ORIGINAL ARTICLE

Effect of Zinc Supplementation on Serum Zinc Level and Micronucleus Frequency in Bangladeshi Adult Females With Poor Socioeconomic Status

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ABSTRACT

Zinc deficiency may result in increased DNA oxidation resulting in DNA breaks which leads to micronuclei formation. Therefore, micronuclei frequency in peripheral blood mononuclear cells has the potential to be an indicator for zinc status. The study was designed to explore the possibility of using micronuclei frequency (MNF) in peripheral blood mononuclear cells (PBMCs), as a biomarker for zinc status. The study was double blind and placebo controlled. Fourteen females with moderately low dietary zinc intake were randomly assigned to receive either zinc (20 mg of zinc as zinc sulfate /day) or placebo for twenty-one days. MNF of peripheral blood lymphocytes were determined by using cytokinesis-block micronucleus (CBMN) assay. Plasma zinc levels were measured using atomic absorption spectrophotometry. Zinc supplementation increased serum zinc levels (P = 0.008) and decreased cells with micronucleus (P =0.054) and micronuleus frequency (P = 0.016) in PBMCs. Individuals with higher zinc status, as achieved with zinc supplementation, have low micronucleus frequency.

Keywords: MNF (Micronucleus Frequency), CBMN (Cytokinesis Block Micronucleus) Assay, PBMC (Peripheral Blood Mononuclear Cells)

Introduction

Zinc deficiency in mammalia is characterized by growth retardation and skin lesions. Zinc deficiency significantly reduced the incorporation of thymidine-methyl-3H into skin DNA of both intact and wounded rats. These findings and the reported reduction in thymidine-labeling index in the skin of zinc-deficient rats support the view that zinc directly regulates DNA synthesis[1]. Poor zinc nutrition may be an important risk factor in oxidant release and the development of DNA damage and cancer. function Zinc as an antioxidant was first proposed in 1990, based largely upon *in vitro* evidence that illuminated two distinct mechanisms. The first is the protection of proteins and enzymes against free radical attack, or from oxidation. Free radicals are very unstable molecules that react quickly and deleteriously with other substances, damaging their normal functions. The zinc molecule in zinc-containing enzymes was found to act as an antioxidant and protect specific regions of the enzyme from free radical attack, thus preserving its stability and activity[9]. Zinc deficiency in cells causes an increase in oxidant production (dichlorofluoroscein fluorescence) and a significant induction of single-strand breaks (Comet assay) and p53 protein expression (Western blot analysis)[2]. Thus, zinc deficiency not only caused oxidative stress and DNA damage, but also compromises the cells' ability to repair this damage. Zinc has been shown to have antioxidant properties and to exhibit inhibitory effects on apoptosis. Zinc ions interfere with the apoptosis process at an early stage, by decreasing DNA damage able to trigger apoptosis[3]. Changes in intracellular zinc dramatically affects DNA damage and repair. Although zinc is an essential mineral in human nutrition, many people have insufficient zinc status due to low dietary intake. Zinc

functions as an antioxidant and is involved in many critical biochemical reactions. It also helps to protect DNA from damage and assists in its repair[4]. Zinc adequacy appears to be necessary for maintaining DNA integrity and may be important in the prevention of DNA damage and cancer. Zinc thus indirectly reduces potential free radical formation and lipid peroxidation, and protein and DNA oxidative injury[2]. Changes in dietary zinc intake affects DNA single-strand breaks. Zinc appears to be a critical factor for maintaining DNA integrity in humans[8].

Zinc deficiency, through increased oxidative stress, may play a role in the formation of micronuclei. Cytokinesis-block micronuclei(CBMN) and conventional cultured micronuclei in peripheral blood lymphocytes, serum levels of lipid peroxide, superoxide dismutase, and the total antioxidation capacity by chemical colorimetry increased significantly in chromosomal damage. So Cytokinesis-block micronuclei(CBMN) can be used as an indicator for measuring zinc status. In this study we explored the possibility of using CBMN as a marker for zinc status that will be more accurate, appropriate and applicable for any third world country like Bangladesh as well as any developed country.

Method

This study were carried out at the clinical research and service center of the International Center for Diarrheal Disease Research, Bangladesh (ICDDR, B). Subjects were enrolled from the Mirpur field sites of the center in Dhaka who were able to read and sign consent form. The supplementation trial was a double–blind, randomized placebo-controlled trial. Healthy subjects (n=14 females) aged between 18 to 45 years was enrolled for this study. Subjects suffering from diarrhea in last seven days and/or infection were not eligible. Study health workers screened for eligible subjects in the Mirpur field sites. The health workers explained the nature of the study to eligible subjects and requested him/her to appear at the field site for study. A physician collected 'informed consent' from eligible subjects to participate in a dietary survey after explaining the nature of the study to them. Trained Field Research Associates (FRAs) collected dietary data from these subjects, using 24-h dietary recall method for five days (including one week-end day). These data were analyzed for zinc content using USDA database. Subjects with marginal dietary zinc intake (for female <5.5 mg/d) were invited to participate in the supplementation trial. After obtaining a new informed consent, an investigator randomly assigned the subjects to one of the two groups (zinc or placebo). In the morning of day 1, blood (10 ml) samples were collected after 10-12 h (overnight) fasting from the subjects at the field office. After collection of the specimens, subjects were requested to take 5 ml PEP-20 (ORION laboratories Ltd, Dhaka, Bangladesh) syrup, containing 20 mg of zinc as zinc sulfate or placebo on that day and were requested to take the same syrup for next 20 days in the morning before breakfast. Ten ml (2+8) venous blood was collected from each individual in a green top vaccutainer tube and in acid washed glass tube after 10-12 hours (overnight) fasting on the morning of supplementation day started and after 21 days of supplementation, by a well-trained physician. The blood collected in acid washed glass tube (2 ml) was used to separate serum for zinc analysis and blood collected at green top vaccutainer tube (8 ml) was used to isolate lymphocyte for the cytokinesis-blocked micronucleus assay (CBMN).

For standard micronucleus measurement the following timetable was applied:

t= 0 hrs	**Setting up of cells at 1 x 10^6 cells/mL with 30 µg/mL PHA (10 µL/750µL)**	**(1pm Monday)**
t= 44 hrs	Adding of Cytochalasin B at 4.5µg/mL (62.4 µL/750µL)	(9am Wednesday)
t= 72 hrs	Harvesting of cells by cyto-centrifugation	(1pm Thursday)

One thousand cells were scored per subject to determine the frequency of the various cell types in the cytome assay. These consisted of binucleated cells, cells containing micronuclei. A total of 1000 binucleated cells were scored in order to determine the frequency of micronuclei in a total of 1000 cells. Only binucleated cells were scored for micronuclei and their scores were

combined to give the overall incidence. Cells were scored using bright field microscope. Statistical analyses were performed using Sigma Stat 3.1 (San Jose, CA). Descriptive statistical analysis was performed to examine the distribution of each of the major baseline and outcome variables. The effects of zinc supplementation on serum zinc levels and genomic damage were analyzed by paired t-test. Correlation of differences was considered significant at P = <0.05

Ethical approval

This study was approved by the Institutional Review Boards, Research Review Committee (RRC) and Ethical Review Committee (ERC) of International Centre for Diarrheal Disease Research, Bangladesh (ICDDR, B). Informed consents were obtained from all study participants

Results

Among the fourteen enrolled subjects, seven were supplemented with 20 mg of zinc as zinc sulfate per day and seven were supplemented with placebo for 21 days. Zinc supplementation lead to increased serum zinc levels from 0.73 ± 0.08mg/L to 0.79 ± 0.25 mg/L, (P = 0.049); whereas, in placebo supplemented group the baseline (0.75 ± 0.09 mg/L) and follow-up (0.72 ± 0.09 mg/L) serum zinc levels were almost similar (P = 0.918) (figure 1).

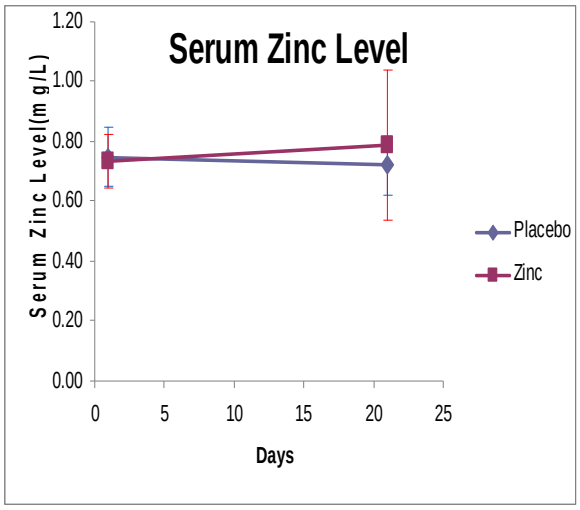


Figure 1. *Serum zinc response with supplementation*

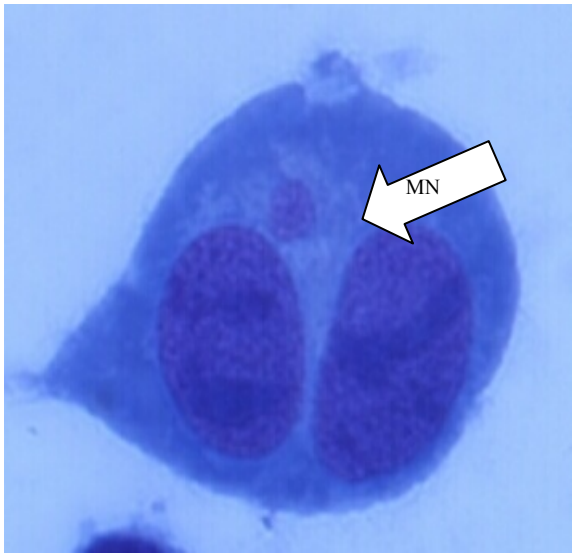
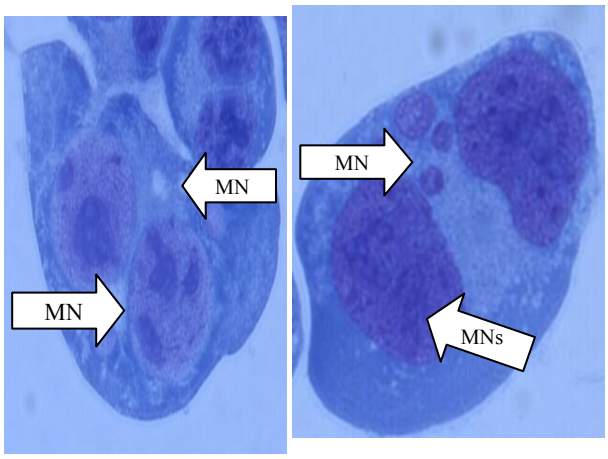


Figure 2: *Photomicrographs of the cells scored in the CBMN assay*

(a) Binucleated cell with micronucleus; (b) Binucleated cell with two micronucleus;(c) BN cell containing four micronucleus;



b. *BN cell with two micronuclei* ***c.****BN cell with four micronuclei*

Twenty-one days of zinc supplementation decreased MNF in PBMCs significantly (P = 0.016). Whereas, the MNF in placebo supplemented group remained unchanged (P = 0.281) (Figure: 3 a). Furthermore, 21 days of zinc supplementation led to decreased frequency of cells with micronuclei (CMN) (P = 0.054) in PBMCs which was very close to statistically significant level, whereas, placebo supplementation could not affect the frequency of cells with micronuclei (P = 1.00) (Figure 3 b).

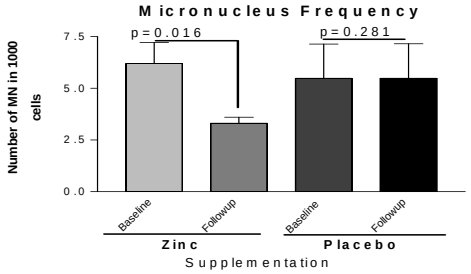


Figure 3 (a): *Micronuclei frequency in PBMCs of zinc and placebo supplemented group before and after supplementation*

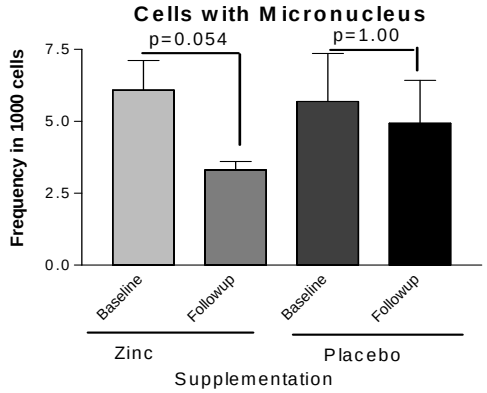


Figure 3 (b): *Frequency of cells with micronuclei in PBMCs of zinc and placebo supplemented group before and after supplementation*

Discussion

One of the most novel roles of zinc in biological system is scavenging reactive oxygen species. Zinc together with glutathione peroxidase and superoxide dismutase, plays a crucial role in antioxidant system and thus protects biomolecules from oxidative damage[6]. Initial laboratory experiments appeared to show two ways in which zinc discharged its anti-oxidant functions. The first is that dozens of vital enzymes within the body contain zinc and in these enzymes the zinc molecule acts directly as an anti-oxidant, protecting the biochemical structure of the enzyme from free radical attack. Secondly, zinc acts to stabilise proteins which may otherwise react with highly unstable minerals, particularly iron and copper, to form free radicals[10]. Therefore, it is expected that zinc deficiency would increases oxidative stress, causes DNA break down, and thus Micronucleus formation. This study was conducted to find out the effect of zinc supplementation on genomic damage in the form of MNF. This is the first study in Bangladeshi population in which lymphocyte micronucleus cytome assay that is based on cellular and nuclear morphology was used to determine the effect of zinc supplementation on micronuclei frequency. There are a number of studies describing serum zinc response with zinc supplementation but very few are conducted in third world country where zinc deficiency is very common. In this study we also tried to find out the effect of zinc supplementation on marginally zinc deficient females.

On the other hand, though zinc deficiency causes a number of biochemical problems including growth retardation, skin lesions, emotional disorders, infections, and delayed puberty in adolescents[7]. It is common in third world counties like Bangladesh. There is no valid biomarker for zinc status. This study also explored the possibility of using MNF as an ideal biomarker for zinc status in human.

Results showed that zinc supplementation, decreased the frequency of micronucleus ($P = 0.016$). Recent literatures suggest the protective effect of zinc on MN formed by scavenging various reactive oxygen species[5] which supports our finding. From this study, it was also found that zinc supplementation decreased the frequency of cells with micronucleus ($P = 0.054$) which was very close to significant. These results suggest that zinc supplementation prevents genomic damage and thus micronuclei formation. It was found that serum zinc concentrations was increased after zinc supplementation and significant higher serum zinc level was maintained up to the end of the study period of twenty one days.

Conclusion

In conclusion, to the best of current knowledge, in this area, this is the first report where it was shown that zinc supplementation led to decreased micronuclei frequency in humans. These findings show that individuals with higher zinc status, as achieved with zinc supplementation, have low micronucleus frequency. On the other hand, individuals with lower zinc status, as indicated by higher dietary phytate zinc ratio, have higher

micronucleus frequency. These findings suggest that MNF and CBMNF have the potentiality to be used as a marker for zinc status in humans. However, more studies with larger sample size are required to verify the repeatability of these findings.

Table I: Frequency of CBMN MN, NPB and Nu Bud among the study cells in 1000 cells

Subject ID	Supplementation type	Number of binucleated cells with MN		Micronucleus Frequency	
		Baseline	Follow-up	Baseline	Follow-up
F012	Zinc	5.46	2.50	5.46	2.50
F019	Zinc	5.80	4.85	5.81	4.85
F020	Zinc	5.27	3.77	6.02	3.77
F025	Zinc	6.11	2.92	6.11	2.92
F032	Zinc	4.35	3.00	4.36	3.00
F096	Zinc	3.64	3.10	3.64	3.11
F015	Placebo	2.48	3.1	2.48	3.10
F035	Placebo	4.19	4.28	4.20	4.28
F021	Placebo	1.56	1.50	1.56	1.70
F023	Placebo	10.74	7.98	10.74	7.98
F056	Zinc	11.96	3.00	11.96	3.00
F098	Placebo	0.87	1.37	0.87	1.37
F064	Placebo	10.88	12.33	11.59	13.96
F067	Placebo	9.10	4.00	6.96	6.00

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