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The Effect of Zinc Consumption on Cell Immunity in Healthy 6 Years Old Children

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ABSTRACT

Introduction: The study aimed to determine the effect of zinc consumption on cell immunity in healthy 6-year-old children.
Methods: In a double-blind clinical trial after the license of parents 40 children 6-7 years old were enrolled. The male healthy and 6-7-year-old children were included and those with chronic disease, failure to thrive, usage of another zinc supplement in the past two months, and refusal to participate were excluded. In the case group (N=20) twenty mg of zinc sulfate syrup orally has been prescribed daily for 6 months. The control group (N=20) received a placebo the same as the case group in the volume, color, bottle size, and shape. Serum zinc level and cellular proliferation were measured before intervention and 12 hours after the last dose of zinc sulfate. No parents and no laboratory staff knew about the intervention. Zinc serum was measured by manual colorimetric method technique. A zinc level of less than 65 ug/dl is considered zinc deficiency. The lymphocyte proliferation before and after zinc treatments have been compared by paired T-test analysis.
Results: The mean weight of children in the case and control group were 20.37±2.21kg and 20.92±1.98 kg respectively (P= >0.05). Serum zinc level was within the normal limit and did not differ between the two groups before and after intervention (P=0.86). After 6 months of supplementation of 20 mg zinc sulfate per day for 6 months, there were no significant improvements in Lymphocyte proliferation (with/without PHA).
Conclusion: This study indicates that moderate supplementation of zinc for six months cannot efficiently improve Lymphocyte proliferation (with/without PHA) in healthy male children.

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Introduction

Zinc is an essential trace mineral that has an important influence on the immune system due to its roles in many general cellular functions like signal transduction, transcription, and replication (1-2). Zinc deficiency affects primarily T cells, resulting in a decreased number of T cells and disturbance of their functions. However, other immune cells have also a relationship with zinc

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deficiency due to a reduced antibody formation, particularly in response to neoantigens, because native B cells are more affected by zinc deprivation than memory B cells (1-3).

Allen et al explained alternations in zinc metabolism may have important implications for human tumor immune surveillance mechanisms (4). Cakman showed dysregulation between TH1 and TH2 cells in the elderly with long-term zinc deficiency (5). This was observed by altered secretion of the typical TH1 cytokines IFN-c and IL-2, whereas TH2 cytokines IL-4, IL-6, and -10 remain unchanged during zinc deficiency (5,6). In a study in Iran (2004) the lymphocyte proliferation response in the zinc group increased relative to that in the control group, but no significant effects in concentrations of cytokines (interleukin 2 and interferon) released from mitogen-stimulated mononuclear cells or on concentrations of cytokines (interleukin 2, interferon, and interleukin 1) in feces (7). However, another study showed that zinc supplementation was effective in decreasing oxidative stress and the generation of inflammatory cytokines such as TNF and IL-1b in elderly individuals and patients with sickle cell disease (8).

Zinc supplementation may have therapeutic or preventive benefits (9) as Bonaventura et al reviewed zinc deficiency affects cells involved in both innate and adaptive immunity at the survival, proliferation, and maturation levels. These cells include monocytes, polymorphonuclear, natural killer, T, and B cells. The cell functions and the balance between the different T helper cell subsets are particularly susceptible to changes in Zn status (10). Lymphopenia is common in zinc-deficient humans and animals and occurs in both the central and peripheral lymphoid tissues because deficiencies in zinc alter endocrine function and lymphogenic process in the mouse (11). It is concluded that Zn in the form of ZnO appears to have specific effects on the innate and adaptive gut-associated immune system of piglets (12). Fernandes et al showed Peripheral blood lymphocyte and macrophage concentrations were eventually reduced by >50% (13). Importantly, even marginal zinc deficiency substantially suppressed peripheral blood lymphoid cell concentrations in mice and humans. Zinc deficiency results not only in decreased lymphocyte concentrations but in depressed T and B lymphocyte function. In general, murine T and B lymphocyte proliferative responses to mitogens were greatly depressed in zinc-deficient compared with pair-fed animals (14). Suppression ranged from 5% to 50% with a tendency for greater suppression in T lymphocytes.

Zinc could become an immunosuppressant in transplantation medicine without toxic side effects which still leaves the immune system with the ability for phagocytosis.(15)

Phaber et al showed that zinc maintains the antigenic potency of the host while blocking the allogenic response (16).

Zinc deficiency is frequently observed during autoimmune diseases and indicates that modulating zinc homeostasis could be a promising approach to counteract inflammation and autoimmunity (17). Bonhan et al found no adverse effect of zinc supplementation on immune status. They supported the US upper level of zinc tolerance at 40 mg/dl (18). While Chvapil et al found that zinc ions inhibit some functions of macrophages(19). A study indicated that 15 ppm zinc supplementation was required to obtain a higher immune response in lambs when fed a basal diet containing 29.28 ppm Zn (20). The severity of immune senescence, which is the age-related decline of immune function, corresponds to the age-dependent decline in zinc status and is counteracted by zinc supplementation (21). The aim of this study was to determine zinc usage on cell immunity in unisex 7 years boys.

Materials and Method

In a double-blind clinical trial (IRCT 138711021162N9) 40 children enrolled in the study. The male healthy and 6-7 years old children were included and those with chronic disease, failure to thrive, usage of another zinc supplement in recent 2 months, and refusal to participate were excluded. After the license of parents and the Education organ of Khorasan Razavi province 6-7 years old male children were enrolled. In our country, male and female children are educated in separate schools. Through cluster random sampling students of grade one had the freedom to take part in the study. The sample size was determined based on Pinna et al study (1). In the case group (20 children) 20 mg zinc sulfate syrup orally has been used daily for 6 months. The control group (n=20) received a placebo as the same case in the volume, color, bottle size, and shape. The samples were coded. Serum zinc levels and cellular proliferation were measured before intervention and 12 hours after the last dose of zinc sulfate. The volume of the blood sample was 5 cc. No parents and no laboratory staff knew about the intervention. Zinc serum was measured by manual colorimetric method technique (24). A zinc level of less than 65 ug/dl was considered zinc deficiency. the sample size was detected based on Kathryn Pinna study(22).

At the end of the study codes were opened and statistical analyses were done.

Lymphocyte proliferation has been performed on peripheral blood lymphocytes (PBL) obtained by centrifugation on Ficoll-Metrizoate (density: 1077). 2 x 10 cells were cultured in triplicate in 0.2 ml culture medium (RPMI 1640; 2.5% pooled human serum, 2 mM glutamine, 20 mM Hepes, 1 g/1 bicarbonate, geomycin 40 /ig/ml) during 72 hours at 37°C in a water saturated atmosphere containing 5% $C0_2$ and 95% air. Final concentrations were 0.2 and 1 /tig/ ml for PHA (Phytohemagglutinin) and 25 /ig/ml for Con A (PHA-P, Welcome; Con a Sigma). Eighteen hours before harvesting, 5 /xCi methyl ^{3}H thymidine (specific activity 3.6 Ci/mA/, Institut des Radioelements, Fleurus) was added to each well. Cells were collected on glass fiber filters (934 AH, Whatman) and the radioactivity was measured in a liquid scintillation counter (Tricarb-Packard). The lymphocyte proliferation was compared before and after an intervention.

Statistical analysis

The lymphocyte proliferation response before and after zinc supplement has been compared by paired t-test analysis. The mean difference between the pre-test and post-test was also compared by t-test, Spss version 16.

Results

The mean weight of children in the case group was 20.37±2.21kg (17.1 minimum and 24.6 maximum) and in the control group was 20.92±1.98 kg (17.2 minimum and 27 maximum). There was no significant difference between the two groups in weight (P= >0.05). As Table 1 shows serum zinc levels were within normal limits between the two groups before and after intervention (P=0.86). In cellular culture (table 2), the impact of in vivo zinc supplementation in healthy children on Lymphocyte culture (with/without PHA) shows the Lymphocyte proliferation (with /without PHA) before and after supplementation of zinc in subjects. After supplementation of 20 mg zinc sulfate per day for 6 months, there were no significant increases in Lymphocyte proliferation (with/without PHA) as a function of lymphocytes. This indicates that moderate supplementation cannot efficiently improve Lymphocyte proliferation (with/without PHA) in children within six months.

Discussion

This study showed zinc supplement do not influence the immune function in normal six-year-old children. The treatment had not significant influence on the lymphocyte response to PHA as indicated by a comparison of post- and pre-test mean differences in the two groups.

The essential of zinc was first documented by Prasad and colleagues in the 1990s. Zinc is a cofactor of more than 300 enzymes. Growth retardation hypogonadism in males, rough skin, impaired immunity, neuro-sensory disorder, and cognitive impairment are some of the clinical manifestations of zinc deficiency (1).

Prasad defined that both epidemiological and clinical experiences indicate an important role of zinc in immunologically mediated host defense. Zinc affects multiple aspects of the immune system. Zinc is required for the normal development and function of cells mediating innate immunity, neutrophils and natural killer cells. Macrophages are also affected by zinc deficiency. Phagocytosis, intracellular killing,

Table 1. Serum zinc level (ug/dl) before and after zinc supplementation

Intervention	Case group	Control group	p- value
Before intervention	90.87±29.20	76.09±36.55	P= 0.86
After intervention	170.04±83.01	117.60±33.48	

Table 2. Lymphocyte proliferation before and after zinc supplementation

Test	Case(N=20)	Control(N=19)	P value
With PHA* (pre test)	1.07±0.45	0.91±0.37	P=0.31
With PHA (post test)	0.26±0.10	0.23±0.05	
Without PHA (pre test)	0.47±0.14	0.41±0.13	P=0.45
Without PHA (post test)	0.09±0.03	0.09±0.03	
With/Without Ratio Pre test	2.3±0.73	2.1±0.49	P=0.46
With/Without Ratio post test	3.2±1.7	2.8±0.97	P=0.38
Difference of ratio	0.93±1.9	0.69±1.13	P=0.64

* Phytohemagglutinin

This Statistical Analysis was done by paired t-Test

and cytokine production are all affected by zinc deficiency. Adversely zinc deficiency affects the growth and function of T and B cells. This occurs through deregulation of basic biological functions at the cellular level. Zinc is necessary for DNA synthesis, RNA transcription, cell division, and cell activation. Apoptosis (programmed cell death) is potentiated by zinc deficiency. Secretion and function of cytokines, the basic messengers of the immune system are adversely affected by zinc deficiency (1). There are many studies about zinc and the immune system some found positive effects such as studies of Mocchegiani et al and Bobat et al who they did on ill babies (23, 24) and others studies the same as us showed negative effects of zinc on the immune system (25, 26) because we did in healthy babies with normal Zinc level.

In children recovering from severe malnutrition, several studies have confirmed the stimulation of the immune capacity by zinc (21). In a study in Shiraz of Iran (27) in 2004 there were no Zinc deficiency in 3- 18-year-old. It is the same as our study.

Lymphocyte production effects include primary immune deficiency and immune deficiency due to malnutrition or zinc deprivation (28)

Sabrina et al showed serum concentrations of zinc are often low in critically ill children early after PICU admission. So low serum zinc levels were associated with lymphopenia (29).

Farida et al also showed serum concentrations of zinc were generally low in critically ill children and were correlated with the severity of illness (30).

Kewcharoenwong et al 2020, studied the effect of zinc supplementation on immune function in young Laotian children. They found zinc supplementation in rural Laotian children did not affect cytokines or T-cell concentrations, on the other hand, it affected lymphocyte and eosinophile concentrations (31).

Jin reviewed zinc intakes and health outcomes in an umbrella study. They found the immune function in humans could be influenced by zinc status as our study was done on healthy babies with normal Zinc level therefore we didnt found any difference. When zinc is reduced humans will suffer depressed T and B lymphopoiesis and impaired maturation and antibody production. Zinc efficiency would compromise the activities of natural killer cells, neutrophils, monocytes, macrophages, and T helper cells (32).

The negative result may be because our children were not zinc deficient. Second, the age of children may be high for this aim because they were not zinc deficient third, it appears more sample size is required for better results.

The side effects of Zinc is vomiting or regurgitation but we did not observe any effects.

Conclusion

In this study, daily administration of 20 mg oral zinc sulfate in healthy children did not affect the lymphocyte proliferation as a cell function. For the detection of the zinc effect on cellular immunity, it appears we should select zinc-deficient children with a larger sample size. On the other hand, for prescription of zinc as supplementation should be paid attention.

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Conflict of interest

The author declares that no financial or other conflict of interest exists in relation to the content of the paper.

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