ORIGINAL RESEARCH ARTICLE

Tanaffos (2003) 2(7), 53-60

The Positive Effect of Oral Zinc Sulphate on Sputum Conversion of Patients with Pulmonary Tuberculosis

Mohammad Reza Boloorsaz [1], Ali Reza Milanifar [1], Soheila Khalilzadeh [1], Mehdi Kazempour Dizaji [1], Heydar Masjedi [2], Seyed Mehdi Mirsaeidi [2], Parisa Farnia [3], Mohammad Reza Masjedi [4], and Ali Akbar Velayati [1]

[1] Department of Pediatrics, [2] Department of Infectious Diseases, [3] Department of Mycobacteriology, [4] Department of Pulmonary Medicine, NRITLD, Shaheed Beheshti University of Medical Sciences and Health Services, TEHRAN-IRAN

ABSTRACT

***Background**: Tuberculosis is still a major health problem around the world. Malnutrition is frequently seen in TB patients. One of the important minerals being investigated in the malnutrition study is zinc.*

Deficiency of minerals in TB patients especially zinc has been reported in many cases. The aim of this study was to determine the effect of oral zinc supplements on the Sputum conversion of TB patients.

***Materials and Methods**: This research was a randomized, double blind, placebo- control trial that was conducted in National Research Institute of Tuberculosis and Lung Disease (NRITLD). Patients with confirmed pulmonary tuberculosis having positive smears and cultures were divided by simple randomization into two groups i.e.: case and placebo groups.*

To the individuals of the case group, 0.5% zinc sulphate solution with a dose of 15mg/day was administered. Meanwhile in the placebo group, distilled water (as a placebo) with the same dose as zinc sulphate was given. Both groups received standard cat-I treatment.

Patients from both groups underwent regular clinical investigation and smear test on days 0, 15, 30 and 60 and results were studied.

***Results**: Out of 24 patients in the treatment group and 20 patients in placebo ,smears of 6 individuals (25 %) in case group and no individual in placebo group became negative at the end of fifteenth day ($p<0.05$).*

Smears of 17 (70.8 %) in intervention group and only 8 (40%) in placebo group became smear negative at the end of first month ($p<0.05$). At the end of second month, all patients in both groups became smear negative.

***Conclusion**: According to the conclusions reached from this study, it seems that sputum conversion among the case group which used oral zinc sulphate supplement was much faster.* **(Tanaffos 2003; 2(7): 53-60)**

***Key words**: Tuberculosis, Zinc, Sputum conversion, Mineral supplements*

INTRODUCTION

Tuberculosis (TB) is a major world-wide health problem. The rate of positive smear pulmonary TB in Iran is 13/100000. Malnutrition is frequently seen in TB patients (1). One of the important minerals being investigated in the malnutrition study is zinc. The deficiency of zinc in TB patients has also been reported in China and India. Since chemotherapy

Correspondence to: Boloorsaz MR
Tel.: +98-21-2803550
E-mail address: Blour_saz@hotmail.com

with anti TB drugs for a duration of 6 month increases the plasma zinc concentration in children suffering from TB, this factor i.e. the concentration of zinc in plasma, could provide valuable information regarding severity of disease and its response to anti-TB drugs (2,3). It is noted that administration of zinc to patients with TB or bacterial pneumonia improves the functional activity of immune system (4).

Previously conducted studies show the existence of a clear and direct relation between malnutrition and zinc (5). Also, the negative effect of zinc deficiency on the growth and development of infants has been reported earlier (6). Researches have shown the positive effect of zinc on the growth velocity of short stature male children (7) and also on various infectious diseases including infectious diarrhea (8,9). In addition the effect of zinc on different upper and lower respiratory tract infections is in such a way that there would be a decrease in the prevalence and severity of this group of diseases in pre-school children (10).

Keeping in view the above facts, we decided to evaluate the positive effect of oral zinc sulphate supplements on smear of TB patients.

MATERIALS AND METHODS

This research was conducted in NRITLD among pulmonary TB patients admitted from July 2002 to July 2003. Sample collection was as follows:

- Age 2-65 years old.
- Early morning, sputum smear (×3) was obtained from all of the adult patients' under-study. In children, gastric aspirate (×3) was collected by the standard method. Also, a single BK culture of sputum was prepared. All the clinical manifestations and radiological findings were noted.
- None of the patients had received any anti-TB drugs or gave such a history.
- Factors and elements that were responsible for sample exclusion were: Multi-Drug Resistant (MDR) TB, pregnancy and lactation, history of corticosteroid and supplemental mineral consumption in the last two months, diabetes mellitus, chronic renal failure, liver cirrhosis, recent surgery, tumor, malignancies, GI mal-absorption, immunodeficiency disorders, and AIDS. Although our topic of discussion is immune system, disorders of this system and infections such as AIDS are included as exclusion factors.

The ethical guideline of the Council for International Organization of Medical Science was followed. This study was approved by the ethic committee of Shaheed Beheshti University of Medical Sciences, Iran.

All of the patients were informed of the indication and procedure of the research. Their related questions were answered and finally a consent letter was obtained from them.

- STUDY PATTERN

This study was a community based, double blind, placebo-control trial with supplementation pattern. Group sample sizes of 24 and 20 achieve 80% power to detect a difference of 0.44 between the null hypothesis that both group proportions are 0.80 and the alternative hypothesis that the proportion in group 2 is 0.35 using a two-sided chi-square test with continuity correction and with a significance level of 0.05.

The participants were randomly assigned to intervention by using random allocation.

- MICRONUTRIENT SUPPLEMENT AND ANTI- TUBERCULOSIS DRUGS

Powder form of zinc sulphate (made in Merck Company) was mixed with distilled water and

contained in 250cc bottles. To the patients in the case group, 0.5% zinc sulphate solution with doses of 15mg/day for adults and 1mg/kg for children were given.

Meanwhile to the placebo group, distilled water with the above mentioned dose was administered.

Duration of the zinc sulphate treatment period was 2 months. In the first four weeks of the treatment, the patients were admitted in the hospital, after that they were discharged but were recommended to return on days 45^{th} and 60^{th}. All the necessary recommendation and advises were given to the patients and their companions. Their cooperation was encouraged. The TB treatment process was according to the WHO/DOTS strategy.

- METHODS

Direct examination of sputum for AFB (Acid Fast Bacilli) and sputum culture for M.tuberculosis were performed before the start of anti-TB treatment and later, on days 15^{th}, 30^{th} and 60^{th} following anti-TB treatment.

- SPUTUM EXAMINATION

Three early morning sputum specimens were obtained from the patients. After being stained with Ziehl-Neelsen stain, the sputum was observed directly under the microscope. Also, for each sample culture was obtained.

Depending on the number of bacilli seen in the microscopic field, sputum smear was divided into the following groups:

(-) In 100 microscopic fields, no bacilli were seen.

(+/-) In 100 microscopic fields, a total of 1-9 bacilli were observed.

(1+) In 100 microscopic fields, a total of 10-99 bacilli were observed.

(2+) In at least 20 microscopic fields, 1-9 bacilli were seen in each field.

(3+) In at least 20 microscopic fields, 10 or more bacilli were seen in each field.

The frequencies of bacilli in sputum smear were as follows:

In 1 cc of negative sputum smear no bacilli are detected.

In 1 cc of +/- sputum smear 5000-10000 bacilli are present.

In 1 cc of 1+ sputum smear 10^4-10^5 bacilli are present.

In 1 cc of 2+ sputum smear 10^5-5×10^5 bacilli are present.

In 1 cc of 3+ sputum smear more than 5×10^5 bacilli are present.

Usually the culture is positive when 100 or more bacilli are seen in each milliliter of sputum.

- STATISTICAL ANALYSIS

Data on the patients age, sex distribution and sputum smear grade before the start of treatment and on 15^{th}, 30^{th}, and 60^{th} days of follow up were summarized and used to compare the patients of the two groups. Independent t-tests were used to compare normally distributed age between the groups. Differences in treatment outcome (negative culture smear proportion) between intervention and placebo groups, before the start of treatment and on 15^{th}, 30^{th}, and 60^{th} days of follow up were tested by chi-square test. Statistical significance was based on a two tailed p-value <0.05. Analyses were performed using the SPSS release 11.5 software.

RESULTS

All of the 24 (54.5%) participants in the intervention group and 20 (45.5%) from the placebo group completed the study.

There were no significant differences in sex and age distribution between the two groups.

Before the treatment all of the participants were smear positive and no significant difference between smear grades of two groups was observed.

At the end of the fifteenth day, out of 24 patient present in the treatment and 20 in the placebo groups, only six individuals (25%) from the case group became negative. Meanwhile none of patients in the placebo group had negative smear at the end of 15th day (p= 0.016).

At the end of first month, smears of 17 (70.8 %) in case group and only 8 (40%) in placebo group became smear negative (p=0.04).

At the end of second month all patients in both groups became smear negative.

Table 1. Charactеrisics of the patients

	Micronutrient group		Placebo group		p_value
	count	**%**	**count**	**%**	
Sample	24	54.5	20	45.5	
At the time of admission					
+	5	20.8	1	5	
++	8	33.3	6	30	
+++	11	45.8	13	65	
The first 15 days					
+	12	50	10	50	
++	3	12.5	8	40	0.016
+++	3	12.5	2	10	
-	6	25	zero	zero	
End of first month					
+	6	25	12	60	0.04
++	zero	zero	zero	zero	
+++	1	4.2	zero	zero	
-	17	70.8	8	40	
End of second month					
+	zero	Zero	zero	zero	
++	zero	zero	zero	zero	
+++	zero	zero	zero	zero	
-	24	100	20	100	
Age	27.13±15.2		37.3±20.24		0.1
Sex					0.45
Female	17	70.8	12	60	
Male	7	29.2	8	40	

DISCUSSION

Zinc is an important mineral and trace element that is present in the body, having significant role in the human immune system. It protects the cells from being destroyed by various free radicals (11, 12). Different studies performed in- vitro shows that in case of zinc deficiency, macrophage function is impaired, which is improved by the addition of this mineral (13, 14).

Administration of this mineral prevents lung parenchyma from being destroyed in hyperoxic

environment (15). Another effect of zinc is its role in the defensive mechanism of the host at the site of infection, which is shown very clearly in the case of Trypanosoma cruzi (16).

During the process of TB disease, there is a decrease in the concentration of minerals in the body. This has been shown clearly in various researches, including those conducted by Taneja (17). It seems that malnutrition is not the only reason for the mineral deficiency seen in TB patients. By performing a study, Ray and colleagues compared the serum zinc concentration of "TB patients" with that of "non-TB malnourished patients". According to the results, it was seen that although the concentration of serum zinc was low in the malnourished individuals, still zinc deficiency was more significant and evident in TB patients, a fact that could not be explained by the malnutrition status seen in these individuals (3).

It is not known whether zinc deficiency seen in TB disease is due to secretion of various cytokines in the body or being a defensive mechanism of the body against M.tuberculosis.

Earlier it was shown that the reason for the serum iron deficiency observed in *Salmonella* and *Yersinia* infections was because of the increased demand of this mineral by these microorganisms resulting in low serum iron concentration. The question whether or not such a phenomenon exists in case of zinc deficiency observed in M.tuberculosis infections arises. Abul and colleagues showed that pulmonary macrophages in the vicinity of zinc, demonstrate an increased secretion of interleukin alpha-1. It seems that the presence of this mineral in cell-mediated immunity, which has an important and major defensive role in pulmonary TB, is important (4).

Another important question is whether M.tuberculosis uses zinc and if so, increasing serum zinc concentration increases it's pathogenicity or not. The answer to this question is difficult. The studies made by Dassurget and colleagues have shown that Zinc containing metalo-protein present in M.Tuberculosis (Cu-Zn superoxide dismutase), which protects the organism against various free oxygen radicals, does not have a major role in the pathogenesis of this bacteria (18). It seems that the same condition exists in other species of mycobacterium as well. Therefore, further research is needed to study the effect of zinc supplements on the pathogenecity of mycobacteria especially M.tuberculosis.

In this research with the aim of initiation of anti TB treatment and/or decreasing the transmission period, which is associated with the break-off of the disease chain, zinc supplementations were given to the patients. Based on the results of this study, it is proved that zinc supplementation causes a faster and quicker sputum conversion in TB patients.

Significant clinical difference exists between the case and placebo groups ($p=0<0.051$). In such a way that 70.8% from case and 40% from placebo groups became smear negative at the end of first month. Since, a positive smear TB patient could transmit infection to an average of 20-28 healthy individual, this investigation has an epidemiological value. The results of this research are comparable to that of Karyadi and co-workers. They showed that simultaneous administration of vitamin A and Zinc supplements resulted in a faster and significant sputum conversion ($p=0.05$) (19). In our study, although the patients used zinc supplementation exclusively, still similar results were achieved. Our research regarding sputum conversion of TB patient was the second one of such kind.

Another research in which zinc supplements was administered, was Cuevas and colleagues study. In this investigation, Cuevas demonstrated that by giving zinc supplementations to patients with zinc

deficiency, the induration of PPD test was increased, showing the fortifying effect of this trace element on cell-mediated immunity (20).

Zinc could also be used as an indicator for diagnosis of tuberculosis.

Rankovic and co-workers showed the diagnostic value of Zinc concentration of pleural effusion in the differential diagnosis of pleurisy (21).

CONCLUSION

According to the conclusions reached from this study, it seems that sputum conversion among the case group which used oral zinc sulphate supplement was much faster.

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