Original Article

Levels of interleukin-8 and catalase have correlations with zinc in healthy adults: Implications for inflammatory conditions

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Abstract

Aim: To determine the relationship between Zinc levels with different aspects of innate immunity [percentage leucocyte migration (%LM), intracellular killing (%NBT), plasma inflammatory cytokines (IL-6) and IL-8), plasma antioxidant enzymes (catalase, superoxide dismutase, myeloperoxidase, hydrogen peroxide) and nitric oxide] among healthy Nigerians.

Material and Methods: In 50 healthy adults, two phagocytic indices [percentage leucocyte migration (%LM) and intracellular killing (%NBT)] were determined by microscopy; two inflammatory cytokines [Plasma interleukin 6 (IL-6) and 8 (IL-8)] were assessed using ELISA; respiratory burst indices [plasma catalase (CAT), superoxide dismutase (SOD), myeloperoxidase (MPO), hydrogen peroxide (H_2O_2) and nitric oxide (NO)] were evaluated by spectrophotometry. Zinc (Zn) was determined using Atomic Absorption Spectrometry. Phagocytic indices, cytokines and respiratory burst indices were correlated with plasma Zn levels using Spearmans Correlation analysis at $P < 0.05$.

Results: The result of the study shows that plasma IL-8 level had negative correlation with Zn level while catalase had positive correlation with Zn level in healthy adults.

Conclusion: These findings suggest that potential mechanism of Zn during innate immunity include production of antioxidant catalase enzyme and reduction of IL-8 inflammatory cytokine.

Recommendation: These findings raise the possibility that Zn supplementation or Zn containing diets may be beneficial to individuals with inflammatory conditions.

Keywords: Cytokines; inflammation; intracellular infections; micronutrient Zn; phagocytosis

INTRODUCTION

The World Health Organization recommends a daily zinc intake of 9.4–10 mg and 6.5–7.1 mg for men and women, respectively (1) so as to meet zinc's daily requirement. In health, human body contains 2–4 grams of zinc (2) as located in the skeletal muscle, bone, liver and the skin, and in other tissues (3). Internal zinc balance is regulated by activities of two metal transporter protein families (Slc39a4 and Slc39a5). Most labile zinc is absorbed via intestinal epithelial cells into the plasma by Slc39a4 (4) while excess zinc is excreted using kidneys (5) and the intestine (6) by Slc39a (5).Zinc levels affect number and function of immune cells(macrophages, neutrophils, dendritic cells, mast cells, T cells and B cells) (7, 8). Zinc also play essential roles in the signaling and inflammatory output of monocytes and macrophages, including activation of mitogen-activated protein kinase and NF-κB (9), reduction of lysosomes integrity (10), activation of inflammasome (11), induction of IL-1β secretion by macrophages (12), reduction of IL-6 and TNF-α in human monocytes (13).

Leucocytes are involved in host defense responses including phagocytosis, antigen presentation and immunomodulation14, cytokine production, phagocytosis and other immune system processes (15). Activation of the immune system results in increased generation of reactive oxygen species (ROS) excess of which damages immune cells and this is neutralized by Zn (16). Zn inbalance is detrimental to health because Zn deficiency increases susceptibility to infectionas much asexcessive zinc (17,18). One studyreported that taking 300 mg of Zn twice daily for 6 weeks causes impaired immune response19. Another study reported that after Zn supplementation (45 mg zinc gluconate for 12 months), the incidence of infections was significantly lower and generation of tumor necrosis factor alpha was significantly lower in adults (55-87yrs of age) (20). Zn supplementation (440mg daily of zinc sulphate for a month) in adults (aged older than 70yrs)

caused raised number of T cells, delayed hypersensitivity reactions, and effective antibody response to a vaccine (21). A systematic review found that use of high doses (75mg) of Zn resulted to a significant reduction (20-42%) in the duration of colds and that 45 mg zinc gluconate, 440 mg zinc sulfate or 45-100 mg zinc acetate daily improved immunity especially in older individuals (22).

Zinc supplementation also decreased oxidative stress biomarkers and decreased inflammatory cytokines. Studies on the experimental model of Zn deficiency in humans showed that zinc deficiency increased the generation of IL-1β and its mRNA in human mononuclear cells following lipopolysaccharide stimulation. Zinc supplementation upregulated A20, a zinc transcription factor resulting in decreased generation of inflammatory cytokines (16-18,23).

Taken together, above literatures suggest that Zn regulates leucocyte phagocytic functions and inflammation in a variety of ways. Also, oxidative stress and chronic inflammation which are contributing factors to several chronic diseases are ameliorated by Zn.

MATERIALS and METHODS

Subject population
Five ml blood was collected from 50 healthy subjects recruited from University College Hospital, Ibadan, Nigeria. The participants did not have hypertension, diabetes mellitus, cardiovascular disease, cerebrovascular disease, cancer, communicable diseases, chronic renal disease or inflammatory conditions. Also excluded were those that drink alcoholic beverages or cigarette smokers or on compulsory medications. Written, informed consent was obtained from all participants and the research was conducted in compliance with the Helsinki Declaration.

Plasma Isolation
Whole blood in a test tube with lithium heparin anticoagulant was centrifuged at 1500 ×g for 10 minutes for the collection of plasma which was immediately transferred into a clean polypropylene tube using a Pasteur pipette and stored at 2−8°C.

Percentage Leucocyte Migration
Percentage leucocyte migration (%LM) was determined as previously described (24). Leukocytes were isolated from whole blood using 6% dextran. After separation of plasma by centrifugation, 6% dextran was mixed with cells sediment (1:1) and incubated for 45 minutes at 37°C. Leukocyte-rich supernatant was obtained and washed 3 times in Kreb-Ringers solution, filled into capillary tubes, and anchored into a migration chamber filled with either Kreb-Ringers solution or antigen (BCG) and Kreb-Ringers solution (1:50). This was incubated for 18 hours at 37oC and the area of LM in the chamber containing antigen was compared with the area of migration in the chamber without antigen. The %LM was calculated as follows:

%LM = (area of migration in antigen solution/area of migration in medium alone) x 100.

Percentage Nitroblue Tetrazolium Dye Reduction
Percentage nitrobluetetrazolium (%NBT) dye reduction was based on a previously described (24). For stimulated NBT procedure, 50 µL of NBT solution (0.2% NBT), 25 µL heparinized blood, and 25 µL of stimulant solution (nonviable bacterial extract) were incubated at 37oC for 10 minutes and at 25°C for 10 minutes. A thick smear of the mixture was prepared, air dry, stained with Wright stain for 15 seconds and flooded with distilled water for 30 seconds before rinsing in water and air-drying. Two hundred leukocytes were counted under oil immersion objective and leukocytes showing dark formazan deposit were recorded as positive. The percentage of bacterially stimulated NBT was calculated as:

%NBT = [leucocyte with dark formazan deposit (positive)/ total leukocytes counted] x 100.

Micronutrient analysis
Plasma concentration of Zn was determined using Atomic Absorption Spectrophotometry (Buck Scientific, 210, Atomic Absorption Spectrophotometer, Connecticut, USA) as previously described (25).

Cytokine analysis
Plasma concentrations of cytokines interleukin-6 (IL-6) and IL-8 were determined by enzyme linked immunosorbent assay (ELISA) as previously carried out (26). Assay protocol was as specified by ELISA kit manufacturer (Life Technologies Corp, USA).

Superoxide Dismutase (SOD), Catalase (CAT), Myeloperoxidase (MPO) activities, Hydrogen Peroxide (H_2O_2) and Nitric Oxide (NO) were determined as previously carried out (27).

Statistical Analysis
Data obtained were presented as mean ± S.D for Zn, %NBT, %LM, IL 6 and IL 8 while SOD, MPO, CAT, NO and H_2O_2 were presented as mean (Interquartile Range). Spearman Rank Correlation was used to establish correlation between %NBT, %LM, IL 6, IL 8, SOD, MPO, CAT, NO and H_2O2 with Zn levels. Values were considered significant at $p<0.05$.

RESULTS
The values of cellular %NBT, %LM, plasma Zn, IL-6, IL-8, SOD, MPO, CAT, NO and H_2O_2 were presented in Table 1.

Table 1. Phagocytic indices, plasma Zn levels, inflammatory cytokines and anti-oxidant enzymes in apparently healthy Nigerian adults	
Variables	**Participants (n=50)**
Zn(µg/dl)	89.53±17.89
NBT(%)	83.33±7.58
%LM(%)	58.00±2.0
IL-6(pg/ml)	8.01±3.92
IL-8(pg/ml)	80.04±15.46
SOD(U/ml)	0.19(0.14-0.26)
CAT(U/mg protein)	0.03(0.02-0.05)
MPO(U/ml)	8.27(7.23-9.59)
H_2O_2(µmol/l)	311.0(228.5-336.0)
NO(µmol/l)	12.75(9.47-16.08)

The values are within normal ranges. In Table 2, Spearman's Rank Correlation analysis showed that IL-8 was negatively correlated with Zn level while catalase was positively correlated with Zn level in healthy Nigerians (p< 0.05).

Table 2. Correlation of phagocytic indices, inflammatory cytokines and respiratory burst enzymes with plasma Zn in healthy Nigerian adults		
%NBT	r	0.018
	P	0.773
%LM	r	-0.066
	P	0.290
IL-8	r	-0.146
	P	0.020*
IL-6	r	-0.036
	P	0.568
SOD	r	-0.085
	P	0.172
MPO	r	0.087
	P	0.160
CAT	r	0.127
	P	0.041*
H_2O_2	r	0.004
	P	0.955
NO	r	0.014
	P	0.821
*Significant at p<0.05		

DISCUSSION

The present study showed that the scope of zinc-mediation of innate immunity among healthy Nigerian adults is likely to be at multiple levels. It is clear from this study that not all aspects of innate immunity are affected equally by Zn intake. For example, IL-8 which is a leucocyte chemoattractant had negative correlation with Zn level while catalase which is a mediator of leucocyte intracellular killing and neutralising antioxidant enzyme was positively correlated with Zn level. Phagocytosis, a hallmark of innate immune defenses that plays important role in protection against microbes was altered by Zn (28), but different aspects of phagocytosis were not specifically studied. Phagocytosis can be divided into phases, which include leucocyte migration to the infected foci, engulfment and intracellular killing. These phases employ various mechanisms that are controlled by a combination of factors (cytokines, oxidants and antioxidants among others) to ensure clearance of foreign body.

Superoxide (O_2^-) produced by NADPH oxidase activity is converted to H_2O_2 through dismutation within the phagosome (29). H_2O_2 which is the first effector molecule that mediates microbicidal effect of phagocytes (30)can further react with O_2^- to generate other reactive oxygen species (ROS)having ability to kill the intra-phagosomal pathogens (31). CAT is a major scavenger of H_2O_2which

protectshost cellfrom oxidative damage by excessive H_2O_2 (32). Positive correlation of catalase activity with Zn level seen in this present study posits that increased host plasma CAT activity might have been induced by Zn intake to reduce tissue damage resulting from excessive reactive radical production. Apart from this, it may also be hypothesized that Zn intake reduces inflammation through catalase breakdown of H_2O_2 and Zn negative correlationwith plasma IL-8 level. H_2O_2 is an inflammatory factor and primary chemoattractant of immune cells(33). IL-8 is primarily responsible for the recruitment of monocytes and neutrophils through a chemotactic gradient to attract, retain and activate cells to site of inflammation (34). Also like H_2O_2, IL-8 was reported to stimulate oxidative burst activity (35).

Foods high in zinc include oysters, beef, chicken, tofu, pork, nuts, seeds, lentils, yogurt, oatmeal, and mushrooms with daily value for Zinc as 11mg. Plant foods like nuts and seeds are good sources of zinc. High zinc fruits include avocados, blackberries, pomegranates, raspberries, guavas, cantaloupes, apricots, peaches, kiwifruit, and blueberries. These fruits provide 2-12% of the daily value per cup. Nuts and seeds high in zinc include squash seeds, pumpkin seeds, pine nuts, cashews, sunflower seeds, pecans, chia seeds, flax seeds, brazil nuts, and almonds. Zinc found in plant foods like fruits is not as bioavailable as zinc in animal foods (36).

CONCLUSION

The vital role that the micronutrient zinc plays in maintaining health and reducing diseases have been known for many years. With respect to innate immunity, levels of IL 8 and catalase were respectively, negatively and positively correlated with Zn levelsindicatingreduced inflammation. Thus, Zn supplementation or Zn containing diet is recommended for subjects experiencing infectionsin which phagocytosis is central to resistance and conditions involving apoptosis, damaging effects of oxygen radicals and inflammation.

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