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Altered Serum Zinc Concentration in Patients with Visceral Leishmaniasis (L. donovani) and Healthy Regional Controls from Bihar, India: A Correlation with Susceptibility to Disease

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Background: Visceral leishmaniasis (VL) is a multiorgan disease of the reticuloendothelial system caused by obligate intracellular protozoan parasites of the Leishmania genus, spread through the bite of the phlebotomine sand fly, and leads to death in nearly all untreated cases. In India, VL is almost entirely caused by the species L. donovani, and is epidemic in the rural Eastern states of that country, most notably the Indian State of Bihar. Mounting a successful CD4-TH1 (CD4+ T-helper lymphocyte type 1) immune response has been shown necessary to combat intracellular pathogens, such as L. donovani, and prevent the development of full-blown VL. Zinc is a micronutrient necessary for T-cell development and preferential activation of a TH1 immune response over the TH2 pathway by modulating cytokine release favoring IFN-γ and IL-2 release. We show that specific zinc deficiency is present in control subjects and VL patients from Bihar, but not urban controls from Delhi, independent of nutritional status, suggesting that zinc deficiency may specifically predispose to VL if patients are infected with the intracellular parasite L. donovani.

Objectives: 1. To optimize a protocol for reliably measuring serum zinc concentration using a standard colorimetric spectrophotometer. 2. To understand the relationship of serum zinc concentrations in VL patients from Bihar, regional controls (from Bihar), and urban controls (from Delhi). 3. To determine whether observed differences in serum zinc between groups were related to nutritional deficiency by measuring serum albumin and total protein. 4. To estimate whether immune function was altered in favor of a TH2 response in any of these groups by measuring serum globulin concentration.

Methods: Serum samples were collected from 18 VL patients from Bihar, 47 healthy urban controls (from New Delhi), and 22 healthy regional controls from Bihar between September 2004 and November 2006. All samples were tested for the presence of anti-L. donovani antibodies using the rKE-16 (recombinant kinesin 16) ELISA. Serum zinc estimation was performed by chelating zinc present in serum samples, and measuring the absorbance of zinc-chelator complex with a colorimetric spectrophotometer, comparing it with the absorbance of chelated zinc in a standard solution to determine concentration. Serum total protein and albumin were measured using a biochemical analyzer with standardized reagents in a colorimetric assay, and serum globulin was estimated using these results. One-way ANOVA and Tukey post-hoc tests were used to compare the means of each group for each category, and a Pearson correlation
was used to evaluate correlation between dependent variables, all using SPSS Statistics Package 10.

**Results:** A decrease in serum zinc (mean ± SD) was observed in VL patients from Bihar as compared to urban controls (from Delhi) (8.96 ± 2.81 µmol / L vs. 11.15 ± 3.39 µmol / L, p=0.064), but not between VL and regional controls (from Bihar) (8.46 ± 3.70 µmol / L, p=0.894). Delhi and Bihar control groups showed significantly different serum zinc levels (11.15 ± 3.39 µmol / L vs. 8.46 ± 3.70 µmol / L, p=0.011). Zinc deficiency (serum zinc < 9.95 µmol / L) was seen in both the VL patients (12 of 17 patients) as well as the controls from Bihar (13 of 20 controls), but not in the Delhi controls (20 of 46 patients). Used as a marker for nutritional status, serum albumin was similar in regional controls (from Bihar) and urban controls (from Delhi) (6.16 ± 0.34 mg/dL and 6.20 ± 0.73 mg/dL, p=0.999), but was significantly lower in VL patients compared with both groups (3.76 ± 1.31 mg/dL, p<0.001). Serum total protein levels were similar in all three groups (13.12 ± 2.86 mg/dL, 14.03 ± 2.27 mg/dL, and 12.98 ± 0.83 mg/dL, p>0.273).

As a marker for immune status, serum globulin was significantly increased in patients with VL (9.36 ± 3.01 mg/dL) as compared to urban (Delhi) controls (7.83 ±1.77 mg/dL, p<0.05), and regional (Bihar) controls (6.81 ± 0.76 mg/dL, p<0.001).

**Conclusions:** The decreased serum zinc observed in both VL patients, as well as controls from the Indian State where VL is epidemic (Bihar), indicates that citizens from this region of India may, in general, have a low baseline serum zinc that predisposes them to visceral leishmaniasis (VL) when infected with *L. donovani*. The majority of these controls from Bihar displayed overt zinc deficiency, while a much smaller proportion of urban controls were clinically deficient in zinc. Serum albumin levels in the regional controls (from Bihar) were within normal limits and comparable to urban controls (from Delhi), indicating that these patients were specifically zinc-deficient at baseline and this deficiency was not due to general malnutrition. The significantly increased serum globulin in VL patients, but not in other groups, is consistent with an increased Th2 response with immunoglobulin production and supports the hypothesis that patients who progress to VL were not able to produce an adequate Th1 response. The role of zinc in T-cell immune function makes oral zinc supplementation a good candidate for treatment and prevention of VL in the Bihar, India, and other regions with a profile of low socioeconomic status and high *Leishmania* species prevalence.