ZINC IN HUMANS: HEALTH DISORDERS AND THERAPEUTIC EFFECTS

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ABSTRACT. Deficiency of zinc is prevalent in human populations throughout the world. Major clinical effects of zinc deficiency in humans include growth retardation, immune system disorders and cognitive impairment. Beside them, zinc deficiency can cause other health disturbances. Zinc supplementation can improve health condition in certain cases. It was found that zinc supplementation resulted in clinically important reductions in the duration and severity of diarrhea in infants and young children. Administration of zinc lozenges is associated with reduced duration and severity of common cold symptoms that may be related to a decrease in pro-inflammatory cytokine levels. Therapeutic levels of zinc improve condition of sickle cell disease patients by inducing copper deficiency in them. This suggests to evaluate zinc as a therapeutic modality for the treatment of Wilson's disease. In case of age related macular degeneration (AMD), zinc reduced the risk of developing advanced AMD and reduced the risk of vision loss. The ability of zinc to function as an antioxidant and stabilize membranes suggests that it has a role in prevention of free radical induced injury during inflammatory processes. On the other hand, acute high-level exposure to zinc compounds can produce respiratory and gastrointestinal toxicity, though these effects are largely self-limiting. Some specific problem is that the excess intake of zinc leads to copper deficiency. Zinc deficiency can be diagnosed by measurement of zinc level in blood plasma, red cells, hair or urine. Zinc determination in granulocytes and lymphocytes, however, reflect the body zinc status more accurately. Quantitative assay of alkaline phosphatase activity in granulocytes, thymulin activity in plasma and IL-2 expression in mononuclear cells are also suggestive useful tools for zinc deficiency evaluation.

KEYWORDS: zinc, deficiency, clinical effects, diagnostics, treatment, supplementation, toxicity.

INTRODUCTION
Essentiality of zinc for humans and its deficiency was recognized in 1963 (Prasad et al., 1963). During the past 50 years, it has become apparent that deficiency of zinc is prevalent in human populations throughout the world; the current estimate is that nearly 2 billion subjects in the developing world may have zinc deficiency. Consumption of cereal proteins high in phytate decreases the availability of zinc for absorption. Conditioned deficiency of zinc is also very common. One would also expect to see a spectrum of zinc deficiency, ranging from severe cases to marginally deficient examples, in any given population. Zinc is involved in many biochemical functions and nearly 2000 transcription factors require zinc for gene expression. Zinc deficiency can result in many serious health problems that is known for nearly fifty years. It is indeed very surprising and somewhat disappointing that major health agencies such as WHO, FAO and UNESCO have ignored this major problem. The solution is relatively simple and very cost-effective and I certainly hope that this nutritional problem is corrected soon.

MAJOR CLINICAL EFFECTS OF ZINC DEFICIENCY IN HUMANS

Growth retardation
We documented zinc deficiency in growth retarded villagers by the following criteria: the zinc concentrations in plasma, red cells and hair were decreased and the $^{65}$Zn in urine and stool was less in the growth retarded subjects than in the control subjects (Halsted et al., 1972; Prasad, 1993). Our studies also documented that the rate of growth was greater in patients who received zinc supplement as compared to those who received iron, or those who received only an adequate animal protein diet. Pubic hair appeared in all subjects within 7 to 12 weeks after zinc supplementation and genitalia increased to normal size and secondary sexual characteristics developed within 12 to 24 weeks in all subjects receiving zinc (Sandstead et
al., 1967). No such changes were seen in a comparable length of time in the iron supplemented group or in the group receiving an animal protein diet. We concluded that the growth retardation and male hypogonadism in these subjects were due to zinc deficiency. The anemia was due to iron deficiency and responded to oral iron treatment but not zinc.

A meta-analysis of 33 studies of the effect of zinc supplementation on children's growth was reported by Brown et al. (2002). Prospective intervention trials were included if they enrolled a control group and provided suitable data on change in height or weight during the period of observation. The pooled study population included 1834 children less than 13 years of age, with representation from most regions of the world. The meta-analysis showed that in all children studied, zinc supplementation had a highly statistically significant positive response in height and weight increments respectively.

Nakamura et al. (1993) screened 220 prepubertal subjects with short stature in a hospital clinic for zinc supplementation trial. In zinc supplemented children, caloric intake (p < 0.01), growth velocity (p < 0.01), serum zinc, calcium and phosphorus concentrations, alkaline phosphatase activity (p < 0.001), percentage of tubular reabsorption of phosphorus (p < 0.05), ratio of maximal tubular reabsorption rate for phosphorus to the glomerular filtration rate (p < 0.05), serum osteocalcin level (p < 0.01), and plasma insulin like growth factor 1 (IGF-1) (p < 0.05) were significantly increased in comparison to control group but urinary excretion of growth hormone (GH) was unchanged. All the above parameters remained unchanged in the controls. Zinc supplementation resulted in a considerable increase in height. Also these investigators reported that urinary GH excretion and other pituitary hormone levels in the plasma were unchanged with zinc supplementation. However, a significant elevation in serum IGF-1 level after zinc supplementation was observed. Increased plasma levels of IGF-1, despite unchanged GH production, may be explained if zinc has a role in binding GH to GH receptor in hepatic cells. Nakamura et al. (1993) recommended that the children with non-endocrinologic short stature should be treated for at least six months with zinc, the children with non-endocrinologic short stature may have a marginal deficiency of zinc and should be treated for at least six months with zinc, the children with non-endocrinologic short stature probably have a significant deficiency of zinc.

Zinc deficiency is associated with diets high in phytate and fiber and low in protein. Because > 80% of the dietary energy intake in Vietnam is derived from rice and intake of animal products is low, the Vietnamese diet places children at risk for zinc deficiency.

In order to determine whether zinc deficiency might be responsible for the failure to thrive observed in Vietnamese children, Ninh et al. (1996) assessed growth, incidence of infections, and circulating IGF-1 concentrations in a double-blind study of zinc supplementation. Zinc supplementation increased weight (+0.5 ± 0.1 kg; p < 0.001) and height (+1.5 ± 0.2 cm; p < 0.001) after five months compared to placebo group. The relative risk of infectious episodes in the zinc supplemented group was reduced 3-fold for diarrhea (p = 0.012) and 2.5-fold for respiratory tract infection (p = 0.057). Plasma IGF-1 concentration increased in zinc treated subjects between one and five months (p = 0.018), whereas no change in placebo group was observed.

This study showed that zinc deficiency was limiting growth in Vietnamese children and the growth stimulating effect of zinc might be mediated through changes in circulating IGF-1. Ninh et al. (1995) investigated the mechanism responsible for the IGF-1 decline due to zinc deficiency in rats. Zinc depletion in comparison to pair-fed animals specifically reduced body weight gain (–22%, p < 0.05), serum IGF-1 concentration (–52%, p < 0.001), hepatic GH receptors (–28%, p < 0.05) and serum growth hormone binding protein (GHBP) levels (–51%, p < 0.05). Both zinc deficient and pair-fed groups of animals had reduced liver IGF-1 and GH receptor/GHBP mRNA levels in comparison with the adlibitum fed controls (p < 0.01). However, only liver IGF-1 mRNA levels were specifically reduced by zinc deficiency (zinc deficient vs pair-fed rats, p < 0.05). Zinc deficiency per se decreased serum IGF-1 independently of the reduction in food intake.

**Zinc and immunity**

Zinc affects multiple aspects of the immune system (Shankar, Prasad, 1998). Zinc is crucial for normal development and function of cells mediating innate immunity, neutrophils and natural killer cells. Macrophages are also affected by zinc deficiency. Phagocytosis, intracellular killing, and cytokine production are all affected by zinc deficiency. Zinc deficiency also affects adversely the growth and function of T and B cells. This occurs through dysregulation of basic biological functions at the cellular level. Zinc is needed for DNA synthesis, RNA transcription, cell division, and cell activation. Programmed cell death (apoptosis) is also potentiated in the absence of ade-
quante levels of zinc. Secretion and function of cytokines, the basic messengers of the immune system are adversely affected by zinc deficiency. The ability of zinc to function as an antioxidant and stabilize membranes suggests that it has a role in prevention of free radical induced injury during inflammatory processes.

It has been known for many years that zinc deficiency in experimental animals leads to atrophy of thymic and lymphoid tissue. Later studies in young adult zinc deficient mice showed thymic atrophy, reductions in absolute number of splenocytes, and depressed responses to both T-cell-dependent (TD) and T cell-independent (TI) antigens (Shankar, Prasad, 1998).

A decrease in vivo generated cytotoxic T-killer activity to allogeneic tumor cells in zinc deficient mice and an impairment in cell mediated response to non-H2 allogeneic tumor cells in zinc deficient mice have been reported (Shankar, Prasad, 1998). Animals maintained on a zinc deficient diet for as little as 2 weeks developed a severe impairment in their ability to generate a cytotoxic response to the tumor challenge. This was totally reversible by zinc supplementation.

**Studies of immune functions in experimental human model**

During our studies in the Middle East, we observed that most of the zinc deficient dwarfs did not live beyond the age of 25 years. The cause of death appeared to be infections. The possibility that zinc deficiency may have played a role in immune dysfunctions in the zinc deficient dwarfs was considered but lack of proper facilities prevented us from gathering meaningful data on immune functions in those patients.

We developed an experimental model, which allowed us to study specific effects of mild zinc deficiency in humans on immune functions (Prasad, 1993). When zinc deficiency was very mild (5.0 mg Zn intake during the zinc-restricted period), the plasma zinc concentration remained more or less within the normal range and it decreased only after 4–5 mo of zinc restriction. On the other hand, zinc concentrations in lymphocytes, granulocytes, and platelets decreased within 8–12 wk, suggesting that the assay of cellular zinc provided a more sensitive criterion for diagnosing mild zinc deficiency (Prasad, 1993).

We assayed serum thymulin activity in mildly zinc-deficient human subjects (Prasad et al., 1988). Thymulin is a thymus-specific hormone and it requires the presence of zinc for its biological activity to be expressed. Thymulin binds to high-affinity receptors on T cells, induces several T-cell markers, and promotes T-cell function, including allogenic cytotoxicity, suppressor functions, and interleukin-2 (IL-2) production.

As a result of mild deficiency of zinc, the activity of thymulin in serum was significantly decreased and was corrected by both in vivo and in vitro zinc supplementation. The in vitro supplementation studies indicated that the inactive thymulin peptide was present in the serum in zinc-deficient subjects and was activated by addition of zinc. The assay of serum thymulin activity with or without zinc addition in vitro thus may be used as a sensitive criterion for the diagnosis of mild zinc deficiency in humans.

An increase in T101-, slg- cells, a decrease in the ratio of T4+ to T8+, and decreased IL-2 activity were observed in the experimental human model during the zinc-depletion phase, all of which were corrected after repletion with zinc (Prasad et al., 1988). We had previously reported that natural-killer (NK)-cells activity was also sensitive to zinc restriction, thus it appears that zinc may play a very important and critical role in the functions of T cells in humans (Prasad, 1993).

In our recent studies we have shown that a mild deficiency of zinc leads to an imbalance of TH1 and TH2 functions, decreases the recruitment of T naive cells (CD4+ CD45RA+), and decreases the percentage of CD73+ cells in the CD8+ subset that are precursors to cytotoxic T lymphocytes (CTL) (Beck et al., 1997a).

Our studies in the experimental human model showed for the first time that the production of IFN-γ was decreased, whereas the production of IL-4, IL-6 and IL-10 was not affected due to zinc deficiency (Beck et al., 1997b). We have reported previously that zinc deficiency decreased IL-2 activity and production in experimental human model subjects and in patients with sickle cell disease and head and neck cancer patients. Taken together, our studies suggest that zinc affects mainly the functions of TH1 cells.

IFN-γ is known to down regulate the TH2 clone, and IL-10 may down regulate the TH1 clone. An imbalance between TH1 and TH2 responses in patients with human immunodeficiency virus infection has been implicated in the immune dysregulation in these patients and it has been proposed that resistance to infection and/or progression to acquired immunodeficiency syndrome is dependent on a TH1 > TH2 dominance. Our data in experimental human model suggest that cell-mediated immune dysfunctions in human zinc deficiency may be due to an imbalance between TH1 and TH2 cell functions.

TH1 cells are known to promote macrophage activation and production of complement fixing and opsonizing antibodies. IFN-γ is the major component of TH1 response panel, and it upregulates major histocompatibility complex class I antigen expression. Our studies, therefore, provide a possible mechanism of zinc on cell-mediated immunity.

T cell subpopulation studies revealed that CD4+ to CD8+ ratio was significantly related to zinc status. A decrease in this ratio was observed during zinc deficiency and this was corrected by zinc supplementation. A borderline significant effect of zinc status on the ratio of CD4+ CD45RA+ to CD4+ CD45R0+ cells was seen in the human volunteers. The newly produced CD4+ T lymphocytes express CD45 isoforms, which are designated CD45RA+ and once
these cells encounter a specific antigen, they become "memory" T lymphocytes, expressing a small isoform of cleaved CD45 designated CD45 R0+ cells. It appears that zinc is required for the regeneration of new CD4+ T cells. Inasmuch as zinc is essential for the activity of thymulin, a thymic hormone, it is possible that zinc may be intrinsically involved in the development of hematopoietic stem cells to T lymphocytes in the thymic microenvironment.

Earlier we have reported that NK cell activity is zinc dependent. In patients with zinc deficiency, NK cell activity is decreased and this is correctable by zinc supplementation (Prasad, 1993). Our studies in experimental human model showed that the percentage of CD8+ CD73+ T lymphocyte are decreased in zinc deficiency and this is corrected by zinc supplementation. CD8+ CD73+ lymphocytes are predominantly precursors to cytotoxic T lymphocytes (CTL), and the presence of CD73 molecule on CTL is required for antigen recognition, the proliferative process, and for generation of cytolytic process. Thus, it is likely that the increased frequency of infections seen in zinc deficient patients may be related to a decrease in NK cell lytic activity and decreased CTL activity.

Our studies in the experimental human model, thus show that even a mild deficiency of zinc in humans may be accompanied by an imbalance of TH1 and TH2 cells, decreased serum thymulin activity, decreased recruitment of T naive cells, decreased percent of T cytolytic cells and decreased NK cell lytic activity. These immunologic consequences of zinc deficiency may be responsible for decreased cell mediated immune functions in zinc-deficient subjects.

Cell culture studies

Nearly 2000 transcription factors require zinc for their structural integrity; however, it is not known if cellular zinc deficiency results in any change in activation of any of the transcription factors. Inasmuch as NF-kB binds to the promoter enhancer area of IL-2 and IL-2Rα genes, we investigated the effect of zinc deficiency on activation of NF-kB and its binding to DNA in HUT-78, a Th0 malignant human lymphoblastoid cell line (Prasad et al., 2001). We showed for the first time that in zinc deficient HUT-78 cells, phosphorylated IkB, and IKK, ubiquitinated IkB and binding of NF-kB to DNA were all significantly decreased. Zinc increased the translocation of NF-kB from cytosol to nucleus. We also demonstrated that the binding of recombinant NF-kB (p50) to DNA in HUT-78 cells was zinc specific. We conclude that zinc plays an important role in activation of NF-kB in HUT-78 cells.

We used a human Th0 malignant lymphoblastoid cell line HUT-78 to study the effect of zinc on IL-2 production in PHA/PMA activated T-cells. In zinc-deficient cells, the gene expression of IL-2 was decreased by 50% compared with that in zinc-sufficient cells (Prasad et al., 2002). The effect of zinc was specific and at the transcriptional level. A significant effect of zinc was also shown on the gene expression of IL-2 receptors α and β. Binding of NF-kB (a zinc-dependent transcription factor) to DNA was decreased in zinc-deficient cells. By utilizing transfection of expression vectors of anti-sense NF-kB p105 (precursor of NF-kB p50) in cells, we showed that a decrease in gene expression of IL-2 and IL-2 Rα may be partly due to decreased activation of NF-kB in zinc-deficient cells. Our studies demonstrate for the first time, the role of zinc on gene expression of IL-2 and its receptors in HUT-78 cells. We also document that the binding of NF-kB to DNA was adversely affected, thereby decreasing the gene expression of IL-2 and IL-2 Rα in zinc-deficient HUT-78 cells.

Role of zinc as an antioxidant and anti-inflammatory agent

Oxidative stress and chronic inflammation are important contributing factors in several chronic diseases, such as atherosclerosis and related vascular diseases, mutagenesis and cancer, neurodegeneration, immunologic disorders, and the aging process. We administered 45 mg zinc as gluconate daily to 10 volunteers and 10 subjects received placebo for 8 weeks (Prasad et al., 2004). The volunteers were healthy and their ages ranged from 19 to 50 years. In subjects receiving zinc, plasma levels of lipid peroxidation products and DNA adducts were decreased, whereas no change was observed in the placebo group. LPS-stimulated MNC isolated from zinc supplemented groups showed reduced mRNA for TNF-α and IL-1β compared to placebo. Ex vivo, zinc protected MNC from TNF-α induced NF-kB activation. In parallel studies using HL-60, a promyelocytic leukemia cell line, we observed that zinc enhances the upregulation of mRNA and DNA-specific binding for A-20, a transactivating factor which inhibits the activation of NF-kB. Our results suggest that zinc supplementation may lead to down regulation of the inflammatory cytokines through upregulation of the negative feedback loop A-20 to inhibit induced NF-kB activation (Prasad et al., 2004).

Zinc deficiency, cell-mediated immune dysfunction, susceptibility to infections, and increased oxidative stress have been observed in elderly subjects (> 55 years old). We conducted a randomized, double-blind, placebo-controlled trial of zinc supplementation in elderly subjects ages 55–87 years (Prasad et al., 2007). Fifty healthy elderly subjects were recruited for this study. The supplementation was continued for 12 months. The zinc supplementation group received zinc gluconate (45mg elemental zinc) orally daily. Compared with a group of younger adults, at baseline the older subjects had significantly lower plasma zinc, higher plasma oxidative stress markers and endothelial cell adhesion molecules. The incidence of infections and ex vivo generation of TNF-α and plasma oxidative stress markers were significantly lower in the zinc supplemented group than in the placebo group. Plasma zinc and PHA-induced IL-2 mRNA in isolated PMNC were significantly higher in
the zinc supplemented group than in the placebo group (Prasad et al., 2007).

In another study, we conducted a randomized, double-blind, placebo trial of zinc supplementation in 40 elderly subjects (aged 56–83 years) and randomly assigned them to two groups (Bao et al., 2010). One group received 45 mg elemental zinc daily as gluconate for 6 months and the other group received placebo. Cell culture studies were also done in order to study the mechanism of zinc action as an atheroprotective agent.

After zinc supplementation plasma high-sensitive C-reactive protein (hs CRP), interleukin-6, macrophage-chemo attractant protein 1 (McP-1), VCAM-1, secretory phospholipase A2, and MDA+ HAE decreased in the elderly subjects in comparison to the placebo group (Bao et al., 2010). In cell culture studies, we showed that zinc decreased the generation of TNF-α, IL-1β, VCAM-1, and MDA+HAE and the activation of NF-kB and increased A-20 and peroxisome proliferator-activated receptor-α in human mononuclear leukemia THP-1 cells and human aortic endothelial cells compared to the zinc deficient cells. These data suggest that zinc may have a protective effect in atherosclerosis because of its anti-inflammatory and antioxidant functions (Bao et al., 2010).

**Zinc deficiency and cognitive impairment**

Penland et al. (1997) conducted a study in elementary schools in low-income districts of three provinces in China, in order to assess the effects of zinc supplementation on growth and neuropsychological functions of children. Three hundred-seventy-two children between the ages of 6 to 9 years old were recruited for this study. Supplementations were 20 mg zinc, 20 mg zinc with micronutrients, or micronutrients alone. The micronutrient mixture was based on guidelines of the US NAS/NRC.

Growth was assessed by the change in length of the lower leg. Neuropsychological functions were tested by using the cognition psychomotor assessment system revised (CPAS-R) developed by Penland. Zinc alone had the least effect on growth, while zinc with micronutrients had the largest effect; micronutrients alone had little effect. Zinc containing treatments improved neuropsychological functions but micronutrients alone had little effect. Performance after zinc alone and/or zinc with micronutrients was better than after micronutrients alone for continuous performance, perception (matching of complex shapes), visual memory (delayed matching of complex shapes), tracking of a cursor on the computer screen, concept formation (identification of oddity) and key tapping.

It is evident that the Chinese children were deficient also in other nutrients besides zinc inasmuch as the group receiving micronutrients with zinc showed the maximum growth. These results are similar to the report published from Iran, which showed that depletion of latent deficiencies was essential for demonstration of the effects of zinc supplementation on growth.

**DIAGNOSTIC CRITERIA FOR ZINC DEFICIENCY**

Measurement of zinc level in plasma is very useful provided the sample is not hemolyzed and contaminated. In the condition of acute stress or infection, or following a myocardial infarction, zinc from the plasma compartment may redistribute to other tissues, thus making an assessment of zinc status in the body difficult. Intravascular hemolysis would also increase the plasma zinc level inasmuch as the zinc in the red cells is much higher than in the plasma.

Zinc in the red cells and hair also may be used for assessment of body zinc status, however, inasmuch as these tissues turn over slowly, and their zinc levels do not reflect recent changes with respect to body zinc stores. Zinc determination in granulocytes and lymphocytes, however, reflect the body zinc status more accurately and is thus a useful measurement (Prasad, 1993). A quantitative assay of alkaline phosphatase activity in the granulocytes is also a useful tool in our experience (Prasad, 1993).

Urinary excretion of zinc is decreased as a result of zinc deficiency. Thus, determination of zinc in 24-hour urine may be of additional help in diagnosing zinc deficiency provided cirrhosis of the liver, sickle cell disease, chronic renal disease, and other conditions known to cause hyperzincuria are ruled out. Hyperzincuria may be associated with zinc deficiency in the above mentioned diseases.

In our experiments, during the zinc-deficient state our subjects showed a marked positive balance for zinc (Lee et al., 1993). Thus, a metabolic balance study may clearly distinguish zinc deficient from zinc-sufficient subjects. One may also suggest that perhaps a test based on oral challenge of zinc and subsequent plasma zinc determination may be able to distinguish between zinc deficient and zinc sufficient states in humans.

Kaji et al. (1998) measured zinc clearance following i.v. injections of zinc sulfate solution (1 μmol/kg) in Japanese children with low stature. The body zinc clearance test was much more useful than serum zinc concentration in diagnosing marginal zinc deficiency according to these investigators.

We assessed the efficiency of zinc absorption as well as endogenous zinc excretion during a 6-month period of dietary zinc restriction in human volunteers by using a stable zinc isotope 70Zn. Efficiency of zinc absorption was not sustained when the zinc-restricted diet was continued for 6 months (Lee et al., 1993). We also observed a decrease in urinary zinc excretion as a result of zinc restricted diet. Our studies indicated that measurement of endogenous intestinal zinc excretion and urinary excretion, both of which are decreased, may be useful for diagnosing marginal deficiency of zinc in humans. Hyperzincuria, however, may be associated with zinc deficiency in certain conditions such as cirrhosis of the liver, sickle cell disease and certain renal disorders.

In our studies in experimental human model where we induced a mild deficiency of zinc by die-
Among the children who received zinc, there was a decrease in serum thymulin activity, decreased production of IL-2, decrease in lymphocyte ecto-5'-nucleotidase activity, decrease in intestinal endogenous zinc excretion and decrease in urinary zinc excretion occurred within eight weeks of the institution of a zinc restricted diet (approximately 5 mg zinc daily intake) (Prasad, 1993). These changes were observed prior to the changes in plasma zinc concentration and changes in lymphocyte and granulocyte zinc concentration. The decrease in plasma zinc was observed at the end of twenty weeks and decrease in zinc concentration of lymphocytes and granulocytes were observed at the end of twelve weeks of zinc-restricted diet.

The activities of many zinc-dependent enzymes have been shown to be affected adversely in zinc-deficient tissues. Three enzymes, alkaline phosphatase, carboxypeptidase, and thymidine kinase, appear to be most sensitive to zinc restriction in that their activities are affected adversely within 3–6 days of institution of a zinc-deficient diet to experimental animals. In human studies, the activity of deoxynucleoside kinase in proliferating skin collagen and alkaline phosphatase activity in granulocytes were shown to be sensitive to dietary zinc intake. As a practical test, quantitative measurement of alkaline phosphatase activity in granulocytes may be a very useful adjunct to granulocyte zinc level determination in order to assess the body zinc status in man. Following supplementation with zinc to deficient subjects, a prompt response in the activities of sensitive enzymes is observed (Prasad, 1993).

We have observed that a decrease in plasma thymulin activity in zinc deficient subjects was corrected by in vitro addition of zinc to the plasma and our recent data show that decreased IL-2 mRNA in PHA stimulated mononuclear cells by RT-PCR is also corrected by in vitro addition of zinc (Prasad et al., 2006). These tests, therefore, may be the most definitive diagnostic tools for marginal zinc deficiency in humans.

**THERAPEUTIC IMPACT OF ZINC**

**Acute diarrhea in infants and children**

Among children in developing countries, diarrhea of prolonged duration is an important cause of growth retardation and death (Sazawal et al., 1995). Episodes of diarrhea, which usually resolve within a few days in healthy children, persist longer in children with malnutrition and impaired cellular immunity. In children with severe zinc deficiency, diarrhea is a common manifestation, which responds promptly to zinc supplementation. Diarrhea also leads to excessive loss of zinc and thus sets up a vicious cycle.

A double-blind, randomized, controlled trial of zinc supplementation (20 mg elemental zinc) involving 937 children, 6 to 35 months of age was conducted in New Delhi, India. All the children also received oral rehydration therapy and vitamin supplements. Among the children who received zinc, there was a 23 per cent reduction in the risk of continued diarrhea. When zinc supplementation was initiated within three days of the onset of diarrhea, there was a 39 per cent reduction in the proportion of episodes lasting more than seven days. In the zinc supplemented group there was a decrease of 39 per cent in the mean number of watery stools per day and a decrease of 21 per cent in the number of days with watery diarrhea. The reductions in the duration and severity of diarrhea were greater in children with stunted growth than in those with normal growth. The authors concluded that zinc supplementation resulted in clinically important reductions in the duration and severity of diarrhea in infants and young children (Sazawal et al., 1995). Similar studies have now been done in many developing countries and the results are strikingly similar.

Possible mechanisms of beneficial effect on diarrhea in children include improved absorption of water and electrolytes by intestines, regeneration of gut epithelium or the restoration of its functions, increased levels of enterocyte brush-border enzymes, and enhanced immunologic mechanisms for the clearance of infections, including cellular immunity and higher levels of secretary antibodies.

**Zinc for the treatment of common cold**

The common cold is one of the most frequently occurring human illnesses in the world (Mossad et al., 1996). More than 200 viruses may cause common cold. These include rhinoviruses (the commonest cause), coronaviruses, adenoviruses, respiratory syncytial virus and parainfluenza viruses. Adults in USA develop an average of two to four colds and children develop an average of six to eight colds per year. The morbidity resulting from this disease and the subsequent financial loss in terms of working hours are substantial. Previously prescribed treatments have not succeeded in providing a consistent or well-documented relief of symptoms.

Eby et al. (1984) were the first to test zinc gluconate lozenges in a double-blind, placebo controlled trial for treatment of cold. One 23 mg zinc lozenges or matched placebo was dissolved in the mouth every 2 wakeful hours or after an initial double dose. After 7 days, 86% of 37 zinc treated subjects were asymptomatic, compared with only 46% of 28 placebo-treated subjects who became asymptomatic ($p = 0.0005$). Side effects included objectionable taste and mouth irritation.

In order to test the efficacy of zinc acetate lozenges in reducing the duration of symptoms of the common cold, we carried out a randomized, double-blind placebo-controlled trial in 50 ambulatory volunteers recruited within 24 h of developing symptoms of the common cold (Prasad et al., 2000). Participants took one lozenge containing 12.8 mg of zinc (as acetate) or placebo every 2 to 3 h while awake as soon as they developed cold symptoms. Subjective symptom scores for sore throat, nasal discharge, nasal congestion, sneezing, cough, scratchy throat, hoarseness,
muscle ache, fever and headache were recorded daily for 12 days. Plasma zinc and pro-inflammatory cytokines were measured on day 1 and after participants were well.

Forty-eight participants completed the study (25 in the zinc group and 23 in the placebo group). Compared with the placebo group, the zinc group had shorter mean overall duration of cold symptoms (4.5 vs 8.1 days), cough (3.1 vs 6.3 days) and nasal discharge (4.1 vs 5.8 days) and decreased total severity scores for all symptoms (p < 0.002). Mean changes in soluble interleukin-1 receptor antagonist level differed non-significantly between the zinc group and the placebo group (difference between changes – 89.4 pg/ml).

Administration of zinc lozenges was associated with reduced duration and severity of cold symptoms, especially cough. Improvement in clinical symptoms with zinc treatment may be related to a decrease in pro-inflammatory cytokine levels; however, in this study the observed differences between changes in cytokine levels in zinc and placebo recipients were not significant statistically.

We have recently published the results of our second placebo-controlled trial of zinc lozenges for the treatment of common cold (Prasad et al., 2008). Compared with the placebo group, the zinc group had a shorter mean overall duration of cold (4.0 vs 7.1 days, p < 0.0001) and shorter duration of cough (2.1 vs 5.0 days, p < 0.0001) and nasal discharge (3.0 vs 4.5 days, p = 0.02). Blinding of subjects was adequate. Symptoms severity scores were decreased significantly in the zinc group. Plasma sIL-1ra and ICAM-1 levels decreased significantly in the zinc group.

sIL-1ra is an anti-inflammatory cytokine which functions as a specific inhibitor of IL-1α and IL-1β inflammatory cytokines. Our results indicate that in zinc supplemented group sIL-1ra decreased, suggesting that overall inflammation was decreased in this group. Plasma ICAM-1 was also decreased in zinc treated subjects. Human rhinovirus type 14 “docks” with ICAM-1 on the surface of somatic cells. Thus, zinc may in effect act as an antiviral agent by reducing ICAM-1 levels. Another possibility is that zinc ions may form a complex with ICAM -1, preventing the binding of rhinovirus to cells.

The effect of zinc lozenges on the duration or severity of common cold symptoms has been examined in at least 14 different studies since 1984. Results of trials in which no effect of zinc was demonstrated were criticized for having inadequate sample sizes or for using inadequate doses of zinc or formulations that reduced the release of zinc ions from the lozenges. Zinc acetate and gluconate are suitable salts, inasmuch as zinc ions are released at physiological pH. Several zinc lozenges use glycine or citrate as ligands which prevent release of zinc ions and therefore, are not effective in curing common cold. In our experience, two other factors are important. One is that zinc lozenges must be started within 24 h of the onset of common cold and the other is that daily total dose of elemental zinc should be at least 75 mg.

**Zinc therapy for Wilson's disease**

Wilson's disease is an inherited autosomal disorder of copper accumulation. The excretion of liver copper in the bile is defective and this leads to a failure of excretion of excess copper in the stool. Eventually excess copper accumulates in the liver and brain and damages these vital organs. Patients typically present in the second to the fourth decades of life with liver disease, a neurological disease of the movement disorder type, or a wide array of psychiatric disturbances. In many cases the diagnosis is either missed or delayed.

The clinical presentation may be that of liver, neurological or psychiatric disease (Brewer, Yuzbasiyan-Gurkan, 1992; Brewer, 1995). One third of the patients present with hepatitis or chronic liver cirrhosis. If the hepatic failure is rapidly progressive and fulminant, only hepatic transplantation will save the patient. Approximately one-third of the patients present initially with neurological signs and symptoms. After the hepatic store of copper is exceeded, accumulation of copper in brain takes place. The areas of brain, which are most sensitive to copper accumulation, are those that control and coordinate movement. The symptoms include abnormalities of speech, difficulty in swallowing, and abnormalities of coordination of hand and limb movement and eventually abnormalities of gait and posture are observed. Tremor is a very prominent clinical manifestation. One-third of the patients present with psychiatric disturbances prior to the onset of neurological manifestations. The younger patients (pre-teenage) usually present with hepatic failure.

The gene for Wilson's disease has been now identified and sequenced. This gene codes for a membrane-bound, copper-binding adenosine triphosphatase type protein, which probably acts as a copper pump, in either the plasma membrane or the intracellular membrane. A large number of mutations in this gene causing Wilson’s disease have been identified. This complicates the development of an easy DNA test for the diagnosis of Wilson's disease.

It is important to establish diagnosis of Wilson's disease as early as possible since effective therapeutic measures may completely prevent serious damage to vital organs such as the liver and the brain. Assay of blood ceruloplasmin level is helpful, inasmuch as 90% of the patients with Wilson's disease have low levels. A better diagnostic test is measurement of 24 h urinary copper, which is always elevated in patients with Wilson's disease who are symptomatic. Urinary copper, however, may be elevated in patients with obstructive liver disease who do not have Wilson's disease. In the plasma, non-ceruloplasmin bound copper is markedly elevated.

A slit lamp examination for corneal copper deposits (Kayser-Fleischer rings) is a very useful non-invasive diagnostic procedure. This is, however, positive in only 50% of the patients who present with liver disease. In the future, it may be possible to develop a direct DNA test that could be used for screening of most of the mutations of Wilson's disease gene.
The objective of initial treatment is to bring copper levels down, or otherwise affect copper such that new copper toxicity is no longer occurring. It is also desirable to prevent copper from shifting from one pool to the other during the process of initial copper control. Initial copper control treatment may last for 2 to 4 months. The objective of maintenance therapy is to reduce copper burden and increase the margin of safety, and to prevent reaccumulation of copper.

The anti-copper drugs used for the treatment of Wilson's disease include penicillamine, trientine, zinc and tetrathiomolybdate. Penicillamine has been used for a long period of time. It acts by chelating copper. It is an aggressive anti-copper drug, and produces a large initial negative copper balance. The urinary excretion of copper, however, decreases as the excess mobilizable copper pool shrinks. Penicillemine, however, is very toxic.

Several years ago, we were using therapeutic levels of zinc (150 mg elemental zinc orally daily) for the treatment of sickle cell disease (SCD) patients (Prasad, 1993). Our hypothesis was that zinc would act as an anti-sickling agent. We discovered that in therapeutic amounts, zinc induced copper deficiency in SCD patients. This led us to evaluate zinc as a therapeutic modality for the treatment of Wilson's disease.

Zinc acts by induction of intestinal cell metallothionein. Metallothionein, once induced, has a high affinity for copper, and prevents the serosal transfer of copper into the blood. The intestinal cells turn over rapidly and take the complexed copper into the stool, where it is excreted. Zinc not only blocks food copper but also the copper, which is endogenously excreted via salivary, gastric, and other gastrointestinal juices. As a result, zinc produces a chronic negative copper balance. Zinc is administered orally. The dosage is 50 mg elemental zinc (as acetate) three times a day, given in a fasting or post-absorptive state. Zinc is a non-toxic drug. The only side effect is that 10% of patients may have gastric discomfort. This is usually confined to the first morning dose, particularly if it is taken before breakfast. This can be mitigated by taking the first dose of zinc between breakfast and lunch.

For maintenance therapy zinc is the treatment of choice. The big advantage of zinc over other drugs is that it has practically speaking no toxic effects. Zinc is also the drug of choice for treatment of pre-symptomatic patients and pregnant women. Whereas, penicillamine and trientine are teratogenic, zinc has no teratogenic effects.

**Prevention of blindness in age related macular degeneration (AMD) by zinc**

Age-related Eye Disease Study group (AREDS report, 2001), supported by National Eye Institute, NIH, conducted an 11-center double-masked clinical trial in patients with AMD. 3640 participants were enrolled. Their ages ranged from 55–80 years and the average follow-up period was 6.3 years. Participants were randomly assigned to receive daily oral tablets containing one of the following: 1) Antioxidants (vitamin C 500 mg; vitamin E, 400 IU; and beta carotene 15 mg), 2) Zinc, 80 mg as zinc oxide and copper 2 mg as cupric oxide, 3) Antioxidants plus zinc, or 4) Placebo. Copper was added to prevent copper deficiency in the zinc supplemented group.

Group taking the antioxidant plus zinc supplements reduced the risk of developing advanced AMD by about 25 percent and reduced the risk of vision loss by about 19 percent. Group taking zinc alone reduced the risk of developing advanced AMD by about 21 percent and vision loss by about 11 percent, whereas the group taking the vitamins alone reduced their risks for developing advanced AMD by about 17 percent and vision loss by about 10 percent. No side effects were noted due to therapeutic levels of zinc supplementation. In therapeutic dosage, zinc is an effective in vivo antioxidant. Another interesting observation was that only the zinc supplemented group showed increased longevity (AREDS report, 2004).

The risk of mortality was reduced by 27% in participants of the Age related eye disease (AREDS) aged 55–81 years who received high dose of zinc (80 mg/d) as oxide during median follow up of 6.5 years (AREDS report, 2004).

**TOXICITY OF ZINC**

Acute high-level exposure to zinc compounds can produce respiratory and gastrointestinal toxicity. However, these effects are largely self-limiting and require only modest medical attention. Exposure to smoke from bombs containing zinc compounds can cause illness, generally transient, as can acute or chronic exposure to fumes from acetylene or electric welding of zinc-containing metals. Exposure to excess zinc tablets intended for human consumption, controllable for the most part can also result in clinical changes, such as decreased activity of erythrocyte superoxide dismutase and increased activity of serum alkaline phosphatase and pancreatic enzymes. Other reported effects such as changes in serum lipoproteins and immunological status are controversial and require more definitive assessment. There is no compelling evidence supporting a cause for concern about zinc in the environment as a putative toxic agent (Walsh et al., 1994). Ingestion of elemental zinc in excess of 50 mg daily for more than 12 weeks causes copper deficiency (Prasad, 1993). This is manifested by hypochromic microcytic anemia and neutropenia, which are easily corrected by administration of 2 mg copper daily.

One schizophrenic patient swallowed a Kg of pennies, which led to zinc toxicity and subsequently copper deficiency. Pennies coined after 1987 are made of zinc with copper coating. In this case, sideroblastic anemia and neutropenia were recorded. Sideroblastic anemia is characterized by the accumulation of ferric iron in mitochondria of erythrocyte precursors (normoblasts). The mechanism of this effect is unknown. One hypothesis is that the excess in-
take of zinc led to copper deficiency. The pathogene-
sis of this effect needs to be studied, which may facil-
itate understanding of mechanisms of sideroblastic anemias and possibly management strategies, both of which are lacking at present (Eby et al., 1984).

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ЦИНК В ОРГАНИЗМЕ ЧЕЛОВЕКА: РАССТРОЙСТВА ЗДОРОВЬЯ И ЛЕЧЕБНЫЕ ЭФФЕКТЫ

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РЕЗЮМЕ. Дефицит цинка у населения широко распространен во всем мире. К числу основных клинических эффектов дефицита цинка в организме человека относятся задержка роста, расстройства иммунитета и когнитивные нарушения. Наряду с этим, дефицит цинка может вызвать и другие нарушения здоровья. В ряде случаев прием цинка может улучшать состояние здоровья. Было обнаружено, что добавка цинка приводит к клинически значимому сокращению продолжительности и тяжести диапер у детей. Прием препаратов цинка ассоциирован с сокращением продолжительности и тяжести симптомов простуды, что может быть связано с уменьшением уровня провоспалительных цитокинов. Терапевтические уровни цинка улучшают состояние пациентов с серповидноклеточной анемией, индуцируя у них дефицит меди. Это говорит о том, что цинк может использоваться как лечебное средство при болезни Вилльсона-Коновалова. В случае возрастной макулярной дегенерации (ВМД) цинк снижает риск развития осложненной ВМД и потери зрения. Способность цинка выполнять функции антиоксиданта и стабилизации мембран позволяет предположить, что он играет важную роль в предотвращении свободнорадикальных повреждений в процессах воспалительных процессах. С другой стороны, при острым воздействии в больших дозах соединения цинка могут проявлять респираторную и аллергическую токсичность, хотя эти эффекты в значительной степени ограничены. Специфическая проблема также состоит в том, что избыточное потребление цинка приводит к развитию дефицита меди. Для диагностики дефицита цинка можно использовать определение цинка в плазме крови, эритроцитах, волосах или моче. Однако более точно состояние обмена цинка отражает его определение в гранулоцитах и лимфоцитах. Количественный анализ активности щелочной фосфатазы в гранулоцитах, активности тимулина в плазме крови и экспрессии ИЛ-2 в моноклональных клетках также представляют собой эффективные диагностические тесты для оценки дефицита цинка.

КЛЮЧЕВЫЕ СЛОВА: цинк, дефицит цинка, клинические эффекты, диагностика, терапевтическое применение, лечебные эффекты, токсичность.