Impact of anti-tuberculosis therapy on plasma zinc status in childhood tuberculosis

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أثر معالجة السل على مستويات الزنك في بلاسما الأطفال المصابين بالسل

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الخلاصـة: تقـارن هذه الدراسـة بين مستويـات الزنـك في البلاسـما لـدى 15 طفلاً مصاباً بسـل رئـوي نـشيط، و15 طفلاً مصاباً بسوء التغذية و15 طفلاً صحيحاً. و لم يكن التـركيز الوسطي للزنك في بلاسما الأطفـال المصابين بالسل (71.7 مكـغ/دل)، مختلفـاً اختلافـاً يُعْتَدُّ بـه إحصائيـاً، عمـا هـو عليـه فـي المجموعتـين الأخريـيّن، إذ بلـغ (72.5 مكغ/دل) في الأطفال المصابين بسوء التغذية و(76.9 مكغ/دل) لدى الأطفـال الأصحاء. وقد قيّم البـاحثون مستوى الزنك لدى الأطفال المصابين بالسل، بعد شهرين وبعد أربعـة أشـهر مـن معـالجتهم باستـراتيجية المعالجة القصيرة الأمـد تحت الإشـراف المباشر، وتبيّـن أن مستوى الزنك في المصل قـد نقـص بعـد شـهر واحد مـن معالجـة السل، ثم عاد إلى مستواه البدئي بعد مرور 4 شهور من المعالجة.

ABSTRACT This study compared plasma zinc levels in 15 children with active pulmonary tuberculosis, 15 malnourished children and 15 healthy children. Mean plasma zinc concentrations in children with tuberculosis (71.7 µg/dL) were not significantly different than the other 2 groups (72.5 and 76.9 µg/dL). The zinc status of the children with tuberculosis was evaluated after 2 months and 4 months of DOTS therapy. The serum zinc level during anti-tuberculosis therapy decreased after 1 month and then recovered to the initial level after 4 months of treatment.

Impact du traitement antituberculeux sur le statut du zinc plasmatique dans la tuberculose infantile

RÉSUMÉ Cette étude a comparé les concentrations plasmatiques de zinc chez 15 enfants présentant une tuberculose pulmonaire évolutive, 15 enfants souffrant de malnutrition et 15 enfants en bonne santé. Il n'est pas apparu de différence significative entre les concentrations plasmatiques moyennes de zinc observées chez les enfants tuberculeux (71,7 µg/dL) et celles enregistrées dans les 2 autres groupes (72,5 et 76,9 µg/dL). Le statut du zinc des enfants tuberculeux a été évalué après 2 et 4 mois de thérapie DOTS [pour *Directly Observed Treatment, Short-course* -traitement de brève durée sous surveillance directe]. Il a été noté une diminution du zinc sérique après 1 mois de traitement antituberculeux, taux qui est revenu à sa valeur initiale après 4 mois de ce même traitement.

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Introduction

Tuberculosis (TB) is on the increase throughout the world and is one of the most important causes of death among adults in developing countries. In 1993, the World Health Organization (WHO) declared TB to be a global health emergency [1]. Although malnutrition has been described in TB patients previously [2–4], contrary to what is commonly believed, little is known about nutritional status with respect to micronutrients especially zinc. Low concentrations of these nutrients may affect the host's defence mechanisms [5,6].

There are studies that have revealed the correlation of low zinc levels and active pulmonary TB in adults [7,8]. However, few studies in children with the same findings are available [9]. Zinc supplementation has been shown to have a positive effect on the incidence of diarrhoea, pneumonia and may even lead to a decrease in the incidence of malaria [10]. Zinc deficiency is known to cause impaired cell-mediated immunity and compromise neutrophil functions [5]. This can increase susceptibility to TB because the cell-mediated immunity plays a major role in the disease. Zinc deficiency also affects host defence in a variety of ways. It results in decreased phagocytes and leads to a reduced number of circulating T-cells and reduced tuberculin reactivity, at least in animals [6].

Although the first reports on zinc deficiency were among children in the Islamic Republic of Iran, Turkey and Egypt in 1960 [11], there is no comprehensive study of zinc deficiency in the Islamic Republic of Iran. Because of the limited data available on the relationship between nutritional status and TB and due to the increasing incidence of TB we decided to compare zinc status in children with active pulmonary TB and 2 control groups (healthy and malnourished children). The present study also measured the variations in zinc levels over the course of anti-TB therapy after 1 and 4 months.

Methods

Setting

This case–control study was conducted between September 2002 and March 2003 at the National Research Institute of Tuberculosis and Lung Disease, a referral centre for TB and lung diseases in Tehran, Islamic Republic of Iran. The DOTS (directly observed treatment, short-course) regimen has been applied for all cases according to the WHO since 1991. Included in the study were 30 children in the age range 2–12 years who attended the outpatient clinic and were admitted to the paediatric ward: 15 children with pulmonary TB and 15 malnourished children. A control group of 15 healthy children were recruited from the outpatient clinic when they were attending for their annual school check-up. Patients with TB were admitted and the other 2 groups were followed up on an outpatient basis.

The study was conducted under the direct observation of the ethical committee of Shaheed Beheshti University of Medical Sciences. Children and their families were informed about the aims of the study.

Clinical data

A history was obtained from all children in group A about previous TB prophylaxis or treatment and whether they had had close contact with an adult with pulmonary TB. Children with pulmonary TB received DOTS chemotherapy for 6 months according to the standard regimen [12]. All the TB cases were treated with isoniazide, rifampicin, ethambutol and pyrazinamide. After completion of treatment, all of the children became bacteriologically negative and were routinely followed up in the outpatient TB clinic.

A detailed history and a physical examination were also taken for all the malnourished children. There was no evidence of active infectious diseases, immune deficiency, human immunodeficiency virus (HIV) or any chronic diseases. The children were defined as malnourished if they had weight and height under the 5th percentile for Iranian weight-for-age tables. Healthy children were defined as children whose weight and height were in the range of ideal growth chart without any diseases.

A 5 mL sample of peripheral blood was collected from each child before the onset of therapy and sent to the reference laboratory in acid-washed and metal-free tubes. Zinc levels in plasma were estimated by atomic absorption spectrophotometer (Chemtech Analytical, USA) using a hollow cathode lamp at 214.1 nm. The instrument was calibrated with Chemlab standard solution (National Bureau of Standards, Washington DC, USA). The serial estimation of plasma zinc was done in all groups at the time of admission. The serum zinc level of children in group A was again assessed at 1 and 4 months after treatment. For ethical reasons serum zinc levels of the children in the control groups were estimated at month 0 only.

Statistical analysis

Continuous variables are expressed as group means and standard deviation (SD). The outcome measures of this study were plasma zinc, serum albumin and total protein. The null hypothesis was that no difference in the mean would be found between the 3 groups. We made a comparison between groups using the Kruskal–Wallis test. For comparing the outcome after month 1 and month 4 in the TB group, the Friedman test was performed. All P-values were 2-tailed. Statistical significance was considered to be demonstrated by a 2-tailed P-value of less

than 0.05. All analyses were made using *SPSS*, version 11.05.

Results

A total of 45 children in 3 groups were evaluated. In each group there were 7 boys (46.7%) and 8 girls (53.2%). The mean age of the participants was 10.1 (SD 3.2) years for TB children, 8.0 (SD 3.5) years for malnourished children and 5.9 (SD 4.2) years for healthy children.

The mean plasma zinc level was 71.7 µg/dL for children with active pulmonary TB, 72.5 µg/dL in malnourished children and 76.9 µg/dL in healthy children. No statistically significant difference existed between the serum zinc levels for the different groups at the beginning of the study ($P >$ 0.05) (Table 1).

Plasma zinc levels were estimated serially at 0, 1 and 4 months of therapy in children with TB. The data analysis showed a decrease in serum zinc level (62.4 µg/dL) after 1 month of therapy in this group. The zinc level rose significantly in month 4 of therapy to a mean zinc level of 71.7 µg/dL ($P <$ 0.05) (Table 1).

Table 2 also shows the mean serum albumin and total protein of children in each group and in the TB group over 4 months of anti-TB therapy. There was a statistically significant difference in serum total protein levels between the 3 groups at the start ($P <$ 0.05) but not for serum albumin (Table 1). In the TB group no changes in total serum albumin were seen over the 4 months of therapy but there were statistically significant changes in total protein ($P <$ 0.05).

Discussion

Previous studied have suggestive a link between TB and low serum zinc levels.

Table 1 **Mean serum zinc, total albumin and total protein values of all children at the beginning and values in children with tuberculosis (TB) after 4-months of treatment**

Variable/month	TB children (*n* = 15) Mean (SD)	Malnourished children (*n* = 15) Mean (SD)	Healthy children (*n* = 15) Mean (SD)	*P*-value
Plasma zinc (µg/dL)				
Month 0	71.7 (13.5)	72.5 (7.7)	76.9 (17.4)	< 0.05[b]
Month 1	62.4 (20.4)	_ _	_ _	
Month 4	71.7 (11.3)	_ _	_ _	
	P < 0.05[a]			
Total protein (µg/dL)				
Month 0	7.33 (1.09)	7.07 (0.92)	7.79 (0.22)	< 0.05[b]
Month 1	7.77 (0.79)	_ _	_ _	
Month 4	7.71 (0.33)	_ _	_ _	
	P < 0.05[a]			
Total albumin (µg/dL)				
Month 0	4.13 (0.44)	4.03 (0.38)	4.12 (0.28)	> 0.05[b]
Month 1	4.15 (0.33)	_ _	_ _	
Month 4	6.58 (0.52)	_ _	_ _	
	P > 0.05[a]			

− = not measured.
[a]2-tailed; [b]Kruskal–Wallis.
SD = standard deviation.

Deveci et al. showed low serum zinc and albumin levels and high serum metalloenzymes in pulmonary TB patients in comparison with the control groups [13]. They found a strong correlation between serum metallo-enzymes and serum zinc levels. The low plasma zinc levels observed in our study among children with active pulmonary TB are similar to the findings of other studies. Ray et al. conducted a similar study in 1998 in India [14]. They showed low zinc levels in children with different types of TB including pulmonary TB. They also showed the low level of serum zinc in patients with malnutrition in comparison with a healthy control group. There are other studies that showed changes of micronutrient levels in TB patients. Liu et al. showed that the level of zinc, copper and selenium in the serum

of TB patients decreased significantly (*P* < 0.01) compared with the control group [15]. The reason for low serum zinc levels in TB could be multifactorial. Firstly a change in distribution of zinc in the body tissues is known to occur in chronic infections, with a net flow of zinc to the liver for the synthesis of acute phase reactants including metalloenzymes. Secondly, zinc may be utilized by *Mycobacterium tuberculosis* for growth and multiplication [15].

Ray et al. showed that serum zinc levels of patients increased gradually after anti-TB therapy and reached normal levels after 6 months [14]. Interestingly, our study shows that the serum zinc level was first decreased after 1 month of therapy and then reached the normal level after 4 months. Another study conducted by Ciftci et al. measured

the level of serum selenium, copper and zinc in patients with pulmonary TB at the beginning and 2 months after therapy [16]. They found that although selenium and copper levels were not affected during the treatment there was a significant increase in the levels of zinc and a decrease in the Cu/Zn ratio. They concluded that a Cu/Zn ratio can be assessed to evaluate the response to therapy. Some studies have not noted significant changes in zinc levels during TB therapy, probably because they assessed the levels too early during the course of therapy [7,17].

TB and malnutrition often co-exist and are among the main causes of childhood death in developing countries. The normal value of serum zinc in children is 70–120 µg/dL (atomic absorption spectrophotometer method) [18]. In our study serum zinc values in all children, including the control group, were low. Many children in the Islamic Republic of Iran suffer from zinc deficiency. In Zahedan, the prevalence of zinc deficiency among school-aged girls was about 43.8% [19]. A study in the capital of the country, Tehran, revealed a zinc deficiency in up to 50% of high-school students [20]. Because of the low levels of zinc in Iranian children we used a control group chosen from the same population to establish the specific effects of TB and malnutrition on our patients. It is well known that malnutrition is a predisposing factor to low zinc levels, which results in reduction of thymulin activity, proliferation response of lymphocytes in the presence of mitogens and neutrophil chemotaxis [21]. It also causes a significant reduction in the number of CD4 helper cells. On the other hand, TB is very closely linked to the cell-mediated immune response of the host, and alterations in lymphocyte and macrophage functions contribute to the natural course of the disease [22].

Whether zinc supplementation would result in earlier recovery from TB or in more rapid multiplication of *M. tuberculosis*, is a question that needs to be addressed. Pant et al. stated that patients receiving zinc sulphate in addition to anti-TB therapy showed faster sputum conversion, radiological improvement and marked rise in plasma zinc levels in comparison to patients receiving anti-TB alone [23]. Karyadi et al. showed that vitamin A and zinc supplementation improves the effect of anti-TB medication after 2 months and results in earlier sputum conversion compared with the placebo group [24]. Another study in our centre showed a positive effect of zinc sulphate on sputum conversion of patients with pulmonary TB [25]. Another study demonstrated that zinc increases the purified protein derivatives (PPD) induration size irrespective of their nutritional state. Hence it can be a booster of immunological mechanisms [26].

Conclusion

The results of this research demonstrate that serum zinc level decreased 1 month after therapy and then increased gradually to reach a normal level in the 4th month. Because of diverse results in studies which demonstrate a pattern of zinc level during therapy, further studies should be done to determine a precise pattern of zinc level changes. In this case zinc level assessment during anti-TB therapy can be used as an indicator for clinicians to assess the response and effectiveness of anti-TB therapy.

Acknowledgements

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