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ROLE OF VITAMIN A AND ZINC SUPPLEMANTATION ON SPUTUM SMEAR CONVERSION TIME IN PULMONARY TUBERCULOSIS PATIENTS

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ABSTRACT

Objective: To see the efficacy of which single micronutrient contributed more to the sputum conversion time.
Methodology: It is double-blind placebo controlled study in which new sputum smear positive pulmonary tuberculosis patients were randomly assigned to receive either placebo or vitamin A or zinc or vitamin & zinc both. Patients were asked to deliver their sputum at weekly interval to measure positivity of bacteria.
Results: A total of 260 patients were enrolled in the study among them 214 patients completed the study. Patient's mean age was almost similar (p>0.05) at enrolment in all 4 groups (Group A=31, Group B=29.9, Group C=30.6 and Group D=30.9 years). Sputum positivity grade +3 was most prevalent. Patients in Group D (Vitamin A & Zinc) showed earlier sputum conversion time (4.1±1.3; mean±sd) compare with that in the other groups. However, the difference was not significant.
Conclusions: Combination of Vitamin A & Zinc supplementation reduces sputum smear conversion time but it failed to show superiority other groups.

KEY WORDS Vitamin A & Zinc, pulmonary tuberculosis

INTRODUCTION

India is the second-most populous country in the world, India has more new TB cases annually than any other country. In 2008, out of the estimated global annual incidence of 9.4 million TB cases, 1.98 million were estimated to have occurred in India, of whom 0.87 million were infectious cases, thus catering to a fifth of the global burden of TB. About 40% of Indian population is infected with TB bacillus. Protein-energy malnutrition frequently occurs in patients with tuberculosis with or without HIV infection, as indicated by reductions in anthropometric variables and serum concentrations of visceral proteins and micronutrients (1). Micronutrient deficiencies in particular may have an adverse effect on components of the immune system for the control of mycobacteria. Several observational studies have shown low concentrations of vitamin A and zinc in adults and children with pulmonary tuberculosis (2-7).

Vitamin A, as found as retinol in plasma, is one of important micronutrient which has specific immune function (8). The presence of vitamin A deficiency in sputum-positive pulmonary TB patients compared with healthy subjects was confirmed (4,6) and associated with the future of pulmonary adult TB patients (9). Zinc is a trace mineral, which is essential for the function of cells of the immune system (10) and a mild deficiency depresses the immune function in humans (11). Zinc is also known as an essential mineral for normal mobilization of vitamin A from the liver to the plasma (12).

As in other programs for the combat against TB, the National Tuberculosis Program in Indonesia states that the disappearance of acid-fast bacilli (AFB) from sputum after treatment is basic in pulmonary TB patients' management. The presence of AFB in sputum can be assessed by direct visualization using the light microscope, and can be confirmed by growth of Mycobacterium tuberculosis in sputum culture. Conversion of sputum smears to AFB-negative status is only used in resource-limited countries (13) while in developed countries treatment success is measured by conversion to no growth of Mycobacterium tuberculosis (MTB) in sputum culture (14). The effectiveness of anti-TB therapy is determined by several factors, including the burden of mycobacteria, underlying immune status, adherence to treatment, and drug susceptibility. In the face of new threats to combat tuberculosis, i.e. multidrug and extensively drug resistance MTB, and also immunosuppressive diseases (HIV, diabetes, malnutrition); the studies on micronutrients as modulator of the immune system are important. It would provide more evidence to the importance of supplementation against tuberculosis. Following the previous finding in Jakarta, the present study aimed to investigate the effect of single supplementation of zinc or vitamin A or the combinations, on the sputum conversion time and the health status of newly diagnosed pulmonary TB patients. The objective of the study is to see the efficacy of which single micronutrient contributed more to the sputum conversion time.

METHODS

Study Design and Subjects

This was a double-blind placebo controlled study. Cases were out-patients with active pulmonary TB patients (new sputum smear positive) attended the DOTS center in the Department of Pulmonary Medicine, CSM Medical University UP, Lucknow. Selection of cases was based on the following criteria: age 18–55 years; at least two sputum specimens positive for acid-fast bacilli by microscopy; and clinical and radiographic abnormalities consistent with pulmonary TB. Exclusion criteria for cases and controls were as follows: previous anti-TB treatment, pregnancy, lactation, use of corticosteroids or supplements containing vitamin A, zinc or iron during the previous month, moderate to severe injury or surgery during the last month and diseases such as abnormal liver function as measured by elevated serum levels of aspartate amino transferase (ASAT) and alanine amino transferase (ALAT), diabetes mellitus as measured by elevated fasting blood glucose levels, neoplasm as determined by clinical examination, chronic renal failure as determined by elevated serum levels of urea and creatinine, and congestive heart failure.

Randomization and Supplementation

Patients were randomly assigned to receive either placebo or vitamin A or zinc or vitamin & zinc both. Each micronutrient capsule contained 1500 retinol equivalents (5000 IU) vitamin A (as retinyl acetate) and 15 mg Zn (as zinc sulfate). The placebo consisted of lactose alone. Supplement and placebo capsules were indistinguishable in appearance both externally and internally. Dosage was based on the recommendation of the World Health Organization (15). Supplements or placebo has been given to the patients with the antituberculosis drugs on DOTS day.

Sample Size

Assuming 0.37 difference with standard deviation of 0.6 increase in mean between treatment group (16) in plasma zinc from 2 month to 6 month, using the formula $2C/\delta^2+1$ Where $\delta = 0.37$ (Mean difference between treatment groups/Standard deviation) C = 10.5 for 90% power and 5% significance level. The calculated sample size per group is 55. After adjusting 20% loss to follow-up, a total of 65 cases will be required for each group.

Collection of sputum sample

The patients were asked to come to deliver their sputum for direct Acid Fast Bacilli (AFB) smear examination at weekly intervals till the end of the intensive phase. Three early morning sputum specimens were taken from the patients and examined by direct microscopy after Ziehl-Neelsen staining. The time was noted when the first of three weeks' consecutive sputum smears of good quality was negative. In the case either, not all sputum samples were delivered, or the sputum was not of adequate quality, the data were considered missing.

Statistical analysis

The collected data was entered in Microsoft Excel computer program and checked for any inconsistency. A one-sample Kolmogorov-Smirnov test was used to determine whether the variables were normally distributed. Data on the characteristics of the subjects at enrollment for their age and gender distribution, nutritional status (weight) and blood concentrations were summarized and used to assess the comparability of the patients randomly assigned to the four treatment groups. Different means between groups were tested for significance using one-way ANOVA when normally distributed, and Kruskal-Wallis test when not normally distributed. Different proportions between groups were tested using chi-squared test. Within group changes were tested using paired student-t test for normally distributed data and the Wilcoxon signed-rank when for not normally distributed. A p value less than 0.05 were considered significant. An intention to treat analysis was applied. Statistical analyses were performed using computer software SPSS for Windows PC version 15.0 (SPSS Inc, Chicago, IL, USA).

Ethical Considerations

The study has been approved by the Institutional Ethics Committee of CSM Medical University UP, Lucknow, India. Informed consent was obtained from each subject before the start of the study.

Results

A total of 260 patients were enrolled in the study and randomly categorized into 4 groups of intervention, i.e. placebo (Group A, n=65), Vitamin A (Group B, n=65), zinc (Group C, n = 65), and combined zinc + vitamin A (Group D, n = 65). After completion of the study i.e. at 6 months 214 patients completed the study and were analyzed.

Patient's mean age was almost similar ($p>0.05$) at enrolment in all 4 groups (Group A=31, Group B=29.9, Group C=30.6 and Group D=30.9 years). Percentage of males was higher in all groups. Sputum positivity grade 3 was most prevalent. There was no difference of baseline characteristics among the 4 groups of patients (Table 1). As shown in table 1, mean sputum conversion time of the Group D intervention was the shortest (4.1±1.3), and that of the Group A the longest. Group D patients showed less weeks to reach 85% of sputum conversion than the other Groups. The Group A supplementation (Placebo) showed the longest time to reach 85% of sputum conversion. However, no significant difference of the sputum conversion time was

Table-1: Baseline characteristics of the patients

Variables	Groups			
	A (n=47)	**B (n=54)**	**C (n=55)**	**D (n=52)**
Age (year), mean±sd*	31.0±11.1	29.9±11.3	30.6±11.4	30.9±12.0
Gender (n, %)**				
Male	25 (53.2)	29 (53.7)	31 (56.4)	28 (53.8)
Female	22 (46.8)	25 (46.3)	24 (43.6)	24 (46.2)
Marital status (n, %)**				
Married	30 (63.8)	32 (59.3)	36 (65.5)	33 (63.5)
Single	17 (36.2)	22 (40.7)	19 (34.5)	19 (36.5)
Sputum smear grade (n, %)**				
+1	13 (27.7)	15 (27.8)	17 (30.9)	15 (28.8)
+2	16 (34.0)	18 (33.3)	16 (29.1)	19 (36.5)
+3	18 (38.3)	21 (38.9)	22 (40.0)	18 (34.6)
Sputum conversion time (week), mean±sd	7.0±1.3	4.3±1.2	5.3±1.8	4.1±1.3

*P>0.05 (One way ANOVA among groups), **p>0.05 (chi-squared among groups)

observed between groups (ANOVA, p > 0.05). At 5 week, sputum smear was negative in 3.8% of patients from the zinc + vitamin A group (Group D), in 3.7% of patients in the vitamin A (Group B) and no conversion was observed in Group A & C. The sputum conversion became 100% in the patients of Group D at 9 week, however, this became 10 week in the patients of Group C (Zinc supplemented). Taking into account, the WHO success rate target of 85% conversion to negative, we observed that patients in the zinc + vitamin A supplementation group reached the target within 8 weeks (Figure -1). After 2 months of treatment, there were significant improvements of micronutrient status within groups compared with the initial levels, particularly in patients given combined vitamin A + zinc supplementation (Group D). At the end of the treatment, significant changes from the baseline were observed for all variables, but significant difference between groups was observed for WBC, Vitamin A and zinc levels (Table-2, Fig. 2 & 3).

Fig.1: Cummulative percentage of the sputum conversion time (weeks)

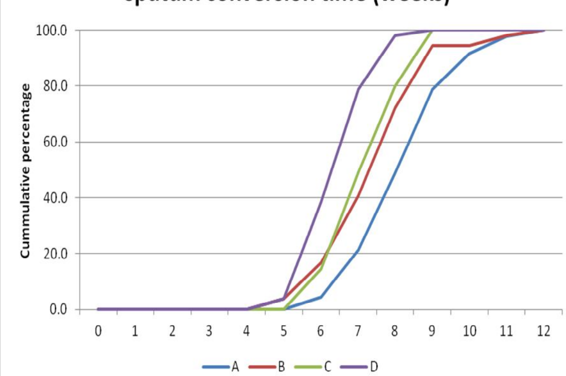

Fig.2: Average percentage change in parameters from 0 week to 2 months

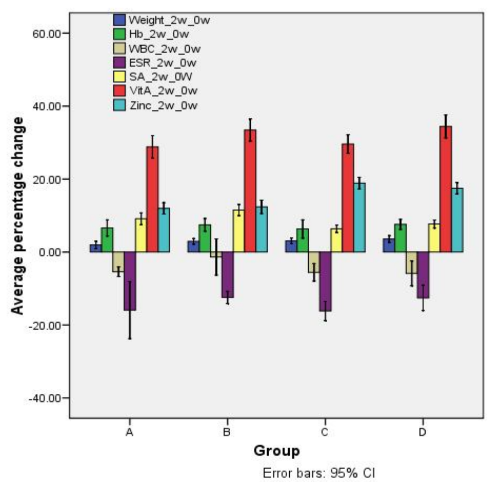

Fig.3: Average percentage change in parameters from 0 week to 6 months

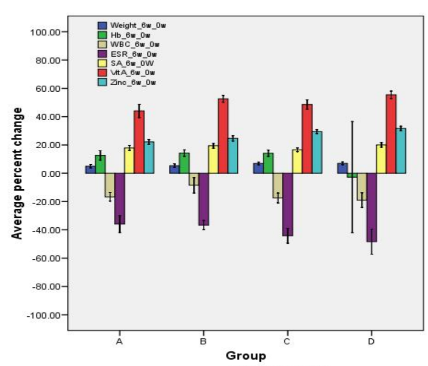

Table-2: Changes in anthropometric and blood parameters prior to, at two and six months of treatment

	Groups			
Variables	**A (n=47)**	**B (n=54)**	**C (n=55)**	**D (n=52)**
Weight (kg), mean±sd				
0 month	45.9±7.1	42.9±5.6	44.1±6.6	44.8±5.9
2 month	46.7±6.4[1]	44.2±5.4[1]	45.4±6.0[1]	46.5±5.8[1]
6 month	48.1±6.0[1, a]	45.3±5.3[1, a]	47.2±5.8[1]	48.1±5.5[1]
Hemoglobin (g/dl), mean±sd				
0 month	10.5±1.3	9.9±1.5	9.9±1.3	9.9±1.2
2 month	11.2±1.1[1, a]	10.6±1.2[1, a]	10.6±1.2[1,a]	10.7±1.2[1]
6 month	12.0±0.8[1]	11.5±1.0[1]	11.5±1.7[1]	11.6±1.7[1]
WBC (cells/mmm^3), median (IQR)				
0 month	9900 (6500-12500)	10500 (750-13200)	9900 (7500-13600)	10950 (4300-14200)
2 month	9500 (5300-11400)[2, b]	9950 (7200-13000)[2, b]	9600 (7300-12100)[2, b]	10400 (6800-11900)[2, b]
6 month	8500 (5200-10600)[2, b]	9100 (7000-12400)[2, b]	8800 (6800-10100)[2, b]	8900 (7200-10600)[2, b]
ESR (mm/hr)				
0 month	14.3±2.9	14.3±2.7	13.9±3.1	15.5±2.9
2 month	12.64±2.4*	12.7±2.3[1]	12.0±2.4[1]	13.8±2.6*
6 month	10.7±2.3[1]	10.5±2.2[1]	9.7±1.8[1, a]	10.7±2.4[1, a]
Serum albumin (g/dl)				
0 month	3.4±0.3	3.3±0.3	3.5±0.3	3.3±0.2
2 month	3.7±0.3	3.8±0.3[a]	3.7±0.3	3.6±0.2[a]
6 month	4.1±0.3[1]	4.2±0.4[1]	4.2±0.3[1]	4.2±0.3[1]
Vitamin A level (µmol/l)				
0 month	0.8±0.3	0.7±0.2	0.8±0.2	0.7±0.2
2 month	1.1±0.3	1.1±0.2	1.1±0.3	1.1±0.2
6 month	1.5±0.3[1, a]	1.5±0.3[1]	1.6±0.2[1]	1.7±0.2[1, a]
Zinc level (µmol/l)				
0 month	9.7±1.2	9.5±0.8	9.7±0.7	9.5±0.6
2 month	11.0±1.2[1, a]	10.9±1.3[1, a]	12.0±0.9[1, a]	11.5±0.9[1, a]
6 month	12.4±1.1[1, a]	12.8±1.6[1, a]	13.8±0.9[1, a]	14.0±1.3[1, a]

[1]p<0.0001, *p<0.05 (change from baseline, paired student-t), [a]significant between groups (One-way ANOVA, p<0.05), [2]p<0.0001 (change from baseline, Wilcoxon signed rank test), [b]p<0.05 significant between groups (Kruskal Wallis test)

DISCUSSION

We analyzed a total of 214 TB patients. This study was double-blind placebo controlled study. In our study, supplementation with zinc and vitamin A, either alone or combined, failed to show superiority over placebo which is contrast to the results of the previous study in Jakarta, in which vitamin A and zinc supplementation was beneficial in terms of sputum conversion time (16). Results similar to ours with regard to zinc supplementation were obtained in a study in Tanzania (20), where such supplementation did not lead to a reduction of sputum conversion time compared to supplementation with a multi-micronutrient or placebo either. Also, zinc supplementation did not improve immune response among TB patients infected with HIV in a study in Singapore (21). The authors further concluded that in the absence of zinc deficiency, additional zinc supplementation was not beneficial. And to the best of our knowledge, the data on the effect of vitamin A supplementation in TB were inconclusive (22-24). The severity of TB in our patients is witnessed by the fact that more 30% had sputum positivity grade 3 prior to treatment whereas in the Jakarta study 68% had grade 1 (for both supplemented and placebo groups) and this can be expected to lead to longer sputum conversion time (25). It may be that the supplementation effect does not become clear in such severe cases.

Our results matched with another study where they found no significant effect of supplementation with a single dose of Vitamin A and daily zinc on the rate of sputum smear or culture conversion by 8 weeks (26). A recent study showed that vitamin D receptor genetic polymorphisms were associated with the time to sputum culture conversion (18). Also, it was shown that vitamin D receptor genotype independently predicted the sputum smear conversion time while on anti-TB therapy (19). One might speculate that ethnic background also plays a role in the response to supplementation with micronutrients.

A study of Tanzanian patients receiving anti-tubercular treatment without any supplementation of vitamin A showed a significant increase in retinol concentrations after 2 month (5). However, in our study, we observed a significant increase in serum retinol level from baseline to 2 and 6 month in all the supplemented groups. Our findings confirm this observation and suggest, therefore, that the low baseline retinol concentrations were most likely due to the presence of active disease rather than underlying deficiency.

In our study, significant increase in serum zinc concentrations occurred in the supplemented groups at 2 and 6 month of treatment. However, data from 2 supplementation trials, both of which failed to show increases in plasma zinc concentrations after 2 month, but noted significant increases at the end of treatment in both supplemented and non-supplemented participants (9,16). Again, the effect of symptomatic disease on zinc homeostasis should be considered. Because the concentration of zinc is much higher in tissues such as the liver, than in serum, small differences in the hepatic uptake of zinc may have a profound effect on serum concentrations. The latter is an intracellular metal-binding protein that is activated by cytokines during the acute phase response (17).

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