INTERNATIONAL JOURNAL OF LEPROSY Volume 52, Number 3
Printed in the U.S.A.

Serum Zinc Levels in Subtypes of Leprosy[1]

Narendra K. Mathur, Mukul Sharma, Hari N. Mangal,
and Suraj M. L. Rai[2]

The role of zinc in the development and maintenance of immunocompetence is now fairly well established. Zinc deficiency in experimental animals causes impaired immune ontogeny ([2]), thymic atrophy ([1, 5, 18]), low thymic hormone levels ([9]), atrophy of T-dependent areas in the lymph node ([18]), decreased mitogen responsiveness ([1]), decreased T cell counts ([5]), and suppressed T helper ([6]) and T killer ([5, 18]) functions. In human beings, zinc deficiency results in atrophy of the thymus ([8]), low thymopoietin levels ([4]), and depressed cell-mediated immune (CMI) reactions ([3, 12, 15]). Since similar alterations in the immune function are known to occur in lepromatous leprosy ([7]), we thought it would be useful to determine the serum zinc levels in different types of leprosy.

MATERIALS AND METHODS

One hundred forty-six leprosy patients were selected from the leprosy clinic and classified into five groups according to the Ridley and Jopling classification ([16]). Out of 146, 80 patients (20 TT, 20 BT, 20 BL, and 20 LL) were on dapsone for about 18 months; 98 patients were males (28 TT, 21 BT, 22 BL, and 27 LL) and 48 were females (12 TT, 15 BT, 10 BL, and 11 LL). All of the patients were in the 16–60 age group. Although patients were selected at random, care was taken not to include patients with evidence of any other cutaneous or systemic illness. Patients who had evidence of lepra reactions were also excluded, as were patients who had taken any antileprosy drug other than dapsone.

Forty healthy volunteers (26 males and 14 females, aged 16–60 years) served as controls. The patients and the healthy control subjects were from the same socio-economic group with similar dietary habits.

Fasting blood samples were collected by venipuncture, using zinc-free, glass syringes and stainless steel needles. All glassware used in the procedure was made zinc free by first treating with chromic acid and then washing several times with demineralized water. Throughout the procedure, care was taken to avoid extraneous zinc contamination and hemolysis. The method described by Meret and Henkin ([11]) for serum zinc estimation, using 6% *n*-butanol as the diluent, was followed. Serum zinc was estimated by means of flame atomic absorption spectrophotometry, using a Techtron model AA-5 atomic absorption spectrophotometer with hollow cathode lamps. Zinc absorbance was measured at 213.8 nm.

RESULTS

The mean serum zinc level (μg/100 ml) in control subjects was 110.2 ± 12.4 (mean $\pm$ S.D.). Mean serum zinc levels in untreated TT, BT, BL, and LL patients were 110 ± 11.2, 99.5 ± 7.4, 83.5 ± 15.4, and 68.7 ± 13.9, respectively (Table 1). The distribution of serum zinc values in the control subjects and untreated cases is shown in The Figure.

The serum zinc levels in LL and BL patients were significantly lower ($p < 0.01$, Student's t test) than those of the BT, TT, and control cases. The differences between the LL and BL levels were also significant ($p < 0.01$). Serum zinc levels in BT patients were also significantly lower ($p < 0.01$) when compared to TT and control cases. There was no significant difference ($p > 0.05$) between the controls and the TT cases. Thus, a gradual lowering of serum zinc levels was observed from the TT to the LL pole.

Serum zinc levels in patients who were on dapsone for 18 months are presented in

[1] Received for publication on 2 September 1983; accepted for publication in revised form on 9 January 1984.

[2] N. K. Mathur, M.D., F.C.A.I., Professor and Head; M. Sharma, M.B., B.S., Resident, Department of Skin, STD and Leprosy; H. N. Mangal, M.D., Professor and Head, Department of Microbiology; S. M. L. Rai, M.D., Lecturer, Department of Pathology, S.M.S. Medical College and Hospital, Jaipur, Rajasthan, India.

TABLE 1. *Serum zinc levels in controls and untreated leprosy patients.*

	No. of cases	Serum zinc μg/100 ml	
		Mean ± S.D.	Range
Controls	40	110.2 ± 12.4	90–130
TT	20	110.0 ± 11.2	80–130
BT	20	99.5 ± 7.4	90–120
BL	20	83.5 ± 15.4	60–100
LL	20	68.7 ± 13.9	50–100

TABLE 2. *Serum zinc levels in leprosy patients treated with dapsone for 18 months.*

	No. of cases	Serum zinc μg/100 ml	
		Mean ± S.D.	Range
TT	15	114.6 ± 12.8	90–130
BT	19	101.9 ± 8.4	90–125
BL	20	85.1 ± 11.4	70–100
LL	12	72.3 ± 10.6	60–100

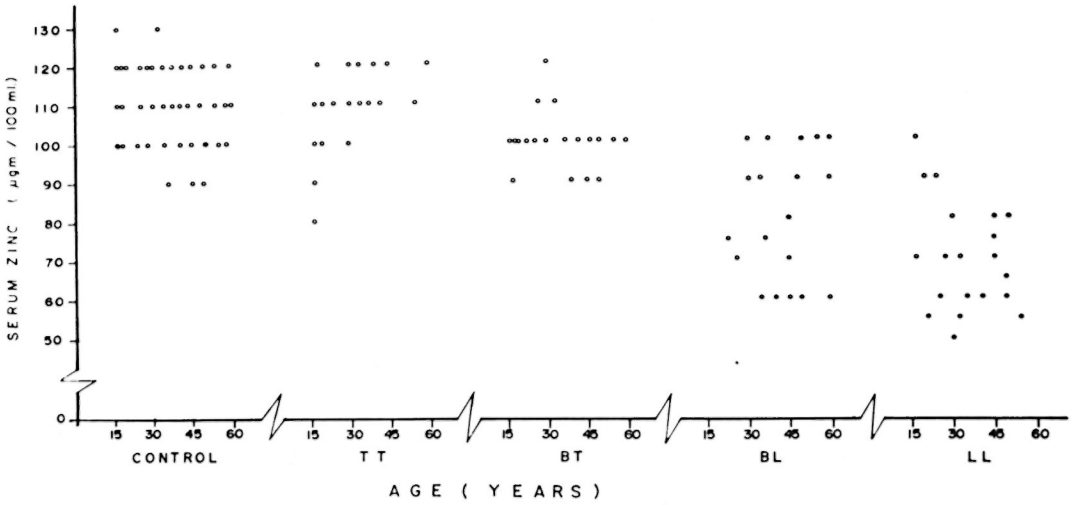
Table 2. A pattern of gradually falling levels from the TT to the LL pole was observed in treated cases also. Although an increase in the mean values after treatment was noted in the LL and BL cases, as well as in the BT and TT patients, the differences were not statistically significant ($p > 0.05$).

DISCUSSION

Low serum zinc levels have been reported in a number of infectious diseases ([14, 20]). Hypozincemia in patients with different types of leprosy with trophic ulcers was first reported by Oon, *et al.* ([13]). Sher, *et al.* ([19]) observed low serum zinc levels only in a lepromatous group of patients. Our results showed gradually falling serum zinc values from the TT to the LL pole. These findings suggest that there could be a correlation in serum zinc levels and the bacillary load. Sher, *et al.* ([19]) hypothesized that bacilli could produce hypozincemia by causing liberation of a leukocyte endogenous mediator (LEM)-like substance from the macrophages where they reside ([20]); LEM causes a redistribution of zinc with decreased serum levels and an increased hepatic uptake. However, LEM has been reported to be liberated transiently from leukocytes only in acute infections ([14]); thus, the hypothesis of its constant liberation from macrophages, in a chronic infection like leprosy, may be untenable.

Another possible explanation is that leprosy bacilli require zinc for their own metabolism. Zinc is essential for at least 90 enzymes which participate in all major metabolic pathways, and 40 metalloenzymes exist in which zinc is bound tightly to the apoenzymes and serves one or more structural, regulatory, or catalytic functions ([17]). It is likely that many of the enzymes in the metabolic pathway of leprosy bacilli are zinc-dependent metalloenzymes and require zinc. The consumption of body zinc by billions of leprosy bacilli in a lepromatous leprosy patient could produce zinc deficiency. However, if hypozincemia is due to an overwhelming bacterial load in lepromatous leprosy, one would expect dapsone to improve serum zinc because of its antileprotic action; dapsone is known to decrease the bacterial index (BI) by one unit per year. But, contrary to expectations, in the present study no significant improvement was observed in the serum zinc levels after 18 months of treatment with dapsone. This suggests that factors other than bacterial load are involved in hypozincemia in lepromatous leprosy.

Dietary factors (low zinc intake, complex formation with phytates, etc.) are unlikely to be responsible because then the extent of the hypozincemia should be similar in all subtypes of leprosy. Malabsorption of zinc from the gut in lepromatous leprosy is not the cause of the hypozincemia because a) there are no structural or functional abnormalities of the gastrointestinal tract, even in extensive lepromatous leprosy ([9]), and b) serum zinc levels improve after oral zinc therapy (220 mg zinc sulfate daily) in lepromatous leprosy patients (unpublished data). Kidney involvement is frequently seen in lepromatous leprosy, and it is possible that increased excretion of zinc in the urine occurs in LL, leading to low serum zinc levels. However, this fails to explain the significantly lower serum zinc levels in BL and BT patients. Zinc is transported in plasma in protein-bound form. Most is bound to albumin and to α_2-macroglobulin. Dysproteinemia of varying degrees is a feature of all subtypes of leprosy. Therefore decreased carrier protein in the plasma could be one of the factors responsible for the lowering of serum zinc levels.



THE FIGURE. Distribution of serum zinc levels in normal subjects and untreated leprosy cases.

Zinc deficiency in experimental animals ([1, 5, 6]) and also in humans ([3, 12, 15]) has been demonstrated to cause suppression of the CMI functions. The presence of low serum zinc levels in lepromatous leprosy patients suggests that zinc deficiency could be one of the various factors involved in the non-specific suppression of CMI, particularly in the late stages of the disease. It would be interesting to explore the potential of zinc as an immunostimulant in lepromatous leprosy.

SUMMARY

Serum zinc levels were estimated in 146 patients with different types of leprosy and 40 control subjects. Tuberculoid patients were found to have normal serum zinc levels. A gradual reduction in serum zinc levels was observed from TT to LL. Serum zinc levels were not significantly higher in patients treated with dapsone for approximately 18 months. The cause of hypozincemia is not clear, but the findings suggest that there are correlations among bacillary load, immune status, and serum zinc. Zinc deficiency could be one of the factors involved in the non-specific suppression of cell-mediated immunity in lepromatous leprosy.

RESUMEN

Se midieron los niveles de zinc sérico en 146 pacientes con diferentes tipos de lepra y en 40 individuos sanos. Se encontró que los pacientes tuberculoides tuvieron niveles normales de zinc en suero y que tales niveles mostraron una reducción gradual del extremo TT al extremo LL. Los niveles de zinc sérico no fueron significativamente mayores en los pacientes tratados con dapsona durante aproximadamente 18 meses. Aunque no está clara la causa de la hipozincemia, los hallazgos sugieren que hay correlaciones entre la carga bacilar, el estado inmune, y el zinc sérico. La deficiencia de zinc puede ser uno de los factores involucrados en la supresión no específica de la inmunidad celular en los pacientes con lepra lepromatosa.

RÉSUMÉ

Chez 146 malades atteints de différents types de lèpre, et chez 40 sujets témoins, on a mesuré les taux sériques de zinc. On a constaté que les malades tuberculoïdes présentaient des taux sériques de zinc normaux. Une diminution graduelle des taux de zinc dans le serum a été observée chez les patients, et ceci tout au long du spectre de la classification de TT à LL. Les taux de zinc dans le serum n'étaient pas significativement plus élevés chez les malades traités par la dapsone pendant environ 18 mois. La cause de l'hypozincémie n'est pas claire, mais ces observations suggèrent qu'il existe une corrélation entre la charge bactériologique, le statut immunitaire, et les taux sériques de zinc. La carence en zinc pourrait être l'un des facteurs qui intervient dans la suppression non spécifique de l'immunité à médiation cellulaire dans la lèpre lépromateuse.

Acknowledgment. We are thankful to Dr. K. N. Mathur, Senior Chemist, Geological Survey of India, for his help in serum zinc estimation.

REFERENCES

1. BEACH, R. S., GERSHWIN, M. E. and HURLEY, L. S. Altered thymic structure and mitogen respon-

siveness in postnatally zinc-deprived mice. Dev. Clin. Immunol. **3** (1979) 725–738.

2. Beach, R. S., Gershwin, M. E., Makishima, R. K. and Hurley, L. S. Impaired immunologic ontogeny in postnatal zinc deprivation. J. Nutr. **110** (1980) 805–815.
3. Björkstén, B., Bäck, O., Gustavson, K. H., Hallmans, G., Hägglöf, B. and Tärnvik, A. Zinc and immune function in Down's syndrome. Acta Paediatr. Scand. **69** (1980) 183–197.
4. Cunningham-Rundles, C., Cunningham-Rundles, S., Iwata, T., Incefy, G., Garofalo, J. A., Menendez-Botet, C., Lewis, V., Twomey, J. J. and Good, R. A. Zinc deficiency, depressed thymic hormones, and T lymphocyte dysfunction in patients with hypogammaglobulinemia. Clin. Immunol. Immunopathol. **21** (1981) 387–396.
5. Fernandes, G., Nair, M., Onoe, K., Tanaka, T., Floyd, R. and Good, R. A. Impairment of cell-mediated immune functions by dietary zinc deficiency in mice. Proc. Natl. Acad. Sci. U.S.A. **76** (1979) 457–461.
6. Fraker, P. J., Hass, S. M. and Luecke, R. W. Effect of zinc deficiency on the immune response of the young adult A/J mouse. J. Nutr. **109** (1977) 1889–1895.
7. Godal, T. Immunological aspects of leprosy—present status. Prog. Allergy **25** (1978) 211–242.
8. Golden, M. H. N., Jackson, A. A. and Golden, B. E. Effect of zinc on thymus of recently malnourished children. Lancet **2** (1977) 1057–1059.
9. Iwata, T., Incefy, G. S., Tanaka, T., Fernandes, G., Menendez-Botet, C. J., Pih, K. and Good, R. A. Circulating thymic hormone levels in zinc deficiency. Cell Immunol. **47** (1979) 100–105.
10. Kumar, N., Malhotra, V., Singh, R., Vij, J. C. and Anand, B. S. Structure and function of the small bowel in lepromatous leprosy. Int. J. Lepr. **50** (1982) 148–151.
11. Meret, S. and Henkin, R. I. Simultaneous direct estimation by atomic absorption spectrophotometry of copper and zinc in serum, urine, and cerebrospinal fluid. Clin. Chem. **17** (1971) 369–373.
12. Oleske, J. M., Westphal, M. L., Shore, S., Gordon, D., Bogden, J. D. and Nahmias, A. Zinc therapy of depressed cellular immunity in acrodermatitis enteropathica. Its correction. Am. J. Dis. Child. **133** (1979) 915–918.
13. Oon, B. B., Khong, K. Y., Greaves, M. W. and Plummer, V. M. Trophic skin ulceration of leprosy: Skin and serum zinc concentrations. Br. Med. J. **2** (1974) 531–533.
14. Pekarek, R. S. and Beisel, W. R. Characterization of the endogenous mediator(s) serum zinc and iron depression during infection and other stresses. Proc. Soc. Exp. Biol. Med. **138** (1971) 728–732.
15. Pekarek, R. S., Standstead, H. H., Jacob, R. A. and Barcome, D. F. Abnormal cellular immune responses during acquired zinc deficiency. Am. J. Clin. Nutr. **32** (1979) 1466–1471.
16. Ridley, D. S. and Jopling, W. H. Classification of leprosy according to immunity. A five-group system. Int. J. Lepr. **34** (1966) 255–273.
17. Riordan, J. F. and Vallee, B. L. Structure and function of zinc metalloenzymes. In: *Trace Elements in Human Health and Disease.* Prasad, A. S., ed. New York: Academic Press, 1976, vol. 1, pp. 227–256.
18. Tanaka, T., Fernandes, G., Tsao, C., Pih, K. and Good, R. A. Effects of zinc deficiency on lymphoid tissue and on immune functions of A/Jax mice. Abstract in Fed. Proc. **37** (1978) 931.
19. Sher, R., Shulman, G., Baily, P. and Politzer, W. M. Serum trace elements and vitamin A in leprosy subtypes. Am. J. Clin. Nutr. **34** (1981) 1918–1924.
20. Wannemacher, R. W., Jr., Pekarek, R. S. and Klainer, A. S. Detection of a leukocyte endogenous mediator-like mediator of serum amino acid and zinc depression during various infectious illnesses. Infect. Immun. **11** (1975) 873–875.