu\text{g/L}$ (characterized by adequate iodine supply) were assigned to the study groups I and III. The experimental group II—included children with urinary iodine contents from 70 to 99 $\mu\text{g/L}$ (which reflects the presence of mild iodine deficiency) [9]. Characterizing indices of thyroid status in boys and girls aged 6-11 years with mild iodine deficiency, it revealed there was a significant decrease of fT_3 contents at 18.5% ($p_{1-2} < 0.05$) and at 19.5% ($p_{1-2} < 0.05$) respectively, and fT_4 at 16.2%, ($p_{1-2} < 0.05$) in boys relative to similar control indices. These changes occurred against the background of an increase of TSH level in blood serum at 98% ($p_{1-2} < 0.01$) in boys and 2.2-fold ($p_{1-2} < 0.001$) in girls with regard to control data. Children of this group aged 12-18 years were found a decrease of fT_3 content at

18.4% ($p_{1-2} < 0.05$) and fT_4 at 13.8% ($p_{1-2} < 0.05$) in boys-adolescents and an increase of TSH in blood serum at 77.4% ($p_{1-2} < 0.05$) in boys and at 73.9% ($p_{1-2} < 0.05$) in girls in relation to control.

As a result of the performed analysis of the indices of iron metabolism, it should be emphasized that in the children of the experimental groups I and II the studied indices were within the reference data. Serum iron contents in studied patients with latent iron deficiency were in the range of 12-10 $\mu\text{mol/L}$, serum ferritin—20-12 ng/mL, total iron-binding capacity of serum exceeded 58 $\mu\text{mol/L}$. Such data confirm the development of latent iron deficiency in children [10]. Analyzing the indices of iron metabolism, the pupils of the experimental group III aged 6-11 years were found a decrease of Hb contents in capillary blood at 12.6% ($p_{1-3} < 0.05$) and at 11.3% ($p_{1-3} < 0.05$), SI—at 44.1% ($p_{1-3} < 0.01$) and at 39.9% ($p_{1-3} < 0.01$), SF—at 39.2% ($p_{1-3} < 0.05$) and at 51.8% ($p_{1-3} < 0.01$), CITS—at 54.4% ($p_{1-3} < 0.01$) and at 57.1% ($p_{1-3} < 0.05$) against the background of the increase of the BSIBA—at 26.7% ($p_{1-3} < 0.05$) and at 33.0% ($p_{1-3} < 0.05$), respectively, in boys and girls regarding the control group data. The same tendency was observed in older school-children, in particular, decrease of Hb content in capillary blood at 15.6% ($p_{1-3} < 0.05$) in boys-adolescents, SI—at 36.8% ($p_{1-3} < 0.01$) in boys-adolescents, and at 36.3% ($p_{1-3} < 0.01$) in girls, SF—at 44.3% ($p_{1-3} < 0.05$) in boys and at 43.6% ($p_{1-3} < 0.05$) in girls, CITS—at 45.5 % ($p_{1-3} < 0.05$) in boys and at 51.3% ($p_{1-3} < 0.05$) in girls against the background of BSIBA growth—at 31.0% ($p_{1-3} < 0.05$) in girls regarding the control group data.

In all children with microelementoses there was a tendency to the increase of the perimeter and area of epitheliocytes of OCMM compared with similar data in the control group (Table 1). In particular, the tendency to the increase of the area of epitheliocytes in school-children aged 12-18 years was more noticeable: in mild iodine deficiency at 24.0% ($p_{1-2} < 0.05$), in latent iron deficiency—at 20.8% ($p_{1-3} < 0.05$) relative to control. In contrast to the changes in cell indices as a

whole, the area of nuclei of epitheliocytes decreased significantly in deficiency of trace elements in children aged 6-11 years. In particular, a decrease of the nuclear-cytoplasmic ratio in young children with mild iodine deficiency was found at 14.3% ($p_{1-2} < 0.05$), with latent iron deficiency at 10.7% ($p_{1-3} < 0.05$); and in older children with mild iodine deficiency and latent iron deficiency at 19.2% ($p_{1-2} < 0.05$, $p_{1-3} < 0.05$) relative to control.

There was determined the increase of integrative optical density of nuclei in children aged 6-11 years with mild iodine deficiency at 29.1% ($p_{1-2} < 0.05$), with latent iron deficiency at 71.0% ($p_{1-3} < 0.05$), and in children aged 12-18 years, the growth of integrative optical density of nuclei was more noticeable only in mild iodine deficiency (at 43.4%, $p_{1-2} < 0.05$) relative to control.

In children with micronutrient deficiencies, a decrease of the light-permeable capacity of nuclei was found, which is caused by the reorganization of interphase chromatin at the level of individual components and the nuclei as a whole, especially in children aged 12-18 years with mild iodine deficiency (Table 1).

In the analysis of morphodensitometric indices of OCMM epitheliocytes' nuclei, with regard to the age and gender peculiarities, the first gender peculiarity was determined—significantly smaller cell area in girls aged 6-11 years of the control group relative to boys at 29.5% ($p < 0.05$). It was found that in boys with trace elements deficiency the cell area was almost unchanged, and in girls there was a tendency to its increase: in mild iodine deficiency at 27.1% ($p_{1-2} < 0.05$), in latent iron deficiency at 45.8% ($p_{1-3} < 0.05$) relative to control. It can be assumed that in the deficiency of trace elements in girls there is a compensatory increase of the area of epitheliocytes.

The integrative optical density of nuclei increases in boys with mild iodine deficiency at 20.5% ($p_{1-2} < 0.05$), with latent iron deficiency at 64.4% ($p_{1-3} < 0.05$) relative to control. In girls, changes in indices of the integrative optical density of nuclei were more

pronounced; in particular, it was recorded an increase at 54.5% ($p_{1-2} < 0.05$) in mild iodine deficiency, in mild iron deficiency at 97.9% ($p_{1-3} < 0.05$) relative to control. Accordingly, the indices of the optical power of the nuclei were reduced, especially in girls (Table 2).

In older school-children, morphodensitometric indices of OCMM epitheliocytes differed from those in younger school-children (Table 3). Thus, a significant increase of the cell area in boys-adolescents in mild iodine deficiency at 38.7% ($p_{1-2} < 0.05$), in latent iron deficiency at 24.2% ($p_{1-3} < 0.05$) relative to the control values, was revealed. In girls, only an insignificant

increase of the cell area was detected in a deficiency of trace elements regarding the control. Nuclei area tended to decrease, especially in boys-adolescents with latent iron deficiency at 28.9% ($p_{1-3} < 0.05$). Index of nuclear-cytoplasmic ratio in boys with mild iodine deficiency decreased at 35.5% ($p_{1-2} < 0.05$), with latent iron deficiency at 41.9% ($p_{1-3} < 0.05$); in girls this index remained almost unchanged.

A significant increase of the integrative optical density of nuclei was observed in boys with mild iodine deficiency at 63.6% ($p_{1-2} < 0.05$) relative to control (Table 3).

Table 1 Cytodensitometric indices of oral cavity mucous membrane epitheliocytes in children with adequate iron and iodine exchange (control group), mild iodine deficiency and latent iron deficiency, aged 6-18 years ($M \pm m$).

Indicators	Group I (control)		Group II (mild iodine deficiency)		Group III (latent iron deficiency)	
	6-11 years ($n = 16$)	12-18 years ($n = 17$)	6-11 years ($n = 18$)	12-18 years ($n = 16$)	6-11 years ($n = 16$)	12-18 years ($n = 16$)
Perimeter of the cell, mkm	332.8 ± 42.6	331.9 ± 39.6	349.4 ± 39.6	380.3 ± 52.2	340.2 ± 37.1	363.1 ± 39.5
Cell area, mkm ²	7,715.3 ± 618.1	6,975.1 ± 716.1	7,870.5 ± 786.2	8,648.1 ± 825.11	8,047.6 ± 854.2	8,429.0 ± 865.2
Perimeter of the nucleus, mkm	55.3 ± 6.52	50.4 ± 7.34	54.3 ± 5.49	51.8 ± 6.18	54.3 ± 6.34	51.2 ± 6.94
Core area, mkm ²	214.1 ± 42.9	184.0 ± 40.8	192.4 ± 38.3	183.7 ± 38.8	204.6 ± 39.4	176.4 ± 44.3
Nuclear-cytoplasmic ratio, U	0.028	0.026	0.024	0.021	0.025	0.021
Transmittance of the nuclei	117.7 ± 7.87	118.0 ± 10.5	107.3 ± 10.8	103.5 ± 15.4	106.1 ± 12.2	109.2 ± 10.9
Integrative optical density of nuclei	384,504.6 ± 84,991.1	475,324.2 ± 75,847.2	496,969.2 ± 98,306.4	681,596.7 ± 75,543.6	657,576.2 ± 81,951.2 $p_{1-3} < 0.05$	544,719.5 ± 91,378.4

p with Arabic numerals—significant difference ($p < 0.05$) between indices in the respective study groups.

Table 2 Cytodensitometric indices of oral cavity mucous membrane epitheliocytes in children with adequate iron and iodine exchange (control group), mild iodine deficiency and latent iron deficiency, aged 6-11 years ($M \pm m$).

Indicators	Group I (control)		Group II (mild iodine deficiency)		Group III (latent iron deficiency)	
	Boys ($n = 8$)	Girls ($n = 8$)	Boys ($n = 9$)	Girls ($n = 9$)	Boys ($n = 8$)	Girls ($n = 8$)
Perimeter of the cell, mkm	351.2 ± 34.2	289.5 ± 25.6	360.1 ± 36.1	336.8 ± 40.0	329.6 ± 31.0	350.8 ± 40.2
Cell area, mkm ²	8,281.1 ± 827.3	5,840.2 ± 301.8 $p < 0.05$	8,165.5 ± 747.8	7,423.6 ± 759.9	7,498.3 ± 635.6	8,513.8 ± 625.1 $p_{1-3} < 0.01$
Perimeter of the nucleus, mkm	55.6 ± 6.74	54.8 ± 6.19	53.9 ± 5.32	55.2 ± 5.81	55.6 ± 4.92	52.1 ± 8.09
Core area, mkm ²	221.5 ± 41.0	202.6 ± 43.9	187.8 ± 38.2	203.7 ± 39.7	200.5 ± 30.5	210.9 ± 51.4
Nuclear-cytoplasmic ratio, U	0.027	0.035	0.023	0.027	0.027	0.025
Transmittance of the nuclei	117.2 ± 8.43	119.0 ± 6.41	108.1 ± 10.9	106.4 ± 10.6	105.9 ± 10.8	106.3 ± 13.8
Integrative optical density of nuclei	404,257.7 ± 89,489.9	328,364.3 ± 63,294.1	487,310.8 ± 52,295.1	507,290.5 ± 80,745.2	664,591.3 ± 92,991.1	649,976.0 ± 88,965.3 $p_{1-3} < 0.05$

p with Arabic numerals—significant difference ($p < 0.05$) between indices in the respective study groups.

Table 3 Cytodensitometric indices of oral cavity mucous membrane epitheliocytes in children with adequate iron and iodine exchange (control group), mild iodine deficiency and latent iron deficiency, aged 12-18 years ($M \pm m$).

Indicators	Group I (control)		Group II (mild iodine deficiency)		Group III (latent iron deficiency)	
	Boys ($n = 9$)	Girls ($n = 8$)	Boys ($n = 8$)	Girls ($n = 8$)	Boys ($n = 8$)	Girls ($n = 8$)
Perimeter of the cell, mkm	329.9 ± 44.3	333.0 ± 36.9	401.2 ± 43.3	331.8 ± 36.5	372.1 ± 40.9	359.1 $\pm$ 39.1
Cell area, mkm ²	6,755.9 $\pm$ 610.4	7,112.2 $\pm$ 774.9	9,367.8 $\pm$ 893.6 $p_{1-2} < 0.05$	7,194.2 $\pm$ 752.1	8,389.7 $\pm$ 891.6	8,445.4 $\pm$ 980.3
Perimeter of the nucleus, mkm	56.4 $\pm$ 5.70	48.1 $\pm$ 6.5	52.0 $\pm$ 6.37	51.3 $\pm$ 5.81	46.2 $\pm$ 5.80	5,228 $\pm$ 6.65
Core area, mkm ²	211.7 $\pm$ 37.1	171.7 $\pm$ 36.1	188.1 $\pm$ 38.6	176.5 $\pm$ 38.1	150.4 $\pm$ 32.1	182.2 $\pm$ 44.7
Nuclear-cytoplasmic ratio, U	0.031	0.024	0.020	0.024	0.018	0.022
Transmittance of the nuclei	115.1 $\pm$ 11.2	120.3 $\pm$ 9.52	99.5 $\pm$ 17.7	107.0 $\pm$ 11.7	102.0 $\pm$ 9.26	111.9 $\pm$ 10.3
Integrative optical density of nuclei	436,372.1 $\pm$ 45,262.2	501,040.2 $\pm$ 89,729.3	713,748.4 $\pm$ 93,090.5 $p_{1-2} < 0.05$	650,646.9 $\pm$ 71,362.4	520,911.4 $\pm$ 53,051.0	552,576.1 $\pm$ 62,108.1

p with Arabic numerals—significant difference ($p < 0.05$) between indices in the respective study groups.

4. Discussion

As a result of analysis of cytodensitometric parameters of OCMM epitheliocytes and their nuclei, the dependence of their changes on the age, gender of children and the type of microelementosis, was revealed. Significant increase of epitheliocyte area and the decrease of the nuclei area were found. This fact can be explained by the swelling reaction of the cytoplasm to provide more optimal conditions for transmission and synthesis of polypeptides under the conditions of micronutrient deficiency [6]. This proved the presence of gender dimorphism, which was manifested by a greater sensitivity of girls aged 6-11 years to the mild iodine deficiency, latent iron deficiency. This can be regarded as a natural process related to the participation of iodine and iron in the important physiological processes occurring at the cellular and molecular levels, in particular, DNA biosynthesis, cellular immunity [8].

With age (in children aged 12-18 years), young men were more sensitive to the deficiency of the studied trace elements. In girls, the most noticeable tendency to chromatin compaction was detected only in mild iodine deficiency, due to the fact that iodine is involved in the activation of gene enhancers and initiates the activity of their expression [7]. A mediated marker of the latter

one can be the obtained result as for the predominance of epitheliocytes' nuclei with condensed inactive chromatin in all children with mild iodine deficiency, which correlated with an increase of the maximum optical density. Therefore, a greater polymorphism of the phenotypes of OCMM epitheliocytes provides functional heterogeneity of the tissue, characterizing changes in morphodensitometric parameters depending on the deficiency of trace elements, age and gender of children [7, 8].

5. Conclusions

Changes of morphodensitometric indices of the oral cavity mucous membrane epithelial cells depended on the age, gender of children and the deficiency of the studied trace elements.

Children aged 6-11 years showed a significant increase in the compaction of nuclei chromatin in pupils with latent iron deficiency. The heterogeneity of the chromatin topography was the most pronounced in children aged 12-18 years with mild iodine deficiency.

Younger school-girls and older school-boys were more sensitive to micronutrient deficiency. A significant growth of cell area and total optical density of nuclei in the decrease of karyoplasmic area, especially pronounced in mild iodine deficiency, has been proved.

References

- [1] Belykh, N. A. 2017. "Prediction of the Risk of the Formation of Pituitary-Thyroid Maladaptation in the Region of Moderate Iodine Deficiency." *Mizhnarodnyy zhurnal pediatriyi, akusherstva i hinekolohiyi* 11 (2): 28-35.
- [2] Mamenko, M. Y., Shleyenкова, H. O., and Dontsova, K. M. 2017. "Influence of Iodine Supplementation on Physical, Neuropsychological Development and Neurological Status of Young Children." *Sovremennaya Pediatriya* 1 (81): 13-9.
- [3] Cassat, J. E., and Skaar, E. P. 2013. "Iron in Infection and Immunity." *Cell Host Microbe* 13 (5): 509-19.
- [4] Milto, I. V., Suhodolo, I. V., Prokopieva, V. D., and Klimenteva, T. K. 2016. "Molecular and Cellular Bases of Iron Metabolism in Humans." *Biochemistry* 81 (6): 549-64.
- [5] Banadyha, N. V. 2016. "Influence of Iron Deficiency Anemia of the Formation of Systemic Immunity in Children." *Journal of Education, Health and Sport* 6 (1): 93-100.
- [6] Nikolovski, D., Dugalic, S., and Pantic, I. 2017. "Iron Oxide Nanoparticles Decrease Nuclear Fractal Dimension of Buccal Epithelial Cells in a Timedependent Manner." *Journal of Microscopy* 1 (1): 1-8.
- [7] Han, R. Z., and Popel, S. L. 2017. "Morphological and Biochemical Mechanisms of Changes in Buccal Epitheliocytes and Erythrocytes in Children with Psycho-Emotional Stress." *Regul. Mech. Biosyst.* 8 (3): 363-8.
- [8] Kocherha, Z. R., Kovalchuk, L. Y., and Herashchenko, S. B. 2015. "Cytodensitometric Indices of Somatic Cells of Healthy Newborns and Newborns with Intrauterine Growth Retardation." *Galician Medical Journal* 22 (2): 53-6.
- [9] Zimmermann, M. B., and Boelaert, K. 2015. "Iodine Deficiency and Thyroid Disorders." *Lancet Diabetes Endocrinol* 3 (4): 286-95.
- [10] Salakh, A. A., Abushanova, O. V., and Kucher, O. V. 2014. "Modern Approaches to the Laboratory Diagnosis of Iron Deficiency Anemia." *Semeynaya Meditsina* 1 (51): 134-42.