Clinical Evaluation

Effect of Zinc Supplementation on Adenosine Deaminase Activity in Leprosy

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Absract

Serum Adenosine Deaminase (ADA) acts as marker of cellular immunity and its activity is found to be altered in various diseases in which there is a cell mediated immune response (CMI) including leprosy. The role of zinc is well established in the development and maintainence of immunocompetence and its supplementation activates the immune response in particular T-lymphocytes and monocytes in several ways. The aim of the study was planned to evaluate the effect of nutritional zinc supplementation on cell mediated immune response by investigating the pre and post intervention serum ADA levels after oral zinc sulphate supplementation in leprosy patients.

A total of 49 cases, 30 Tuberculoid Leprosy (TT) and 19 Lepromatous Leprosy (LL) patients, within the age group of 25-60 years were enrolled in the study along with 30 age matched healthy controls. Serum ADA was estimated in all the subjects before and after (2 months and 4 months) oral zinc supplementation. Pre intervention serum ADA level was observed to be significantly increased in both the TT and LL ($p<0.001$) groups as compared to controls, revealing raised immunological activity in the patients. After oral zinc sulphate supplementation serum ADA re-evaluation was done in 38 cases. A highly significant ($p < 0.001$) rise in ADA level was registered in the post intervention period (4 months supplementation) in TT cases with a moderately significant ($p< 0.05$) increase in LL cases, indicating the ability of oral zinc therapy to affectively alter the cell mediated immune response in leprosy.

Keywords

adenosine deaminase (ADA), cell mediated immunity (CMI), zinc

Introduction

Leprosy is a chronic infectious disease that affects the skin and the peripheral nerves, but has a wide range of possible clinical manifestations. This mycobacterial infection in man results in a spectrum of disease, the type of which depends upon the CMI response of the host[19]. At one end of the spectrum, the Lepromatous (LL) form of leprosy is characterized by a heavy and widely disseminated bacterial load, with poor cell mediated immune response to the *Mycobacterium leprae* antigens, while at the other end a comparatively effective cell mediated immunity of the Tuberculoid (TT) patient restricts bacillary growth[3].

ADA participates in the purine salvage degradative pathway, catalyzing the hydrolytic cleavage of adenosine to inosine and ammonia. It is a selective marker of immune stimulation in patients of leprosy and its serum levels positively correlated with cell mediated immune response of the affected patient attributing to increased lymphocyte proliferation resulting from antigenic stimulation[13]. Its activity also reflects monocyte/macrophage activity or turnover in different diseases[20]. Studies have demonstrated that ADA activity was directly correlated with zinc ion concentration, it being the only ion amongst several other cations capable of serving as a cofactor and restoring its catalytic activity[4]. Zinc affects multiple aspects of the immune system[15]. It is essential for cell mediated immunity, phagocytosis and for

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functions of neutrophils, natural killer cells and macrophages.

Keeping in view the above facts, this study was designed to find out the pre and post intervention serum ADA level after oral zinc sulphate supplementation and made an attempt to explore the beneficial effects of zinc and its potential as an nutritional immunostimulant in leprosy cases.

Materials and Methods

Forty nine (49) untreated patients visiting the OPD of the leprosy home at Naya Bazaar, Cuttack, with definitive evidence of hypopigmented anaesthetic patches and enlarged peripheral nerves were included in this study. They were categorized according to the Ridley Jopling classification[16] into thirty (30) Tuberculoid Leprosy (TT) and nineteen (19) Lepromatous Leprosy (LL) cases. Slit skin smear and skin biopsy was carried out in all patients to confirm the diagnosis. Thirty (30) age, sex and socio economic status matched healthy personnel working at the leprosy home, not on any type of medication were taken as controls. Seum was estimated in all the leprosy cases and controls. After obtaining informed consent, the patients received zinc sulphate formulation (220 mg/ day) for 4 months. During the treatment period they were advised not to take various drugs or any food products along with the supplement that would interfere with zinc absorption. Patients with history of Multi-Drug Therapy, cases with chronic conditions such as leukemia, lymphoma, hepatitis, cirrhosis or any other chronic illness were excluded from the study.

The present study was conducted in the department of Biochemistry, Hi-Tech Medical College, Bhubaneswar, Orissa. Both cases and controls were subjected to routine biochemical investigations. Serum ADA activity was estimated by the method of Guisti and Galanti[11]. Ammonia liberated by reaction of ADA on adenosine forms an intensely blue indophenol with sodium hypochlorite and phenol in alkaline solution. The intensity of colour formed was measured spectrophotometrically at 620nm. One unit of ADA is defined as the amount of enzyme required to release 1 mmol of ammonia/min from adenosine at standard assay conditions. Results were expressed as international unit of enzyme activity of serum. Estimation was done in the pre-intervention period, and after 2 months and 4 months oral zinc therapy after which results were tabulated after statistical analysis. There were no drop-outs during 2 months supplementation period, however, after 4 months, ADA measurement could be done in 38 cases as 4 TT and 7 LL subjects withdrew their consent, either because of GI side effects or due to lack of motivation. Results were expressed in Mean ± SD. Statistically analysis of results was done using student's 't' test.

Observations

As the cases belonged to a low socio-economic status, routine biochemical parameters like Hb% FBS and Total serum protein revealed a moderate fall ($p< 0.05$) indicating a state of hypoproteinemia and anaemia while TLC and ESR showed a rise ($p<0.05$) in cases in comparison to controls (**Table 1**). Pre-supplementation mean serum ADA level was observed to be significantly high ($p< 0.001$) in both TT (47.04 ± 5.37 IU/L) and LL (30.23 ± 7.52 IU/L) groups in comparison to controls (20 ± 2.31 IU/L) as depicted in **Table 2**. The values in TT cases was greater than LL cases indicating a more effective cell mediated

Table 1
Routine Biochemical Parametrers in Controls and Leprosy Cases

Parameter	Controls (n=30)	TT (n=30)	LL(n=19)
Hb (g/dl)	15.5 ± 1.94	11.3 ± 2.33*	9.8 ± 1.55*
TLC (x 10^9/L)	7.54 ± 2.43	10.2 ± 1.86*	8.8 ± 2.09*
ESR (mm)	10.06 ± 2.5	14.22 ± 4.12*	15.84 ± 1.47*
FBS (mg/dl)	87.3 ± 4.8	75.8 ± 9.3*	75.1 ± 10.7*
Serum Total Protein (Gm/dl)	7.8 ± 1.18	6.2 ± 2.18*	6.0 ± 1.74*

Results are expressed as mean ± SD
*$p < 0.05$ (moderately significant)

Table 2
Baseline ADA (IU/L) Levels in the Study Group

Controls (n=30)	TT (n=30)	LL (n=19)
20 ± 2.31	47.04 ± 5.37*	30.23 ± 7.52*

*(p< 0.001), highly significant

immune response against the invading mycobacteria. However raised ADA activity seen in an immune hyporresponsive state like LL may be due to abnormal proliferation of different subsets of lymphocytes. **Table 3** showed variable changes after zinc sulphate supplementation in the diseased groups. After 2 months, there was no significant change (NS) and a moderate rise ($p<0.05$) in the mean ADA values in the LL (32.93 ± 4.82 IU/L) and TT (52.42 ± 7.96 IU/L) groups respectively as compared to their pre-supplementation status. However, after 4 months of zinc supplementation TT cases registered a significant raise ($p<0.001$) (64.11 ± 5.11 IU/L) in serum ADA level as compared to control. Whereas LL cases revealed moderately significant raise ($p< 0.05$) (39.80 ± 6.48 IU/L) in ADA level than control pointing towards the ability of nutritional zinc in influencing the immune response in leprosy especially in TT.

Discussion

A number of parameters, including ADA activity have been used to asses cell mediated immune response in different diseases. ADA activity originates from the action of the principle isoenzymes, ADA-1 and ADA-2. ADA-1 although found in most tissues, increased amounts are produced by T- lymphocytes at some stage of their activation[2]. ADA-2, is found in monocytes and macrophages is released by these cells when they are stimulated by the presence of live micro-organisms in their interior. Active macrophages have higher ADA activity than normal macrophages[22]. Increased ADA levels that were attributed by various workers due to increase in lymphocyte proliferation, runs parallel with the conventional parameters of cell mediated immunity in patients of leprosy correlating with their immunological status[17]. Suri Babu *et al* and Nirupama Devi *et al* have reported significantly raised activity of the enzyme in all types of leprosy, with a trend of increasing activity from lepromatous to the tuberculoid end of the disease spectrum[18,14]. Our observations corroborated with the above authors showing a significant rise in serum ADA activity in cases as compared to controls.

The enzyme contains a parallel α/β barrel motif, with a zinc ion cofactor bound to the innermost region of the active site. Pharmacological evidence suggested a need for dietary levels of zinc for proper enzymatic activity[8]. It is a potent mediator of host resistance to infection and is required as a cofactor for numerous metalloenzymes involved in continous production of the immune system cells. Zinc is an essential component of thymulin, a thymic hormone involved in T-cell maturation, cytokine production and expression of IL-2 receptor on T-cells[5]. T-cell proliferation induced by zinc is not due to its direct action rather an indirect effect through (zinc induced) monocyte derived cytokines. Effective clearance of mycobacterial infection requires a Th1 (T-helper cells) mediated activation of infected macrophages by IFNγ (Interferon Gamma). It acts by inducing T- cell activation or alteration of lymphokine production, which in turn may activate macrophages to promote bacterial clearance[7]. Despite having such important functions, the body does not store zinc and requires a constant dietary intake. Oral supplementation to leprosy patients have shown beneficial effects relating to cell mediated immunity as observed by some authors[1,9,12]. Others have shown an increase in ADA

Table 3
Ada Level (IU /L) after Oral Zinc Sulphate Supplementation

Study Group	Baseline Level	After 2 Months Supplementation (n=49)	After 4 Months Supplementation (n=38)
TT	47.04 ± 5.37	52.42 ± 7.96 *	64.11 ± 5.11 **
LL	30.23 ± 7.52	32.93 ± 4.82	39.80 ± 6.48 *

* ($p< 0.05$, moderately significant)
** ($p< 0.001$, highly significant)

activity in LL cases by the effect of oral zinc therapy indicating an improvement in several immune parameters[6]. Some experimental studies have concluded that it is possible to restore the enzyme activity in aged skin fibroblasts to normal levels by raising the zinc concentration in the culture medium to four or five times the control normal plasma zinc level[10] while others suggested that though $ZnCl_2$ has enhancement effects on ADA activity in a dose dependent manner, it might show toxicity with deleterious effects in higher concentrations[21]. The above results matched with the present study which depicts that oral zinc therapy plays an important role in specific immune defences and microbicidal activity in both types of leprosy more so in the TT than LL group by significantly enhancing the ADA activity in the cases.

Summary

ADA as a marker of CMI, consistent with lymphocyte and macrophage activity was found to be raised in leprosy patients in comparison to controls. Its concentration runs parallel with the immunological response of the patients against antigenic stimulation. Administration of zinc brings about stimulation of a variety of immunological functions involved in host defense like T-lymphocyte and macrophage activation indicated by a rise in serum ADA levels after supplementation in leprosy patients. Zinc having the ability to enhance cell mediated immunity, could reduce the incidence of reaction and minimize the morbidity of the disease. In addition to various drugs and other primary immunomodulators, it has an immense immunotherapeutic role in treating leprosy cases and maybe used as an adjunct to chemotherapy to enhance bacterial killing as well as bacterial clearance and hence shorten the treatment period.

References

1. Ananda S. Prasada. — Zinc : role in immunity, oxidative stress and chronic inflammation: *Clin Nutr and Metabolic Care*: **12**: 646-652; 2009.

2. Barton R. W. and Goldschneider — Nucleotide metabolizing enzymes and lymphocyte differentiation. *Mol Cell Biochem*. **28**: 135-147: 1979.

3. Bullock W.E. — Studies of immune mechanisms in leprosy. *N Engl J Med*. **278**, 298-304, 1968.

4. Cooper B. *et al*. — The role of divalent cations in the structure and function of murine adenosine deaminase. *Protein Sci*. **6**: 1031-1037: 1997.

5. Coto J.A., E.M. Hadden, M. Sauro, N. Zom, J.W. Hadden. — Interleukin regulate secretion of zinc-thymulin by human thymic epithelial cells and its action on T-lymphocyte proliferation and nuclear protein kinase C. *Proc. Natl. Acad. Sci. USA*: **89**: 7752-7758: 1992.

6. El Shafei M.U., Kamal A.A., Soliman H., El Shayeb F., Abdel Baqui, M.S. Faragull S., Sabry M.K. — Effect of oral zinc supplementation on cell mediated immunity in lepromatous leprosy. *J Egypt Public Health Assoc*. **63**: 311-336: 1988.

7. Fraker P.J., De Pasquale, Jardieu R., Zwick L., Luecke R.W. —Regeneration of T-cell helper function in zinc deficient adult mice. *Proc Natl Acd Sci USA*. **75**: 5660-5664: 1978.

8. Frick L. — Transition state stabilization by adenosine deaminase. Structural studies of its inhibitory complex with deoxycoformycin. *Biochem*. **25**: 1616-1621: 1986.

9. George J., Bhatia V.N., Balkrishnan S., Ramu G. — Serum zinc/copper ratio in subtypes of leprosy and effect of oral zinc therapy on reactional states. *Int J Lepr Other Mycobact Dis*. **59**: 20-24: 1991.

10. Ghneim H.K., Al-Saleh S.S., Al-Shammary F.J., Kordee Z.S. — Changes in adenosine deaminase activity in ageing cultured human cells and the role of zinc; *Cell Biochem Funct*. **21**(3): 275-82: 2003.

11. Guisti G. Galanti B. — Adenosine deaminase. Colorometric Method. In Bergmeyer HU Methods of Enzymatic Analysis: 3rd edn: Vol. **IV**: 315: 1984.

12. Gupta, Sharma, Vohra and Ganguly. — Inhibition of apoptosis by ionomycin and zinc in peripheral blood mononuclear cells of leprosy patients. *Clin. Exp. Immunol*. vol **17**: I; 56-64: 1999.

13. Hovi T., Smyth J.F., Alleson A.C. — Role of adenosine deaminase in lymphocyte oroliferation. *Clin Exp Immunol*. **23**: 395-403: 1976.

14. Nirupama Devi, S.K. Mohapatra, S. Patnaik, Mangala

Ch Das. — Serum ADA activity in relation to Multi Drug Therapy in different types of leprosy. *Ind J Med Biochemistry*. Vol **7**: No1 and 2: 7-11: 2003.

15. Prasad A.S. — Clinical, immunological, anti-inflammatory and antioxidant roles of zinc. *Exp Gerontology*. **43**: 370-377: 2008.

16. Ridley D.S., Jopling W.H. — Classification of leprosy. *Int J Lepr*. **34**: 255: 1996.

17. Shende R.K., Bardapurker J., Patil V., Gaikwad A. — Adenosine deaminase activity in leprosy. *Ind J Lepr*. Vol **65** (2): 201-205: 1993.

18. Suri Babu S.S., Kannan K.B., Katoch V.M., Bharadwaj V.P. — Adenosine deaminase activity in leposy: *Ind J Lepr*. **62**: 437-477: 1990.

19. Turk J.L. — Cell mediated immunological process in leprosy. *Bull*. *WHO*: **41**, 779, 1969.

20. Ungerer J.P., Oosthuizen H.M., Bisbori S.H., Uermaar W.J. — Serum adenosine deaminase: isoenzymes and diagnostic applications: *Clin Chem*. **38**: 1322-1326: 1992.

21. Z. Hussein. — Efects of zinc on the activity of immune system in male albino mice: IBN Al- Haitham. *J for Pure and Appl Sci*. Vol **23** (1): 2010.

22. Zukerman S. H., Olson J.M., Douglas S.D. — Adenosine deaminase activity during in vitro culture of human peripheral blood monocytes and pulmonary alveolar macrophages. *Exp Cell Res*. **129**: 281-287: 1980.