

A double-blind, placebo-controlled study of vitamin A and zinc supplementation in persons with tuberculosis in Indonesia: effects on clinical response and nutritional status¹⁻³

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ABSTRACT

Background: The results of cross-sectional studies indicate that micronutrient deficiencies are common in patients with tuberculosis. No published data exist on the effect of vitamin A and zinc supplementation on antituberculosis treatment.

Objective: Our goal was to investigate whether vitamin A and zinc supplementation increases the efficacy of antituberculosis treatment with respect to clinical response and nutritional status.

Design: In this double-blind, placebo-controlled trial, patients with newly diagnosed tuberculosis were divided into 2 groups. One group ($n = 40$) received 1500 retinol equivalents (5000 IU) vitamin A (as retinyl acetate) and 15 mg Zn (as zinc sulfate) daily for 6 mo (micronutrient group). The second group ($n = 40$) received a placebo. Both groups received the same antituberculosis treatment recommended by the World Health Organization. Clinical examinations, assessments of micronutrient status, and anthropometric measurements were carried out before and after 2 and 6 mo of antituberculosis treatment.

Results: At baseline, 64% of patients had a body mass index (in kg/m^2) < 18.5 , 32% had plasma retinol concentrations $< 0.70 \mu\text{mol}/\text{L}$, and 30% had plasma zinc concentrations $< 10.7 \mu\text{mol}/\text{L}$. After antituberculosis treatment, plasma zinc concentrations were not significantly different between groups. Plasma retinol concentrations were significantly higher in the micronutrient group than in the placebo group after 6 mo ($P < 0.05$). Sputum conversion ($P < 0.05$) and resolution of X-ray lesion area ($P < 0.01$) occurred earlier in the micronutrient group.

Conclusion: Vitamin A and zinc supplementation improves the effect of tuberculosis medication after 2 mo of antituberculosis treatment and results in earlier sputum smear conversion. *Am J Clin Nutr* 2002;75:720-7.

KEY WORDS Tuberculosis, vitamin A, zinc, sputum smear conversion, X-ray lesion area, tuberculosis transmission, micronutrient supplementation, Indonesia

INTRODUCTION

Tuberculosis remains a major public health problem throughout the world. Most cases of and deaths from tuberculosis occur in developing countries. In Indonesia, the prevalence of tuberculosis is 0.24% on the basis of positive sputum

smears (1) and the disease is regarded as a priority disease requiring proper treatment.

Malnutrition is frequently observed in patients with pulmonary tuberculosis. Several studies reported that patients with active pulmonary tuberculosis are malnourished as indicated by reductions in visceral proteins, anthropometric indexes, and micronutrient status (2, 3). We previously reported results from a case-control study conducted in an urban area in central Jakarta, Indonesia, in which we found poor micronutrient status among patients with tuberculosis (4).

In our case-control study, the proportions of tuberculosis patients and control subjects with plasma retinol concentrations $< 0.70 \mu\text{mol}/\text{L}$ were 33% and 13%, respectively (4). A study from Rwanda reported vitamin A deficiency among adults with tuberculosis (5). Vitamin A deficiency increases bacterial adherence to respiratory epithelial cells (6). It has been known since the 1940s that vitamin A is excreted in the urine in patients with fever (7), and this has since been confirmed in subjects with acute infections, including pneumonia (8). In addition, the requirement for vitamin A during infection is raised by its

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²Supported by grants from Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH, Eschborn, Germany; the Neys-van Hoogstraten Foundation; the Directorate General of Communicable Disease Control and Environmental Health, Indonesia; and the Integrated Excellent Research project from the Ministry of Research and Technology Indonesia. PT Kimia Farma, Indonesia, provided the micronutrient supplements and placebo, and PT Indo Farma, Indonesia, provided the standard antituberculosis drugs.

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Received September 20, 2000.

Accepted for publication March 20, 2001.

increased rate of excretion and metabolism (8). Studies have shown that vitamin A has an immunoprotective role against human tuberculosis. This finding has a historical basis in that cod liver oil, which is rich in vitamins A and D, was used regularly for the treatment of tuberculosis before the introduction of modern chemotherapy (9). In addition, vitamin A supplementation results in a modulation of the immune response in patients with tuberculosis (10).

In our case-control study, the prevalences of low plasma zinc concentrations ($<10.7 \mu\text{mol/L}$) among tuberculosis patients and control subjects were 21% and 5%, respectively (4). Zinc deficiency was reported in patients with pulmonary tuberculosis in India (2) and China (11). Chemotherapy with antituberculosis drugs for 6 mo increased plasma zinc concentrations in childhood tuberculosis patients in India. Thus, it was suggested that plasma zinc status is likely a marker for monitoring the severity of disease and the response to therapy (12). In addition, zinc supplementation of patients with pulmonary tuberculosis and bacterial pneumonia was shown to increase immune function (13). Studies in humans and animals have shown that zinc deficiency impairs the synthesis of retinol binding protein (14) and reduces plasma retinol concentrations (15). Therefore, it appears that zinc supplementation has a beneficial effect on vitamin A metabolism (14).

It is not known whether vitamin A and zinc supplements given together with antituberculosis drugs would increase the efficacy of the antituberculosis treatment. Therefore, we investigated the effect of vitamin A and zinc supplementation on tuberculosis treatment among Indonesian patients with pulmonary tuberculosis. We assessed the following indexes: clinical response, nutritional status, resolution of radiologic signs (cavities and infiltration), and conversion of sputum smears to negative.

SUBJECTS AND METHODS

Subjects

The study was carried out in the pulmonology outpatient clinics in 1 public hospital and 3 health centers in Jakarta, Indonesia, from December 1997 to December 1998. Cases were outpatients with newly diagnosed, active pulmonary tuberculosis. The subjects were selected on the basis of the following criteria: age 15–55 y, 3 sputum specimens positive for acid-fast bacilli by direct microscopy and culture, clinical and radiologic signs consistent with pulmonary tuberculosis, and no history of previous antituberculosis treatment. Exclusion criteria were drug resistance at baseline or during the follow up; extrapulmonary tuberculosis; 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 previous month; presence of diabetes mellitus as measured by elevated fasting serum glucose concentrations; chronic renal failure as indicated by elevated serum urea or creatinine concentrations; liver disease as determined by elevated serum aspartate aminotransferase and alanine aminotransferase concentrations; and clinical signs of neoplasm and congestive heart failure.

The ethical guidelines of the Council for International Organizations of Medical Sciences (16) were followed. This study was approved by the Committee on Health Research Ethics, Faculty of Medicine, University of Indonesia, Jakarta. All subjects received and signed an informed consent form.

Study design

The study was designed as a community-based, double-blind, placebo-controlled supplementation trial. The sample size was based on the ability to determine a difference with an $\alpha = 0.05$ and $1 - \beta = 0.95$ with use of a one-tailed test for concentrations of hemoglobin in blood and of retinol and zinc in plasma. Because the plasma retinol concentration was the variable requiring the largest sample size, it was calculated that with a sample size of 22 in each group, a between-group difference of $0.31 \mu\text{mol/L}$ in plasma retinol concentration (17) could be detected. Therefore, each group had to include ≥ 35 patients to account for a 30% dropout rate and for a 25% failure to meet the inclusion criteria. We used a table with randomly assorted digits to allocate the patients into 2 groups. In total, we recruited 110 subjects, who were assigned into 2 groups proportionally. One group received the antituberculosis drugs and vitamin A and zinc supplementation (micronutrient group) and the other group received the antituberculosis drugs and placebo (placebo group).

Micronutrient supplement and antituberculosis drugs

Supplements and placebo were prepared by PT Kimia Farma, Indonesia, in the form of capsules. Each micronutrient capsule contained 1500 retinol equivalents (5000 IU) vitamin A (as retinyl acetate) and 15 mg Zn (as zinc sulfate) in a lactose matrix. The placebo consisted of lactose alone. Supplement and placebo capsules were indistinguishable in appearance both externally and internally. All capsules were stored in dark bottles, and the patients were instructed to store the bottles in a refrigerator at $4-6^\circ\text{C}$ or in a cool, dark place. Standard antituberculosis drugs were provided by PT Indo Farma, Indonesia, and the dosage was based on the recommendation of the World Health Organization (18). The antituberculosis therapy for patients with body weights between 33 and 50 kg comprised 300 mg isoniazid, 450 mg rifampicin, 1500 mg pyrazinamide, and 750 mg ethambutol daily for 2 mo, followed by 600 mg isoniazid and 450 mg rifampicin 3 times each week for the next 4 mo. The regimen for patients with body weights >50 kg comprised 300 mg isoniazid, 600 mg rifampicin, 2000 mg pyrazinamide, and 1000 mg ethambutol daily for 2 mo, followed by 600 mg isoniazid and 600 mg rifampicin 3 times each week for the next 4 mo.

We assessed patient compliance by comparing the number of remaining capsules with the number recorded in a logbook provided to the patients. During the first 2 mo of the study, the patients were asked to come to the clinic every week to collect the antituberculosis drugs and supplement or placebo capsules for the ensuing week, and health staff checked compliance at home each day. At least one family member or neighbor was asked to help in monitoring patient compliance. This is in line with the directly observed treatment, short-course (DOTS) strategy recommended by the World Health Organization (19). Patients who did not take their medication regularly, missing even 1 d in the first 2 mo, were dropped from the study. From 2 to 6 mo, health staff visited the patients at home every week to provide antituberculosis treatment and to monitor compliance. At the end of the antituberculosis treatment, all patients were requested to come to the clinic, at which time all measurements were repeated. The authors, health staff, and patients were unaware of the treatment code until the study was completed.

Patients who had severe adverse drug effects were excluded from the study and received further treatment under the guidance

of clinicians in each participating clinic. Patients with strains of *Mycobacterium tuberculosis* resistant to one or more drugs after 2 mo of antituberculosis treatment were placed on a modified drug regimen and their data were excluded from the study.

Methods

A clinical examination, chest X-ray, direct sputum examination and culture, anthropometric measurements, and blood collections were carried out before and 2 and 6 mo after antituberculosis treatment began.

Clinical examination

Outpatients were interviewed with the use of a structured questionnaire to determine whether they were eligible for inclusion in the study. Eligible patients were thoroughly examined at the time of diagnosis by 2 of the authors with either basic training (EK) or specialist training as a pulmonologist (ZA). The clinical assessments performed included completion of the Karnofsky performance status scale to assess health, measurement of body temperature, and assessment of the presence of a bacille Calmette-Guérin (BCG) scar. Scores on the Karnofsky scale range from 0 (dead) to 100 (normal) (20).

Chest X-ray

Chest X-rays of all patients were taken at the time of diagnosis and were evaluated by one of the authors (ZA) by calculating the visible lesion area in both lungs. In patients with cavities, the total area of exposed cavity wall was calculated from the radius of visible cavities (πr^2) as described previously (21). Chest X-rays were randomly assessed twice by the same investigator for 23 of 240 chest X-rays (10%), and 91% of the differences in total lesion area and cavity wall between the 2 assessments were within ± 2 SDs.

Sputum examination

Three specimens of early morning sputum were taken from the patients and each specimen was examined by direct microscopy after Kinyoun-Gabbett staining (22) and was cultured in Kudoh medium (22). Sputum smears were graded according to the Bronkhorst scale, which is based on the number of acid fast bacilli (AFB) visible in oil immersion by microscopy. The categories of this scale are as follows: grade +1, ≥ 3 AFB found in 15 min; grade +2, 1–20 AFB in 10 fields; grade +3, 20–60 AFB in 10 fields; grade +4, 60–120 AFB in 10 fields; and grade +5, > 120 AFB in 10 fields (23). We collected 3 sputum specimens twice weekly to assess sputum conversion from positive to negative. The time was noted when the first of 3 consecutive sputum smears was negative. Drug susceptibility was determined by the indirect absolute concentration method (24).

Tuberculin skin test

Delayed-hypersensitivity skin test responses were assessed by injecting Purified Protein Derivative (0.1 mL, 5 tuberculin units; Perum Biofarma, Pasteur Institute, Bandung, Indonesia) into the skin of the volar area of the forearm. Reactions were assessed 48–72 h after injection by measuring the mean diameter of induration to the nearest millimeter. An induration ≥ 10 mm was considered positive. Each skin test was read independently by 2 investigators who were unaware of each patient's treatment group.

Anthropometric measurements

Body weight was measured with an electronic platform weighing scale (model 770 alpha; SECA, Hamburg, Germany) to the nearest 0.1 kg. Height was recorded to the nearest 0.1 cm by using a microtoise. Body mass index (BMI) was calculated as body weight divided by height squared (kg/m^2). Midupper arm circumference was measured with a plastic measurement tape (25). Skinfold thickness was assessed at 4 sites (biceps, triceps, subscapular, and suprailiac) with the use of a Holtain skinfold caliper (Holtain Ltd, Crosswell, United Kingdom) and was recorded to the nearest 0.2 mm. Each site was measured 3 times on the left side of the body (25). The proportion of total body fat and fat-free mass were based on anthropometric data and were calculated with use of the Durnin and Womersley equations (26).

Blood collection and analyses

Blood samples were collected between 0800 and 1000 at each visit. About 20 mL fasting whole blood was withdrawn by venipuncture into 8 separate 3–5-mL vacutainers (Becton Dickinson, Rutherford, NJ). For zinc analysis, the tubes were covered with plastic stoppers and were cleaned and rinsed with an acid solution (27). Measurements of blood hemoglobin, white blood cell count, erythrocyte sedimentation rate (ESR), serum albumin, and free erythrocyte zinc protoporphyrin were carried out on the same day.

Plasma was separated by centrifugation at $750 \times g$ for 10 min at room temperature and was then stored at -20°C until analyzed for C-reactive protein (CRP), plasma retinol, and zinc. Hemoglobin concentrations, white blood cell count, ESR, and albumin concentrations were analyzed at Multilab Laboratory, which collaborates with the pulmonary clinic in Cipto Mangunkusumo Hospital. Hemoglobin concentrations and white blood cell counts were measured with an automatic analyzer (Microdilutor F-800; Sysmex Kobe, Japan). The intra- and interassay CVs for hemoglobin were $< 5\%$. The ESR was measured by the Westergreen technique (28). Albumin was determined with use of the bromocresol green method (29). Free erythrocyte protoporphyrin (expressed as zinc protoporphyrin) was measured by using a portable front-face hematofluorometer (AVIV Biomedical Co, Lakewood, NJ) at the SEAMEO-TROPED University of Indonesia laboratory as described previously (30). Between-duplicate variability was 3.9%. CRP was measured by an immunoturbidimetric method (31) (Behringwerke, Marburg, Germany) at the University Medical Center, Nijmegen. Variability based on duplicate analysis of 30 samples was 2.4%. Plasma retinol concentrations were measured by HPLC as described previously (32). Twenty samples of vitamin A were analyzed in duplicate and the between-duplicate variability was 5.2%. Plasma zinc was measured by flame atomic absorption spectrophotometry (33) at the Laboratory of Clinical Chemistry and Hematology, University of Bonn, Germany. All zinc samples were analyzed in duplicate and the between-duplicate variability was 1.4%.

Statistical analysis

A one-sample Kolmogorov-Smirnov test was used to investigate whether the variables were normally distributed. Data on the patients' age and sex distributions, nutritional status, blood concentrations, clinical signs and symptoms, and results of radiologic signs at enrollment were summarized and used to assess the comparability of the patients randomly assigned to the 2 treatment groups. Independent *t* tests were used to compare

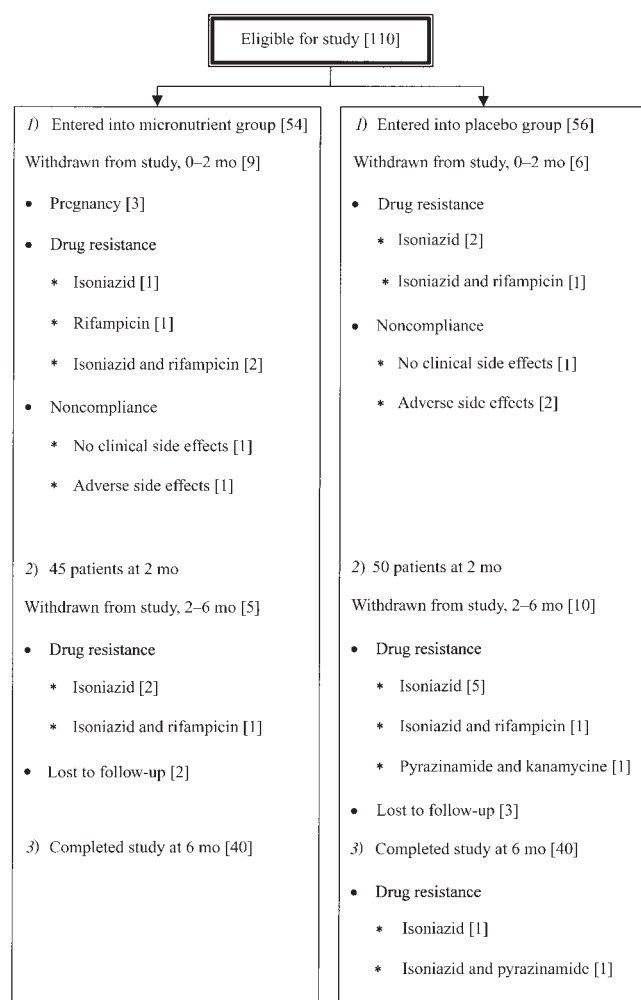


FIGURE 1. Reasons for the loss of eligible study participants. *n* in brackets.

normally distributed variables between the groups. Differences in treatment effects within groups and between the micronutrient and placebo groups were tested by a multivariate analysis of variance repeated-measures design with supplement type as a between-subject factor (2 groups) and treatment effect (baseline compared with 2 and 6 mo) as a within-subject factor. A significant *P* value for the treatment effect indicated a change over time in the combined values of the 2 groups and was further investigated by using a paired *t* test for each individual group. Between-group differences in treatment effect were indicated by significant interactions between treatment effect and supplement type. Baseline values for anthropometric indicators, body weight, ESR, CRP, Karnofsky score, cavity surface area, mean lesion area, blood hemoglobin, zinc protoporphyrin, plasma retinol, and plasma zinc were also included in the analysis as between-subject factors to correct for their possible confounding influence on the changes after 2 and 6 mo. None of the 3-way interactions (treatment effect × supplement type × baseline value) were significant. The logistic regression test was used to investigate the changes in prevalence between groups and by time. The McNemar test was applied to measure the differences in prevalence within groups after 2 and 6 mo. The Pearson test was applied to determine the correlations between variables. The

sputum conversion of the 2 treatment groups was compared by the Kaplan-Meier method and treatment differences were tested by the log-rank test. Cox proportional-hazards regression models were fitted to investigate the following significant covariates as factors in sputum conversion: age, sex, presence of a BCG scar, and variables at baseline with respect to BMI, sputum grade, mean lesion area, and presence of cavity. Statistical significance was based on a two-tailed *P* value < 0.05. Analyses were performed by using the SPSS software package (WINDOWS version 7.5.2; SPSS Inc, Chicago).

RESULTS

Forty of 54 patients (74%) in the micronutrient group and 40 of 56 (71%) in the placebo group completed the study (**Figure 1**). There were no significant differences in sex distribution, age, clinical and biochemical status, and radiologic signs between the 2 groups at baseline (**Table 1**). The women tended to be older than the men but this difference was not significant. Symptoms in both groups were similar before antituberculosis treatment. The symptoms of patients in the micronutrient and placebo groups, respectively, were as follows: hemoptysis (48% and 43%), chest pain (80% and 75%), dyspnea (85% and 83%), and night sweats (85% and 93%).

After 6 mo of antituberculosis treatment, 2 patients in the placebo group had positive sputum smears and mycobacteria resistant to isoniazid, including 1 patient who was also resistant to pyrazinamide (**Table 2**). Decreases in white blood cell count and increases in serum albumin concentrations were found in both groups after 2 and 6 mo of antituberculosis treatment, but there were no significant differences between the groups (data not shown). A significant increase in body weight, which was similar in both groups, occurred between 0 and 2 mo and between 2 and 6 mo. At 2 and 6 mo, there were significant within-group decreases in ESR and CRP, but the difference between groups was not significant. By 6 mo, the Karnofsky score in the micronutrient group was greater than that in the placebo group.

TABLE 1

Baseline characteristics of the patients who completed the study¹

Characteristic	Micronutrient group (<i>n</i> = 25 M, 15 F)	Placebo group (<i>n</i> = 24 M, 16 F)
Age (y)		
Men	24.6 ± 6.7 ²	29.5 ± 11.5
Women	28.5 ± 7.9	31.6 ± 8.8
BMI < 18.5 kg/m ² (<i>n</i>)	25	26
Body temperature (°C)	37.3 ± 0.8	37.0 ± 0.6
Presence of a BCG scar (<i>n</i>)	11	10
Tuberculin skin test induration (mm)	17.8 ± 6.1	18.1 ± 5.9
Sputum smear grade (<i>n</i>)		
+1	25	29
+2	11	9
+3	4	2
Radiologic signs		
Infiltration (<i>n</i>)	40	40
Cavities (<i>n</i>)	17	13
Serum albumin (g/L)	38.1 ± 5.0	36.7 ± 5.5
White blood cell count (× 10 ⁹ cells/L)	10.4 ± 2.7	9.7 ± 2.8

¹ BCG, bacille Calmette-Guérin. There were no significant differences between groups.

² $\bar{x} \pm SD$.

TABLE 2

Clinical status of patients with pulmonary tuberculosis at 0, 2, and 6 mo of antituberculosis treatment

Clinical status and time of assessment	Micronutrient group (n = 40)	Placebo group (n = 40)
Body weight (kg)		
0 mo	44.7 ± 0.8 ¹	43.6 ± 0.9
2 mo	46.9 ± 0.9 ²	45.8 ± 0.9 ²
6 mo	51.6 ± 0.8 ²	48.5 ± 0.9 ²
Erythrocyte sedimentation rate (mm/h)		
0 mo	37.0 (19.3–88.8) ³	33.5 (21.0–70.0)
2 mo	24.5 (17.3–38.0) ⁴	28.5 (19.3–40.8) ⁴
6 mo	13.0 (5.0–23.0) ²	19.5 (7.0–28.8) ²
Plasma C-reactive protein (mg/L)		
0 mo	53.0 ± 5.0	44.2 ± 5.8
2 mo	7.5 ± 1.9 ²	8.6 ± 2.0 ²
6 mo	1.5 ± 0.7 ²	2.1 ± 1.0 ²
Sputum and culture positive (n)		
0 mo	40	40
2 mo	0	0
6 mo	0	2
Drug resistance (n)		
0 mo	0	0
2 mo	0	0
6 mo	0	2 ⁵
Karnofsky score ⁶		
0 mo	80.8 ± 0.6	82.0 ± 0.7
2 mo	90.0 ± 0.6 ²	90.2 ± 0.3 ²
6 mo	97.8 ± 0.7 ²	95.3 ± 0.8 ^{2,7}

¹ $\bar{x} \pm \text{SEM}$.

^{2,4}Significantly different from baseline within the same group: ² $P < 0.001$, ⁴ $P < 0.05$.

³Median (25th–75th percentiles).

⁵Resistant to isoniazid (n = 1) and to isoniazid and pyrazinamide (n = 1).

⁶Range: 0 (dead) to 100 (normal).

⁷Change from baseline significant different between groups, $P < 0.05$.

Micronutrient supplementation resulted in an earlier elimination of tubercle bacilli from sputum (**Figure 2**). After 2 wk, the number of patients with sputum smears negative for tubercle bacilli was higher in the micronutrient group (23%) than in the placebo group (13%). This difference was maintained for up to 7 wk ($P < 0.01$). After adjustment for age, sex, BMI, presence of a BCG scar, sputum grade, presence of cavities, and mean lesion area on the chest X-ray in the Cox regression model, there was still a significant difference in time to sputum smear conversion between groups ($P = 0.026$).

The mean reduction in lesion area on the chest X-ray was significantly greater in the micronutrient group than in the placebo group after 2 mo (**Table 3**). There was no significant difference in cavity surface area between the micronutrient and placebo groups. In the micronutrient group, the increase in plasma retinol concentration was correlated with the reduction in mean lesion area after 6 mo of antituberculosis treatment ($r = -0.367$, $P = 0.020$). Antituberculosis treatment resulted in progressive and significant improvements in all anthropometric indicators (**Table 4**), but there was no significant effect of micronutrient supplementation.

After 2 and 6 mo of antituberculosis treatment, there were significant increases in hemoglobin and plasma retinol concentrations as well as decreases in zinc protoporphyrin concentrations in both groups (**Table 5**). After 6 mo of antituberculosis treatment, the increase in plasma retinol concentrations was signifi-

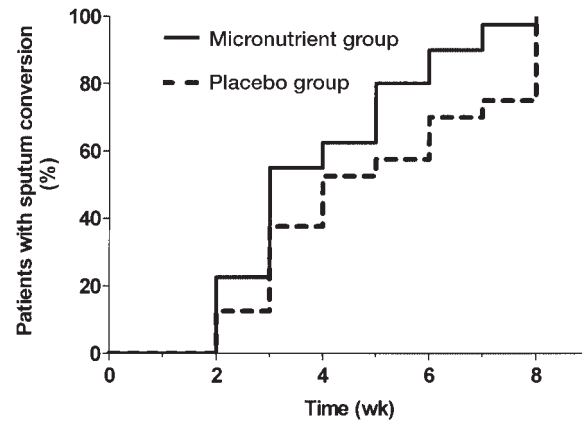


FIGURE 2. Proportion of patients in the micronutrient (n = 40) and placebo (n = 40) groups with sputum smears converting to negative during the first 8 wk of antituberculosis treatment. There was a significant difference between groups, $P < 0.05$ (log-rank test).

cantly higher in the micronutrient group than in the placebo group. Plasma zinc concentrations were not significantly different between the groups after 2 and 6 mo of antituberculosis treatment.

Before antituberculosis treatment, the prevalences of underweight (BMI < 18.5), anemia (hemoglobin < 120 g/L in women and < 130 g/L in men), low plasma retinol concentrations (< 0.70 $\mu\text{mol/L}$), and low plasma zinc concentrations (< 10.7 $\mu\text{mol/L}$) in all patients were 64%, 57%, 32%, and 30%, respectively. The prevalences of patients with anemia, low plasma concentrations of zinc and retinol, and elevated concentrations of zinc protoporphyrin (> 40 $\mu\text{mol/mol}$ heme) were not significantly different between groups after 2 and 6 mo of antituberculosis treatment (**Figure 3**).

DISCUSSION

This study is the first to report an effect of concurrent supplementation with vitamin A and zinc on the treatment outcome of

TABLE 3

Radiologic signs in patients with pulmonary tuberculosis at 0, 2, and 6 mo of antituberculosis treatment

Radiologic sign and time of assessment	Micronutrient group (n = 40)	Placebo group (n = 40)
No. with cavities		
0 mo	17	13
2 mo	14	11
6 mo	9	5
Cavity surface area (cm ²)		
0 mo	25.40 ± 4.89 ¹	7.29 ± 3.43
2 mo	7.47 ± 1.83 ²	6.85 ± 1.63 ³
6 mo	1.58 ± 0.53 ²	1.91 ± 0.89 ²
Mean lesion area (cm ²)		
0 mo	233.91 ± 21.89	221.32 ± 19.08
2 mo	92.94 ± 13.31 ³	123.92 ± 13.98 ^{3,4}
6 mo	21.19 ± 5.70 ⁵	20.63 ± 4.97

¹ $\bar{x} \pm \text{SEM}$.

^{2,3,5}Significantly different from baseline within the same group: ² $P < 0.001$, ³ $P < 0.01$, ⁵ $P < 0.05$.

⁴Change from baseline significantly different between groups, $P < 0.01$.

TABLE 4

Anthropometric indicators in patients with pulmonary tuberculosis at 0, 2, and 6 mo of antituberculosis treatment¹

Nutritional status and time of assessment	Micronutrient group (n = 40)	Placebo group (n = 40)
BMI (kg/m²)		
0 mo	17.6 ± 0.3	18.1 ± 0.5
2 mo	18.4 ± 0.3 ²	19.0 ± 0.5 ²
6 mo	19.4 ± 0.4 ²	20.0 ± 0.5 ²
Midupper arm circumference (cm)		
0 mo	22.8 ± 0.3	21.8 ± 0.6
2 mo	23.4 ± 0.4 ²	22.8 ± 0.6 ²
6 mo	25.2 ± 0.3 ²	24.0 ± 0.6 ²
Biceps skinfold thickness (mm)		
0 mo	4.2 ± 0.2	4.2 ± 0.3
2 mo	4.9 ± 0.3 ³	4.9 ± 0.3 ²
6 mo	5.7 ± 0.5 ²	5.5 ± 0.4 ²
Triceps skinfold thickness (mm)		
0 mo	5.8 ± 0.6	5.5 ± 0.5
2 mo	6.7 ± 0.7 ²	6.6 ± 0.5 ²
6 mo	8.0 ± 0.8 ²	7.5 ± 0.6 ²
Subscapular skinfold thickness (mm)		
0 mo	6.6 ± 0.3	7.0 ± 0.4
2 mo	7.5 ± 0.4 ²	7.7 ± 0.4 ³
6 mo	8.6 ± 0.6 ²	8.6 ± 0.5 ³
Suprailiac skinfold thickness (mm)		
0 mo	5.2 ± 0.4	5.7 ± 0.5
2 mo	6.2 ± 0.5 ²	6.7 ± 0.5 ²
6 mo	7.2 ± 0.6 ²	7.8 ± 0.7 ²
Body fat (%)		
0 mo	11.5 ± 1.0	13.0 ± 1.0
2 mo	13.1 ± 1.1 ²	14.6 ± 1.0 ³
6 mo	14.2 ± 1.2 ²	16.0 ± 1.0 ²
Fat mass (kg)		
0 mo	5.1 ± 0.5	5.7 ± 0.5
2 mo	6.1 ± 0.5 ²	6.7 ± 0.5 ²
6 mo	6.9 ± 0.5 ²	7.7 ± 0.5 ²

¹ $\bar{x} \pm \text{SEM}$.

^{2,3}Significantly different from baseline within the same group: ² $P < 0.001$, ³ $P < 0.01$.

tuberculosis patients. The supplementation improved the effectiveness of the antituberculosis drugs in the first 2 mo. During this period, the antituberculosis treatment aims at killing active bacilli; the subsequent treatment aims at killing dormant bacilli. The use of the DOTS strategy in monitoring treatment compliance and variations in drug absorption may have contributed to the changes observed in sputum positivity and other clinical outcomes. The improved outcome in the micronutrient group was indicated by the higher number of patients with sputum negative for bacilli and the significantly lower mean lesion area in the lungs. This improvement was associated with higher mean plasma retinol concentrations.

Although vitamin A supplementation in conjunction with antituberculosis treatment was shown to have some negative effects on clinical response in South African children with tuberculosis (17), supplementation with vitamin A and zinc in our study was of great benefit, particularly during the first 2 mo of antituberculosis treatment. Moreover, antituberculosis treatment was successful and led to increases in body weight, fat mass, and concentrations of blood hemoglobin and plasma zinc after 6 mo. The clinical benefit of supplementation was less impressive at 6 mo, probably because the effect of supplementation was over-

shadowed by the potent antimycobacterial drug treatment. In addition, the sample size calculation in this study was based on the ability to examine changes in micronutrient status, but the sample size may not have been sufficient to examine effects on clinical outcomes. However, differences may be found when patients with multidrug resistance and HIV infection are studied.

A previous study in India showed that a 4-drug tuberculosis regimen for 3 mo in patients with smear-positive tuberculosis resulted in unacceptably high relapses rates (34). We did not assess the relapse rate in our patients; however, micronutrient supplementation showed an effect on clinical outcome in the first 2 mo and an improvement in the Karnofsky score after 6 mo. The results of our study suggest that it would be useful to adapt current tuberculosis treatment regimens by including micronutrient supplements. It may be possible to reduce the dosage of antituberculosis drugs either in the first or second phase of treatment or to introduce a shorter regimen. Such a shorter regimen would lead to a higher completion rate, fewer adverse drug effects, and a lower cost of antituberculosis treatment.

Conversion of positive sputum smears was significantly faster in the micronutrient group than in the placebo group after 2 mo of antituberculosis treatment. The role of vitamin A and zinc in the conversion of sputum has not been studied. However, retinoic acid was reported to inhibit the multiplication of virulent tubercle bacilli in cultured human macrophages (35). In addition, in vitro cellular killing by macrophages was found to be reduced during zinc deficiency and rapidly restored after zinc supplementation (36). The major benefit of faster sputum conversion would be seen at the community level, because it would reduce the risk of tuberculosis transmission: a person with active tuberculosis will infect an average of 20–28 other persons before recovering from the disease or dying (37).

Radiologic resolution also occurred earlier in the micronutrient group than in the placebo group after 2 mo of antituberculosis

TABLE 5

Micronutrient concentrations in patients with pulmonary tuberculosis at 0, 2, and 6 mo of antituberculosis treatment¹

	Micronutrient group (n = 40)	Placebo group (n = 40)
Hemoglobin (g/L)		
0 mo	124.0 ± 2.0	123.3 ± 2.8
2 mo	130.1 ± 2.4 ²	133.4 ± 2.6 ²
6 mo	141.1 ± 2.2 ³	139.7 ± 2.6 ³
Zinc protoporphyrin (μmol/mol heme)		
0 mo	58.9 ± 4.7	55.0 ± 3.2
2 mo	48.4 ± 4.1 ⁴	39.9 ± 3.6 ³
6 mo	31.3 ± 2.6 ³	29.7 ± 2.8 ³
Plasma retinol (μmol/L)		
0 mo	0.82 ± 0.04	0.90 ± 0.04
2 mo	1.14 ± 0.05 ³	1.08 ± 0.04 ²
6 mo	1.39 ± 0.06 ^{3,5}	1.27 ± 0.05 ³
Plasma zinc (μmol/L)		
0 mo	11.52 ± 0.26	11.15 ± 0.28
2 mo	11.22 ± 0.38	10.22 ± 0.36 ⁴
6 mo	12.50 ± 0.35 ²	12.13 ± 0.31 ²

¹ $\bar{x} \pm \text{SEM}$.

^{2–4}Significantly different from baseline within the same group: ² $P < 0.01$, ³ $P < 0.001$, ⁴ $P < 0.05$.

⁵Change from baseline significantly different between groups, $P < 0.05$.

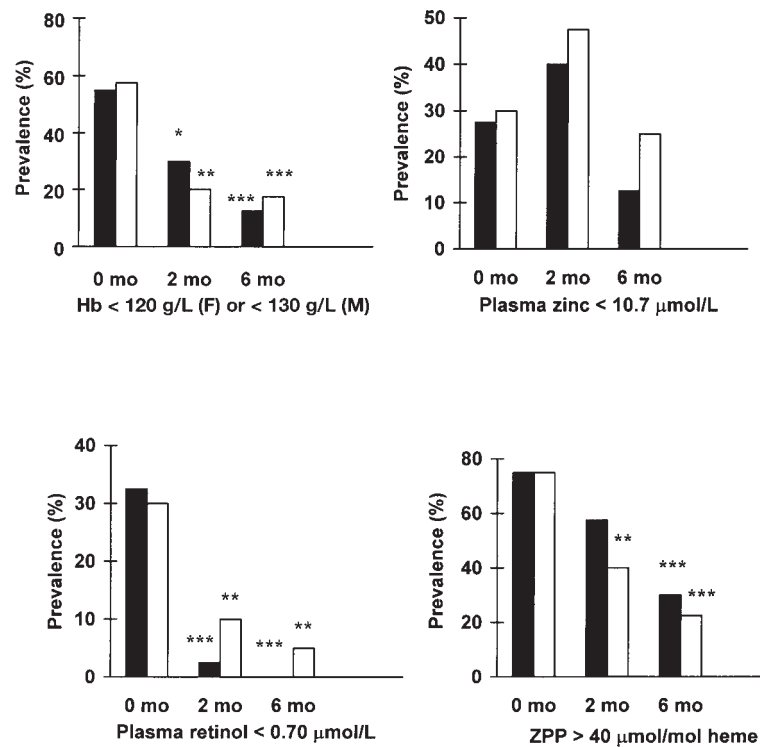


FIGURE 3. Prevalence of low concentrations of blood hemoglobin (Hb), plasma zinc, and plasma retinol and high concentrations of free erythrocyte zinc protoporphyrin (ZPP) before and after 2 and 6 mo of antituberculosis treatment in the micronutrient (■) and placebo (□) groups. ****Significantly different from the respective baseline values: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

treatment. In addition, a correlation between radiologic resolution and increases in plasma retinol concentrations after 6 mo of antituberculosis treatment was found in the supplemented group. There are several potential explanations for this finding. First, retinol is utilized directly by pulmonary tissue, which is rich in nuclear retinoic acid receptors (38). Second, zinc has a role in protecting cells from the damaging effects of free radicals. Nitric oxide was shown to induce zinc release from metallothionein in membranes; thus, an adequate zinc supply may limit free radical membrane damage during inflammation (39). Indeed, zinc supplementation was shown to prevent pulmonary pathology due to hyperoxia-induced lung damage in rats (39).

The mechanism of decrease in plasma retinol concentrations during infection can be explained by the impairment of hepatic release of vitamin A as a result of the reduced synthesis of retinol binding protein, which is a negative acute phase protein. In addition, urinary loss of retinol may also play a role. Patients with pneumonia and sepsis lose up to 50% of their daily requirement of vitamin A in urine (8), and children with shigellosis lose 15% of their daily requirement (40). In this study, vitamin A supplementation resulted in an acute phase shift in hepatic protein synthesis, as indicated by the greater increase in plasma retinol concentrations after 6 mo of antituberculosis treatment in parallel with the reduction in ESR and CRP concentrations.

Interestingly, there was no similar trend in plasma zinc concentrations. Reductions in plasma zinc concentrations were shown in both groups after 2 mo of treatment. This phenomenon may be because during the intensive phase of antituberculosis treatment (in the first 2 mo), the antituberculosis drugs were used to kill the population of replicating bacilli. Zinc plays an

important role in the macrophage contribution to host defense at the site of infection, as was reported in *Trypanosoma cruzi* infection (41). This agrees with the results of a study showing redistribution of zinc to the liver and bone marrow after injection of inflammatory cytokines while acute phase protein synthesis and hemopoiesis were also increased (42). The other possible mechanism is the effect of the antituberculosis drugs on zinc absorption. Ethambutol was shown in rats to increase not only zinc absorption but also urinary zinc losses, resulting in reduced circulating zinc concentrations (43). Although there were no significant differences between the groups in our study, plasma zinc concentrations increased over 6 mo of antituberculosis treatment.

This present study showed that the effectiveness of antituberculosis treatment was improved during the first 2 mo by vitamin A and zinc supplementation. The conclusions drawn from this study should now be tested in larger trials. ⚡

We thank Lisa M Parkkali for her support in carrying out this study and Klaus Pietrzik for arranging the zinc analysis.

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