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Prolonged Impairment of Polymorphonuclear Cells Functions in One Infant with Transient Zinc Deficiency: a Case Report

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Abstract

Background: Zinc is an essential trace element for the immune system. The zinc deficiency diminishes antibody- and cell-mediated responses in man. Lymphopenia and thymic atrophy are usually the early hallmarks of zinc deficiency. Surprisingly, only scarce data are available about polymorphonuclear cells (PMNs) functions in infants with zinc deficiency. We present the results of immunological analyses in one infant with transient zinc deficiency due to decreased zinc concentration in mother milk resulting in severe lactogenic acrodermatitis enteropathica.

Material/Methods: Nine repeated examination of oxidative burst of PMNs and immunoglobulin levels using nitroblue tetrazolium dye test, chemiluminescence, flow cytometry and nephelometry were performed in the infant with severe zinc deficiency during 28 months period.

Results: The unusual prolonged but transient impairment of PMNs respiratory burst accompanied with hypogammaglobulinaemia developed since the age of 2.5 months. Dramatic improvement of the skin was observed within days with total resolution of skin lesions on the 9th day of zinc therapy, but decreased PMNs respiratory burst persisted until the age of 23 months.

Conclusions: We conclude that zinc deficiency may lead to prolonged impairment of polymorphonuclear cells functions and hypogammaglobulinaemia.

Introduction

Acrodermatitis enteropathica (AE) as the clinical consequence of inherited or acquired zinc deficiency is characterized by pustular and bullous dermatitis with an acral and periorificial distribution, alopecia, and diarrhoea [1, 2]. Failure to thrive, growth retardation and secondary infections are frequently observed in affected infants [3–5]. The early diagnostics of zinc deficiency is important because the zinc therapy may be life-saving [2, 6–8].

Zinc is an essential trace element for the immune system. Zinc deficiency may cause the impairment of immune function with reduced humoral and cellular responses and reduction in the number of circulating lymphocytes [9–11]. Surprisingly, only scarce data are available about polymorphonuclear cells (PMNs) functions in infants with zinc deficiency.

We discuss here an unusual prolonged impairment of polymorphonuclear cells respiratory burst in the breast-fed infant with transient zinc deficiency due to low concentration of zinc in the mother's milk manifested as acrodermatitis enteropathica at the age of 2.5 months. The purpose of our study is to extend our knowledge about immune function in zinc deficient infant. The fact that we study a single child prevents us from drawing general conclusions which could be statistically relevant. However, we still think that such results, even in this limited scale, are interesting and have a value of their own, in particular in terms of future research concerning the role of zinc in human organism.

Material and Methods

Material

The girl was born in the 31st week of gestation per *sectio ceasarea* indicated for mother's preeclampsia with birth weight of 1280 g (15. percentile) and length of 41 cm (15. percentile). The parents are healthy; the mother is of Caucasian and the father of sub-Saharian origin. The early postnatal adaptation was uneventful with Apgar score of 9-9 in the fifth and tenth minute, respectively. Parenteral nutrition because of prematurity was gradually exchanged by oral feeding with mother's milk. The girl was than fully breast-fed for 7 months and partially breast-fed until her age of 18 months.

The first skin problems manifested at the age of 2 months with vesicular exanthema on the face and the perianal area, the local antimycotic therapy was without benefit. Two weeks later she was admitted to the hospital with skin bacterial superinfection. The cutaneous lesions were characterized by

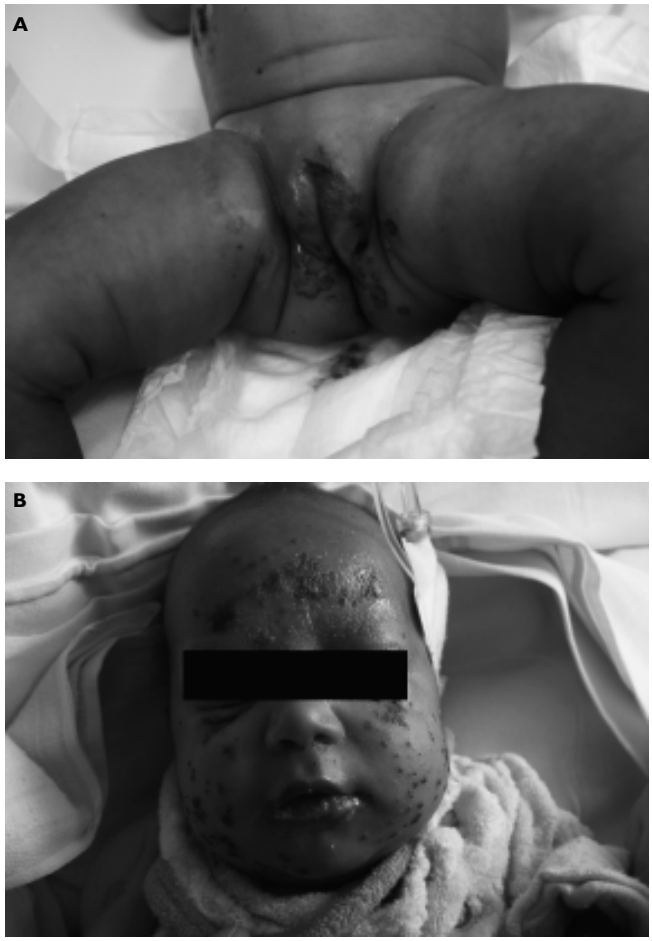

Figure 1 – The erythematous, scaly, erosive patches, papules and tiny vesicles on the perianal and perigenital region (A), on the face (B) in 2.5 months old girl with lactogenic acrodermatitis enteropathica.

erythematous, scaly, erosive patches, papules and tiny vesicles on the face and perilabial region, acral parts of upper and lower limbs, lower back, buttocks, and these lesions were especially severe in perigenital and perianal area (Figure 1A, 1B). Her fingers showed erosion of the finger tips and paronychia involvement. There were mild angular cheilitis and oral mucosal erosions. The girl was restless, refusing drinking, suffering from abdominal pain and diarrhoea with more than 10 watery black-green stools per day.

The routine biochemical and haematological tests including C-reactive protein, aminotransferases and urinary analyses were normal. *Staphyloccocus aureus* and Enterococcus species were cultivated from pustular secret. Profound zinc deficiency was observed in blood (B-Zn 1.6 μmol/l, reference range 9–16 μmol/l) and urine (U-Zn 1.3 μmol/l, reference range > 4.6 μmol/l). In the mother, zinc concentration was normal in blood 14.7 μmol/l but low in mother's milk (2.4 μmol/l, controls 3.8–10.2 μmol/l).

The girl was initially treated with oral zinc (zinci sulfas) supplementation (4 mg/kg/day). Dramatic improvement of the skin was observed within days with total resolution of skin lesions on the 9th day of therapy (Figure 2). The zinc treatment was discontinued after initiation of mashed and solid foods (after 7 months of age).

At present the girl is 30 months old, her weight is 11950 g (SD –1.57), length 90 cm (SD –1.05) and head circumference 47.5 cm (SD –1.48), her psychomotor development is normal.

Methods

The serum zinc level in deproteinized blood serum was determined using atomic absorption spectrophotometer (AAS) (SPECTR AA240SS, Varian). The haemolysis was excluded, and the separator of the serum and gel was used. The

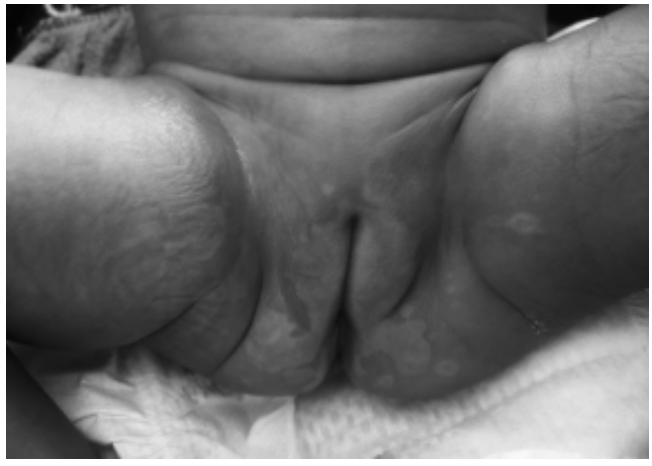

Figure 2 – Complete resolution of dermatitis on the perigenital and perianal region after 9 days of zinc supplementation in 2.5 months old girl with lactogenic acrodermatitis enteropathica.

immunoglobulin levels were determined by using nephelometry (Nephelometer, Boehring). Oxidative burst of polymorphonuclear cells was evaluated by nitroblue tetrazolium (NBT) dye test, chemiluminescence and by flow cytometry. Briefly, nitroblue tetrazolium dye test was evaluated after incubation of PMNs with zymozan and dying with neutral red. A hundred cells were observed under the microscope. Luminol-enhanced chemiluminescence of PMNs was measured by the chemiluminimeter LM 01, Imotect after stimulation of PMNs with PMA (phorbol myristate acetate) [12]. Flow cytometry test using dihydrorhodamine 123 fluorescence (DHR test) (FC500 Beckman Coulter) was used as an alternative investigation of PMNs production of reactive oxygen intermediates [13].

Results

Immunological analyses at the time of diagnosis revealed hypogammaglobulinaemia (IgG 1.6 g/l, IgA 0.07 g/l, age related reference range IgG 2.5–7.5, IgA 0.08–0.8) and decreased polymorphonuclear cells functions. The respiratory burst using nitroblue tetrazolium (NBT) dye test reached only 1% of normal values. Impaired PMNs production of reactive oxygen intermediates during respiratory burst was confirmed by chemiluminescence, which was decreased to the level of 321 (index stimulated/spontaneous chemiluminescence-day control 1228). IgM level was normal 0.31 g/l (age related reference range IgM 0.1–0.7 g/l) likewise adhesion molecules of CD11/18 complex and the cell immunity parameters. Age-appropriated results were found in immunophenotypical and histological investigation of bone marrow aspiration provided at the age of 9 months.

Repeated immunological investigation revealed persistent abnormality of PMNs respiratory burst despite the normal zinc concentrations in blood and urine. Results of immunological data are summarized in Table 1 and Table 2. Nitroblue tetrazolium (NBT) dye test normalized at the age of 19 months and remains

Table 1 – Results of nitroblue tetrazolium (NBT) dye test, chemiluminescence and flow cytometry during follow up in the girl with lactogenic acrodermatitis enteropathica

Age Months	NBT dye test Controls >9%	Chemiluminescence Patient	Chemiluminescence Control**	Neutrophils $x10^9$/l	Lymphocytes $x10^9$/l
2.5	**1**	**321**	1228	2.73	14.6
4	**5**	**295**	1026	2.63	8.17
5	**7**	**154**	1050	1.28	6.45
7.5	**3**	nd	nd	1.95	6.42
10	**9**	**245**	1068	1.02	5.06
13.5	**5**	**220**	1100	1.82	4.03
19	32	**188**	739	2.8	5.58
23	32	88%*	> 76%	2.3	3.46
30	18	85%*	> 76%	1.82	4.4

* DHR burst test was used instead of chemiluminescence, ** day control for the test, nd: not determined

normal thereafter (until present age 30 months). Normal production of reactive oxygen intermediates during respiratory burst using DHR test was documented at the age of 23 months and remains normal thereafter. Hypogammaglobulinaemia persisted until 13.5 months of age and decreased level of IgA until 23 months of age. The control sample obtained at the age of 30 months showed normal immunoglubulines levels. Peripheral blood smear showed intermittent neutropenia, but concentration of PMNs in peripheral blood was normal at the time of immunological analyses. No lymphopenia was documented. Apart from the skin problems she did not suffer from any infections.

Discussion

Zinc is an essential mineral for humans and the second one after iron quantitatively [14]. The biological role of zinc is now recognized in structure and function of proteins, transcription factors, hormonal receptor sites, and biologic membranes. Zinc has numerous central roles in DNA and RNA metabolism and it is involved in signal transduction, gene expression, and apoptosis. Zinc metalloenzymes and zinc-dependent enzymes have been identified and are involved in nucleic acid metabolism and cellular proliferation, differentiation, and growth [14–17].

Acrodermatitis enteropathica (AE) is a rare genetic or acquired disorder of hypozincemia. Some of the genetic facts of AE have been determined. AE is an autosomal recessive inherited disorder of zinc malabsorption with impairment of zinc transporting protein and/or genetic defect in the production, structure, or function of a low molecular weight zinc-binding ligand secreted by the pancreas. It binds to zinc in the intestinal lumen and transports it into the mucosa [18]. The gene encoding zinc transporting protein has been identified on 8q24.3 and in 2002, it was shown to be a member of the solute carrier 39A superfamily (Slc39a) [19, 20]. AE can also be acquired by poor consumption or poor

Table 2 – Longitudinal follow up of immunoglobulin levels in the girl with lactogenic acrodermatitis enteropathica

Age	IgG (g/l)		IgA (g/l)		IgM (g/l)	
Months	Patient	Control*	Patient	Control*	Patient	Control*
2.5	**1.6**	2.5–7.5	**0.07**	0.08–0.8	0.31	0.1–0.7
4	**2.0**	2.5–7.5	**0.08**	0.08–0.8	0.46	0.1–0.7
5	nd		nd		nd	
7.5	**2.5**	2.5–7.5	**0.07**	0.3–1.2	**0.27**	0.4–1.4
10	**2.3**	2.5–7.5	**0.13**	0.3–1.2	**0.34**	0.4–1.4
13.5	3.3	2.5–7.5	**0.21**	0.3–1.2	0.48	0.4–1.4
19	4.8	2.5–7.5	**0.21**	0.3–1.2	0.62	0.4–1.4
23	6.37	2.5–7.5	0.31	0.3–1.2	0.84	0.4–1.4
30	8.4	5–13	0.42	0.3–1.2	0.64	0.4–1.4

* age related controls, nd: not determined

absorption of zinc, leading to a state of hypozincemia. Chronic diseases such as gastrointestinal disorders, chronic diarrhoea, renal disease, sickle cell anaemia, cirrhosis, some cancers, cystic fibrosis, and pancreatic insufficiency have been shown to lead to suboptimal zinc status [9, 21–24].

Breast milk provides sufficient amount of zinc for the first 4–6 months of life. However, as lactation progresses, the physiologic decline in breast milk zinc concentration is notable [25] and dropped by 40% between the first and third month [26]. The average concentration of zinc in mature human mother's milk is 0.46 ± 0.21 mg/l (7 ± 3.2 μmol/l) [27]. Low birth weight or premature infants, who have higher zinc requirements, may develop symptoms when receiving milk with low-normal levels of zinc and it does not always mean that there is impaired secretion by the mammary gland [28–30]. Lactogenic AE indicates defect of mammary zinc secretion [28, 29, 31–33].

In our proband the diagnosis of lactogenic AE was reached by demonstrating extremely low mother's milk zinc levels with her normal blood zinc status, characteristic skin lesion in the infant with diarrhoea, rapid clinical improvement after enteral zinc supplementation and exclusion of other pathology. Inappropriate zinc reserves and higher zinc requirements due to prematurity might be additional risk factor.

The most serious complication of AE is the high morbidity and mortality caused by secondary infections. Studies have shown impaired immune function and reduced humoral and cellular responses with reduction in circulating lymphocytes and poor delayed-type hypersensitivity in patient with AE [34]. Zinc deficiency induces lymphocytic apoptosis mediated by glucocorticoids with resultant decrease in lymphopoiesis [35]. The thymic atrophy and decline in the number of peripheral and splenic lymphocytes in the zinc-deficient mouse paralleled observations made in zinc-deficient humans [9, 36–39]. The decrease in B- and T-cell precursors in bone marrow makes patients prone to infections. The period of zinc deficiency might enhance the possibility of autoimmune disease by contributing to the inefficient removal of anti-self or nonsense clones that are routinely generated in the bone marrow [10]. The influence of phagocytic activity on the early stage defence mechanisms in zinc deficient mouse was studied using active oxygen production at work of Tone et al. [40] showing impaired active oxygen production in zinc deficient mouse. The essential role of zinc finger transcription factor Gfi-1 in myeloid development was documented by Hock et al. [41]. Gene-targeted Gfi-1(-/-) mice lack normal neutrophils and were highly susceptible to abscess formation by gram-positive bacteria. In summary Gfi-1 not only promotes differentiation of neutrophils but also antagonizes traits of the alternate monocyte/macrophage program [41]. Surprisingly, only a few studies assessed zinc and macrophage phagocytosis in human. In two, decreased human monocyte phagocytosis and diminished phagocytic activity of neutrophils were observed as a result of zinc deficiency [42, 43]. On the other hand, large amounts of oral zinc

supplementation significantly impaired polymorphonuclear leukocytes function in one study [44].

The possible duration of polymorphonuclear cells functions impairment in the patient with zinc deficiency has not been published yet.

In our study we present the above-discussed infant with lactogenic AE with severe prolonged impairment of PMNs respiratory burst and hypogammaglobulinaemia lasting 21 months. During this period the zinc level in blood was regularly monitored and kept within the reference interval. The supplementation with oral zinc in standard doses was necessary until the age of 7 months when mashed and then solid foods were initiated. Although we are aware that we draw our conclusion from the facts based on a single patient, and hence these facts may not be statistically relevant, we do think that a complete and thorough analysis of a single patient with zinc deficiency may enhance our understanding of such conditions, and help to guide future research.

Conclusion

We conclude that zinc deficiency may lead to prolonged impairment of polymorphonuclear cells functions and hypogammaglobulinaemia. We recommend including the evaluation of zinc status in every breast-fed infant with skin lesion and chronic diarrhoea and/or immunopathological abnormalities.

References

1. SEHGAL V. N., JAIN S.: Acrodermatitis enteropathica. *Clin. Dermatol*. 18: 745–748, 2000.
2. PERAFAN-RIVEROS C., SAYAGO-FRANCA L. F., FORTES A. C.: Acrodermatitis enteropathica: a case report and review of the literature. *Pediatr. Dermatol.* 19: 426–431, 2002.
3. WALRAVENS P. A., HAMBIDGE K. M., KOEPFER D. M.: Zinc supplementation in infants with a nutritional pattern of failure to thrive: a double-blind, controlled study. *Pediatrics* 83: 532–538, 1989.
4. CAPUTO R., ACKERMAN A. B., SISON-TORRE E. Q. (EDS).: Pediatric dermatology and dermatopathology. Lea and Febiger, Philadelphia, 67–71, 1990.
5. OZKAN S., OZKAN H., FETIL E., CORAPÇIOGLU F., YILMAZ S., OZER E.: Acrodermatitis enteropathica with Pseudomonas aeruginosa sepsis. *Pediatr. Dermatol.* 16: 444–447, 1999.
6. MICHAELSSON G.: Zinc therapy in acrodermatitis enteropathica. *Acta Derm. Venereol.* 54: 377–381, 1974.
7. THYRESSON N.: Acrodermatitis enteropathica: report of a case healed with zinc therapy. *Acta Derm. Venereol.* 54: 383–385, 1974.
8. HERSON V. C., PHILIPPS A. F., ZIMMERMAN A.: Acute zinc deficiency in premature infant after bowel resection and intravenous alimentation. *Am. J. Dis. Child* 135: 968–969, 1981.
9. FRAKER P. J., KING L. E., LAAKKO T., VOLLMER T. L.: The dynamic link between the integrity of the immune system and zinc status. *J. Nutr.* 130: 1399–1406, 2000.
10. FRAKER P. J., KING L. E.: Reprogramming of the immune system during zinc deficiency. *Ann. Rev. Nutr.* 24: 277–298, 2004.
11. RINK L., HAASE H.: Zinc homeostasis and immunity. *Trends Immunol.* 28: 1–4, 2007.
12. METCALF J. A., GALLIN J. I., NAUSEEF W. M., ROOT R. K.: Laboratory manual of neutrophil function. Raven Press, New York, 1986.

13. EMMENDÖRFFER A., HECHT M., LOHMANN-MATTHES M. L., ROESLER J.: A fast and easy method to determine the production of reactive oxygen intermediates by human and murine phagocytes using dihydrorhodamine 123. *J. Immunol. Methods* 131: 269–275, 1990.
14. AGGETT P. J., HARRIES J. T.: The current status of zinc in health and disease states. *Arch. Dis. Child* 54: 909–917, 1979.
15. CHESTERS J. K.: Biochemical functions of zinc in animals. *World Rev. Nutr. Diet* 32: 135–164, 1978.
16. MACDONALD R. S.: The role of zinc in growth and cell proliferation. *J. Nutr.* 130 (55 Suppl): 1500–1508, 2000.
17. HAMBIDGE K. M., KREBS N. F.: Zinc deficiency: A special challenge. *J. Nutr.* 137: 1101–1105, 2007.
18. WEISMANN K., HOE S., KNUDSEN L., SORENSEN S. S.: Zinc absorption in patients suffering from acrodermatitis enteropathica and in normal adults assessed by whole-body counting technique. *Br. J. Dermatol.* 101: 573–579, 1979.
19. WANG K., ZHOU B., KUO Y. M., ZEMANSKY J., GITSCHIER J.: A novel member of a zinc transporter family is defective in acrodermatitis enteropathica. *Am. J. Hum. Genet.* 71: 66–73, 2002.
20. KURY S., DRENO B., BEZIEAU S.: Identification of SLC39A4, a gene involved in acrodermatitis enteropathica. *Nat. Genet.* 31: 239–240, 2002.
21. BLACK R.: Therapeutic effects of oral zinc in acute and persistent diarrhea in children in developing countries. *Am. J. Clin. Nutr.* 72: 1516–1522, 2000.
22. DUTTA S., PRCACCION F., AAMODT R.: Zinc metabolism in patients with exocrine pancreatic insufficiency. *J. Am. Coll. Nutr.* 17: 556–563, 1999.
23. KREBS N., SONTAG M., ACCUISO F., HAMBIDGE M.: Low plazma zinc concentration n young infants with cystic fibrosis. *J. Peadiatr.* 133: 761–764, 1998.
24. TAPAZOGLOU E., PRASAD A. S., HILL G., BREWER G. J., KAPLAN J.: Decreased natural killer cell activity in patients with zinc deficiency with sickle cell disease. *J. Lab. Clin. Med.* 105: 19–22, 1985.
25. KREBS N. F.: Zinc transfer to the breastfed infant. *J. Mammary Gland Biol. Neoplasia* 4: 259–268, 1999.
26. SIMMER K., AHMED S., CARLSSON L., THOMPSON R. H. P.: Breast milk zinc and copper concentrations in Banglades. *Br. J. Nutr.* 63: 91–96, 1990.
27. DOMELLÖF M., LÖNNERDAL B., DEWEY K. G., COHEN R. J., HERNELL O.: Iron, zinc, and copper concentrations in breast milk are independent of maternal mineral status. *Am. J. Clin. Nutr.* 79: 111–115, 2004.
28. AGGETT P. J., ATHERTON D. J., MORE J., DAVEY J., DELVES H. T., HARRIES J. T.: Symptomatic zinc deficiency in breast-fed, preterm infants. *Arch. Dis. Child* 55: 547–550, 1980.
29. ZIMMERMAN A. W., HAMBIDGE K. M., LEPOW M. L., GREENBERG R. D., STOVER M. L., CASEY C. E.: Acrodermatitis in breast-fed premature neonates: evidence for a defect of mammary gland secretion. *Pediatrics* 69: 176–183, 1982.
30. BLOM I., JAMESON S., KROOK F., LARSSON-STYMNE B., WRANNE L.: Zinc deficiency with transitory acrodermatitis enteropathica in a boy of low birth weight. *Br. J. Dermatol.* 104: 459–464, 1980.
31. BARNES P. M., MOYNAHAN E. J.: Zinc deficiency in acrodermatitis enteropathica: multiple dietary intolerance treated with synthetic diet. *Proc. R. Soc. Med.* 66: 327–329, 1973.
32. MANCINI J., TUNNESSEN W. W.: Acrodermatitis enteropathica-like rash in a breast-fed full term infant with zinc deficiency. *Arch. Pediatr. Adolesc. Med.* 152: 1239–1240, 1998.
33. HALIASOS E. C., LITWACK P., KRISTAL L., CHAWLA A.: Acquired zinc deficiency in full-term newborns from decreased zinc content in breast milk. *Cutis* 79: 425–428, 2007.
34. FRAKER J. P., JARDIEU P., COOK J.: Zinc deficiency and immune function. *Arch. Dermatol.* 123: 1698–1700, 1987.

35. COHEN J. J., DUKE R. C., FADOK V. A., SELLINS K. S.: Apoptosis and programmed cell death in immunity. *Annu. Rev. Immunol.* 10: 267–293, 1992.
36. FERNANDES G., NAIR M., ONOE K., TANAKA T., FLOYD R., GOOD R. A.: Impairment of cell mediated immunity function in dietary zinc deficiency in mice. *Proc. Natl. Acad. Sci. USA* 76: 457–461, 1979.
37. OLESKE J. M., WESTPHAL M. L., SHORE S., GORDEN D., BOGDEN J. D., NAHMIAS A.: Correlation with zinc therapy of depressed cellular immunity in acrodermatitis enteropathica. *Am. J. Dis. Child* 133: 915–921, 1979.
38. ALLEN J. I., PERRI R. T., MCCLAIN C. J., KAY N. E.: Alterations in human natural killer cell activity and monocyte cytotoxicity induced by zinc deficiency. *J. Lab. Clin. Med.* 102: 577–589, 1983.
39. KEEN C., GERSHWIN M.: Zinc deficiency and immune function. *Annu. Rev. Nutr.* 10: 415–431, 1990.
40. TONE K., SUZIKI T., TODOROKI T.: Influence on zinc deficiency on phagocytosis in mice. *Kitasato Arch. Exp. Med.* 64: 263–269, 1991.
41. HOCK H., HAMBLEN M. J., ROOKE H. M., TRAVER D., BRONSON R. T., CAMERON S., ORKIN S. H.: Intrinsic requirement for zinc finger transcription factor Gfi-1 in neutrophil differention. *Immunity* 18: 109–120, 2003.
42. KRUSE-JARRES J. D.: The significance of zinc for humoral and cellular immunity. *J. Trace Elem. Electrolytes Health Dis*. 3:1–8, 1989.
43. KARZAKOVA L. M.: Laboratory manifestations of asymptomatic zinc deficiency. *Klin. Lab. Diag*. 12: 39–41, 2005.
44. CHANDRA R., NEWBERNE P.: Nutrition, immunity and infection. Plenum Press, New York, 1977.