VARIATION OF SERUM ZINC CONCENTRATIONS AMONG KALA-AZAR ENDEMIC AND NON-ENDEMIC POPULATION IN BANGLADESH

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Abstract

Background & objectives: *Visceral Leishmaniasis (VL) or Kala-azar is a deadly disease putting 350 million people from 88 countries at risk. In Bangladesh, 45 districts are endemic. Zinc is an essential trace element and its relevance for proper functioning of the entire immune system is already well documented. Though low serum zinc levels have been reported in many parasitic diseases, limited information is available regarding zinc status in human leishmaniasis. This study was conducted to evaluate the zinc level in endemic and non-endemic population of kala-azar.*

Methods: *This cross-sectional study was conducted to assess the serum zinc concentrations in kala-azar endemic and non- endemic population of 06 to 60 years of age range during the period of July 2014 to June 2015.Venous blood was collected from 92 subjects to estimate serum zinc concentrations.*

Results*: Mean Serum zinc levels were found 63.66 microgm/ dl in kala-azar patients of endemic area, 69.44 microgm/ dl in kala-azar endemic populations and77.34 microgm/ dl in non-endemic populations.*

Conclusion: *The average serum zinc concentrations is lower in populations of kala-azar endemic zone compared to populations from kala-azar nonendemic zone. It was also found that kala-azar patient had lower zinc concentrations compared to non-kala-azar patients. There may have potential association of low zinc level and occurrence of kala-azar.*

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Introduction:

Visceral Leishmaniasis (VL) is also called Kala-azar which is fatal in 95% cases if it is left untreated and an estimated 50 000 to 90 000 new cases occurs annually worldwide[1]. Of all the cases, 90% occur in India, Bangladesh, Nepal, Sudan and Brazil. These countries have one thing in common that a substantial number of ultra-poor population living substandard lives. This allows the breeding of the vector 'sand fly' (Phlebotomus argentipes) in the crevasses of the mud house built as shed for the cattle or dwelling of the family.[2] VL, commonly called as Kala-azar (KA), is a tropical neglected disease caused by protozoan parasite, Leishmania donovani complex and transmitted by sand flies. Currently 139 upazilas in 45 districts are endemic for VL and 65 million are at risk. Eight upazilas of the county, 5 of them in Mymensingh district are hyper endemic (>2.5 cases per 10,000). Trishal and Fulbaria of Mymensingh district have 60% of all cases in the country.[3,4]

About 81% of patients with Kala-azar (KA) were below 30 years of age, fever was present in 91% with double peak or triple peak of temperature in 39%. Splenomegaly, hepatomegaly and anemia are very common and found in about

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99%, 92% and 97%.[5] Zinc is an essential mineral that is naturally present in some foods, added to others and available as a dietary supplement. Zinc is involved in numerous aspects of cellular metabolism. It is required for the catalytic activity of approximately 100 enzymes. Zinc has a major role in immune function, protein synthesis, wound healing, DNA synthesis and cell division. Zinc also supports normal growth and development during pregnancy, childhood and adolescence. Zinc is required for proper function of taste and smell. A daily intake of zinc is required to maintain a steady state because the body has no specialized zinc storage system.[6,7,8] Dietary zinc deficiency is widespread in developing countries. Deficiency is aggravated by inter current acute and chronic infections. Recent studies have demonstrated that zinc supplementation can significantly reduce the morbidity and mortality. It also shortens the time to recovery from acute infectious diseases[9]. Since 1990, South Asia had experienced a re-emergence of kala-azar (visceral leishmaniasis). To determine risk factors for kala-azar, Bern et al. performed cross-sectional surveys over a 3-year period in a Bangladeshi community. They found 155 cases out of 2356 residents from 2000-2003. The case was detected by history and serologic test. Risk was highest for persons 3-45 years of age and no significant difference by sex was seen. In age-adjusted multivariable models, several factors were identified: a) proximity to a previous kala-azar patient b) bed net use in summer c) cattle per 1,000 m2 and d) malnutrition due to dietary deficiency[10].

The role of zinc in proper functioning of the entire immune system is well documented. Low serum zinc levels have been reported in parasitic diseases in which immune system was affected but cause or effect relationship could not be established[8]. In a murine model short periods of zinc deficiency resulted in rapid atrophy of the thymus, with preferential involution of the cortex, reduced production of thymulin, along with substantial reductions in the total numbers of lymphocytes and phagocytes[11]. Antibody and cell-mediated immune responses were significantly reduced in zinc deficient mice infected with Trypanosoma cruzi. However, in regards to human leishmaniasis, only limited information is available on this aspect. Interleukin-1 (IL-1) is incapable of stimulating a strong Th1 response in zinc deficiency[12]. Thus, severe zinc deficiency impairs host immune response and leads to Th1 deficiency and strong Th2 response. This shift results in susceptibility of infection and better parasite survival inside the host. In a study in Bihar of India, Serum zinc levels were found to be significantly lower in visceral leishmaniasis (VL) patients than non-endemic controls[13]. There is a scarcity of data on serum zinc concentration among kala-azar endemic and non-endemic population in Bangladesh. So, this study was conducted to evaluate the zinc level in endemic and non-endemic population of kala-azar.

Materials and Methods

This was a cross-sectional and observational study which was done from July 2014 to June 2015 in Department of Medicine, BSMMU, Dhaka.

Patients: Solimpur of Trisal Upazila, Mymensingh was selected as kala-azar endemic are and Joaria of Tungipara Upazila, Gopalganj was selected as kala-azar non-endemic area. Individual of both sex with 6 to 60 year of age were included in the study. Individuals not willing to participate, Pregnant ladies and Individuals who have known co-morbidities (DM, HTN, IHD, CKD, Chronic Diarrhoea) were excluded from the study.

Sampling: For assessing the variation of the serum zinc concentration in kala-azar endemic and non- endemic population a total 69 subjects were targeted in each group. Sample size was calculated considering 2% margin of error and means and SD (12 and 8.8) and SD (3 and 3.1) of previous study on low serum zinc level in endemic and non-endemic population of kala-azar[13]. Calculated sample size was 23 where calculation was done by cross-sectional sample size determination formula and considering non response 20% inflation on the estimative sample. To increase the validity of the study, three times of actual sample was taken. So, sample size in each group is 69. But due to time constraint and availability of the study subjects ultimately 92 subjects were selected,

46 from endemic zone and 46 from non-endemic zone. Sampling was purposive.

Data collection: After taking approval of the Institutional Review Board of BSMMU, Dhaka. Individuals who fulfilled the inclusion and exclusion criteria were enrolled in this study. At first data was collected from population of endemic area. Then data was collected from population of non-endemic area in the same way. Informed written consent was taken from the participant after explaining all the facts potential dangers to the subjects in case of primary data collection. Primary data was collected by face-to-face interview of the patients from both endemic and non-endemic areas, upon their consent and convenience. Socio-demographic and personal information was recorded from patient through interview, with a semi-structured pre-tested questionnaire. Then these subjects were given a code. Venous blood was collected by a trained technician who had proper knowledge about the procedure and safety of venipuncture. Test tubes were made zinc free in ICDDR'B laboratory prior to use. Blood was placed immediately after collection in zinc free test tube, wait for 30 minutes then centrifuged. The serum was separated and stored at "20°C in cryovial. Serum zinc levels were measured by atomic absorption spectrophotometry in nutritional biochemistry lab. In ICDDR, B, Dhaka.

Measures of variables: Main outcome variable was serum zinc level with Demographic variable such as Age, Gender, BMI, Marital status, Education, Occupation, Monthly income.

Statistical analysis of data: All data was collected, coded, revised and entered into the statistical package for social science (SPSS) version (22). The qualitative data was presented as number and percentages while the quantitative data was presented as mean, standard deviations and ranges. Comparisons between groups (continuous parameters) were done by unpaired t test. Categorical parameters were compared by Chi-square test, Pearson's Correlation Coefficient test. The result of the clinical study and statistical analysis were presented in the form of text, table, bar, graph and chart. Level of significance was considered at 0.05 level.

Result:

To assess the serum zinc concentrations of patients in kala-azar endemic and nonendemic region, 92 patients were enrolled. Two cases from nonendemic area and one case from endemic area were discarded due to haemolysis of specimens. So finally, a total of 89 cases were included in the study. There was no difference in mean ages and sex between two groups.

Table I

Age and Sex distribution of the study subjects (n=89)

Age group (years)	Group-I (n=45) No. (%)	Group-II (n=44) No. (%)	p value
6-17	12 (26.67)	11 (25.00)	
18-40	17 (37.78)	18 (40.90)	
41-60	16 (35.55)	15 (34.10)	
Mean ±SD	31.86±24.46	32.11±25.29	0.867
Male	24 (53.33)	23(52.27)	
Female	21(44.67)	21(47.73)	0.920

Group-I: Subjects in endemic area
Group-II: Subjects in non-endemic area
There was no difference of BMI in patients of Kala-azar, endemic and non-endemic subjects.

Table II

BMI of the study subjects (n=89)

BMI in Kg/m^2	Total	Group-I (n=45) No. (%) Subjects with Kala-azar (n=09)	Subjects without Kala-azar (n=36)	Group-II (n=44) No. (%)	P value
<18.5	18 (40.00)	06 (66.67)	12 (33.33)	17 (38.63)	0.847 [a]
18.5–24.9	23 (51.11)	03 (33.33)	20 (55.55)	22 (50.00)	0.756 [b]
25–29.9	04 (08.89)	00 (00.00)	04 (11.12)	05 (11.37)	0.472 c

Chi-square test was performed to see the association between Group I and Group II
Group-I: Subjects in endemic area Group-II: Subjects in non-endemic area
[a]Comparison between group I and group II subjects having BMI < 18.5
[b]Comparison between group I and group II subjects having BMI 18.5–24.9
[c]Comparison between group I and group II subjects having BMI 25–29.9

Table III

Distribution of the study subjects by low zinc level (n=89)

Study subjects	Total subjects	Low No	Serum zinc level Percentage	p value
Subjects in endemic zone	45	16	35.55	0.026
Subjects in non-endemic zone	44	10	22.72	

Chi-square test was performed to see the association between the subjects of endemic and non-endemic zone of kala-azar.

Serum low zinc level was considered when the level was less than 65 microgram/dl.[14.] Low serum zinc level was more frequent in endemic zone.

Table IV

Zinc level of the study subjects according to endemicity (n=89)

Variable	Group-I Patient in Kala-azar zone	Group-II: Subjects in Kala-azar Zone	Group-III Subjects in Non-endemic
Zinc level(microgm/dl)	63.66±12.12	69.44±13.28	77.34±14.06
Statistical analysis			
Group			P value
Group I vs Group III			0.001
Group II vs Group III			0.024

Unpaired student t-test was performed to compare between two groups

Table V

Mean zinc level of the study subjects according to BMI (n=89)

BMI in Kg/m^2	Mean ± SD zinc level in endemic area (n=45)			Mean ± SD zinc level in non (n=44)
	Total Kala-azar	Subjects with without Kala- (n=09)	Subjects endemic area azar (n=36)	
<18.5	68.98±12.64	62.23±10.43	70.84±13.85	75.98±11.77
18.5–24.9	70.85±12.59	65.95±12.83	71.37±11.23	74.93±11.38
25–29.9	74.84±12.56	-	74.84±12.56	77.14±12.05

BMI was expressed in Kg/m^2, Serum zinc level was expressed in microgm/dl.

There is significant difference of serum zinc level in kala-azar patient group with low and normal BMI group.

Serum zinc level was significantly low in kala-azar patient than normal subjects of both kala-azar endemic and non-endemic population. It was also observed that serum zinc level was low in endemic subjects than subjects with non-endemic areas.

Table VI

Correlation with BMI and Zinc level (n=89)

Region	n	Pearson's Correlation Coefficient test	
		r value	p value
Endemic with kala-azar	9	0.453	0.221
Endemic (non-kala-azar)	36	0.040	0.818
Non-endemic	44	0.407	0.006

BMI had moderately positive correlation with zinc level in endemic area and weakly positive correlation in non-endemic area.

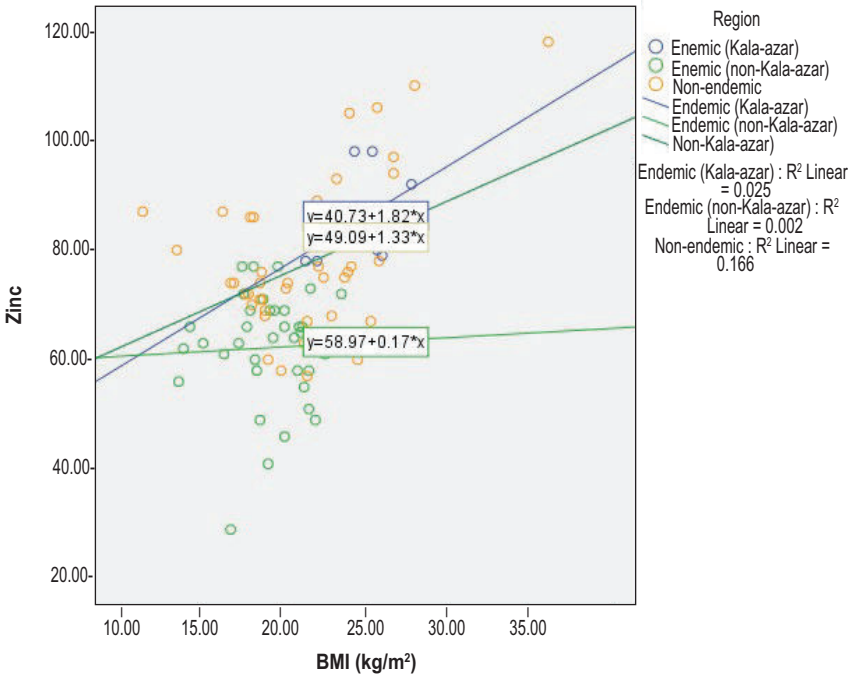

Fig-1: *Scatter diagram showing correlation between BMI and serum zinc level*

Discussion:

This Cross-sectional study with 92 cases was done to assess the serum zinc concentrations in kala-azar endemic and non-endemic population. Mean age ± SD among total subjects were 33.57±25.79 years. There was no difference in mean ages between two groups (Table-I). These findings correlate with previous study[13]. It was observed that 52.8% (47) were male and 47.2% (42) were female among total subjects. There was no difference in gender distribution of two groups (Table-I). So, the study subjects were age and sex matched. There was no significant difference in endemic and non-endemic group in relation to BMI (Table-II). So, both groups have same nutritional status. These findings were congruent with the observation of a previous study[15]. Correlation with BMI and Zinc level showed that BMI had positive correlation with serum zinc level. In a study Weyenbergh et al. (2004) investigated that significant decrease in plasma Zn was observed in all three patient groups (VL, ML, CL) as compared to controls, but only VL and ML patients displayed overt Zn deficiency[16]. Bern et al. (2007) in Bangladesh observed zinc level was lower in current kala-azar patients and those who later developed kala-azar compared with uninfected and asymptomatically infected subjects[4]. But both infected and uninfected group had low serum zinc level in kala-azar endemic area. They found 52% of uninfected subject and 75% of infected subject had low serum zinc level. We found about 35% of uninfected subject and about 44% of infected subject had low serum zinc level. In another comparable study Pourfallah et al. (2009) evaluated the levels of zinc (Zn), copper (Cu), iron (Fe) and zinc/copper ratio in the serum of patients with cutaneous leishmaniasis and revealed that Serum Zn level was lower in patients with cutaneous leishmaniasis than the control group[15]. Mishra et al. (2010) evaluated serum zinc level in kala azar endemic and nonendemic region. It was observed that serum zinc levels were found to be significantly lower in VL patients than non-endemic controls[13]. The serum zinc levels in VL endemic controls were also significantly lower than non-endemic controls but these values were not significantly different from VL patients. However, all samples from endemic region (VL patients and controls) had lower serum zinc levels than non-endemic controls. It was concluded that low serum Zn levels, in healthy subjects from Bihar and more significantly in VL patients of this region, are possibly associated with vulnerability of visceral leishmaniasis in the region. In present study, a significant decrease in serum zinc levels was observed in kala-azar patients as compared to non-endemic subject. However, endemic subject also showed low levels of serum zinc. While comparing the results of endemic and non-endemic, the serum zinc values were significantly lower in endemic population (Table-IV). This indicated that the average resident of endemic area will have lower zinc levels than those who live in non-endemic area. So, our findings support their observations. To understand whether the low serum zinc levels were either due to protein energy malnutrition or zinc intake, we measured BMI all three groups. There was no difference among endemic and non-endemic population in relation to BMI. It indicated that these subjects were specifically zinc-deficient at baseline and the deficiency was not due to BMI. Improved micronutrient status in the population through supplementation and improved dietary intake may have the potential to decrease the proportion of infected individuals who progress to kala-azar and to decrease the burden of kala-azar in highly affected areas.

Limitations

Sample size was small.

Study was done only in two villages so study result may not be generalized to whole population.

Conclusion

This study shows that the average serum zinc concentrations is lower in populations of kala-azar endemic zone compared to populations from Kala-azar non-endemic zone. It was also found that kala-azar patient had lower zinc concentrations compared to non-Kala-azar populations. There may have potential association of low zinc level and occurrence of kala-azar. Further case control analysis could reveal the association.

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