Deficiency of Micronutrient Status in Pulmonary Tuberculosis Patients in North India

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Abstract

Malnutrition is observed frequently in patients with pulmonary tuberculosis (TB), but their micronutrient status, especially of Vitamin A and Zinc, is still poorly documented. The objective of this study was to investigate the micronutrient status of patients with active pulmonary tuberculosis, admitted in the Department of Pulmonary Medicine, CSM Medical University UP, Lucknow. In this case-control study, 43 patients aged 18–55 year with active pulmonary TB were enrolled and blood sample was taken. Cases had clinical and radiographic abnormalities consistent with pulmonary TB and at least two sputum specimens showing acid-fast bacilli. Micronutrient status data were collected. Compared with healthy control cases, TB patients had significantly lower concentrations of blood haemoglobin, WBC count, serum albumin, serum retinol and zinc, whereas the concentration of free erythrocyte zinc protoporphyrin concentration, was greater. In conclusion, the micronutrient status of patients with active pulmonary TB was poor compared with healthy subjects. Low concentrations of haemoglobin and of serum retinol and zinc were more pronounced in malnourished TB patients.

Introduction

India is tuberculosis burden country of the world. Tuberculosis is a major barrier to social and economic development and it is one of the most important causes of death in developing countries. Vitamin A deficiency has been found to be associated with many infectious diseases. A high prevalence of vitamin A deficiency has been observed in patient with pulmonary tuberculosis, which is more pronounced in those co-infected with HIV and this indicates an association between vitamin A deficiency and tuberculosis [1].

Hanekom *et al* found a low plasma Vitamin A levels to be associated with more extensive or severe disease, and low levels of retinol binding protein, prealbumin and albumin [2]. They also found that a high dose of vitamin A supplementation had no effect on the out come of disease. Ramachandran *et. al*, also found a lower serum vitamin A level in patient with pulmonary tuberculosis [3]. Koyanagi *et al*, found a lower serum concentration of retinol and zinc in patients with pulmonary tuberculosis as compared with healthy volunteers [4].

These studies have shown that in developing countries patients with tuberculosis have low serum Vitamin A levels. This could be because patients with Vitamin A deficiency have an increased risk of developing tuberculosis or because of development of active tuberculosis which may decrease the plasma Vitamin A levels Getz *et al,* [5].

Karyadi *et. al,* in their study in Indonesia observed that the Vitamin A and Zinc supplementation improves the effect of tuberculosis medication which is seen usually after 2 months of anti-tubercular treatment and results in earlier sputum smear conversion [6]. Vitamin A and zinc supplements given together with anti-tubercular drugs would increase the efficacy of the anti- tubercular treatment. As there is no evidence available on this issue from India, the present study is planned to investigate the micronutrient status.

Subjects and Methods

Subjects

Cases were out-patients with untreated active pulmonary TB admitted to the Department of Pulmonary Medicine, CSM Medical University UP, Lucknow, which is a tertiary care hospital located in north India. Controls were healthy subjects with no history of pulmonary TB, matched with cases for sex and age, and selected randomly from nonfamily neighbours of the patients. One person was selected at random as a control from the list of 3–7 persons proposed. Selection of cases was based on the following criteria: age 18–55 y; 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.

Study design

The study was designed as a case-control study. The sample size was based on the ability to determine a difference with $\alpha = 0.05$ and $1\text{-}\beta = 0.95$ using a one-tailed test for concentrations of serum retinol and zinc and of blood haemoglobin. Because serum zinc concentration was the variable requiring the largest sample size, we calculated that with a sample size of 35 in each group, a between-group difference of 0.46 µmol/L in Zn could be detected [7]. We recruited 43 subjects for each group because we assumed that 25% of patients might not meet the inclusion criteria.

Data collection

Potential cases and controls were interviewed using structured questionnaire requesting information related to the inclusion and exclusion criteria. Those apparently eligible were then screened clinically including a chest X-ray. All patients had evidence based on a chest X-ray of lung infiltration indicating active TB at the time of data collection. From those with evidence of TB, three specimens of early morning sputum were examined by direct microscopy after Ziehl-Nielsen staining and specimens were cultured in LJ medium. Subjects were weighed without shoes using an electronic platform model weighing scale (SECA 770 alpha; SECA, Hamburg, Germany).

Blood samples (5 ml) were collected from fasting subjects via vein puncture to determine haemoglobin, white blood cell count, erythrocyte sedimentation rate (ESR), serum albumin, serum retinol and zinc concentration. All biochemical tests above were carried out on the same day. Haemoglobin concentration and white blood cells were measured directly using an automatic analyzer (Sysmex Microdilutor F-800, Kobe, Japan). ESR was determined directly using the Westergreen technique [8]. Albumin was determined by the bromcresol green method [9]. Serum retinol was measured using RBP4 (human) ELISA Kit (Cat. No. AG-45A-0011EK-KI01) and zinc concentration was measured using simple colorimetric method [10].

Ethical considerations

The study was 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.

Statistical analysis

A one-sample Kolmogorov-Smirnov test was used to check whether data were normally distributed. Mean and standard deviation (SD) are used for reporting normally distributed data, and median and 25th–75th percentiles are used for reporting non-normally distributed data. An independent sample t-test was used to assess the differences between patients and controls for normally distributed parameters, whereas differences in non-normally distributed parameters were tested using the Mann-Whitney test. A multiple stepwise regression analysis was performed to predict concentrations of serum retinol and zinc by using age, sex, body weight, presence of pulmonary cavity, white blood cell count, ESR and serum albumin as independent variables. Differences in prevalence were tested with a chi-square test. The SPSS software was used for all statistical analyses and a P-value 0.05 was considered significant.

Results

Total 43 pulmonary tuberculosis (27 men & 16 women) age 18 to 55 enrolled for study. All the patients have fever, cough, haemoptysis, chest pain and loss of appetite. Of these cases 59% patients had three sputum smear positive and 41% patients had two sputum smear positive. Concentration of haemoglobin, serum albumin, serum retinol and serum zinc was significantly lower in pulmonary tuberculosis patients rather than in control. Erythrocyte sedimentation rate and WBC count was higher in pulmonary tuberculosis patients rather in control (Table 1).

Table 1. *Biochemical variables in pulmonary tuberculosis patients and healthy controls*
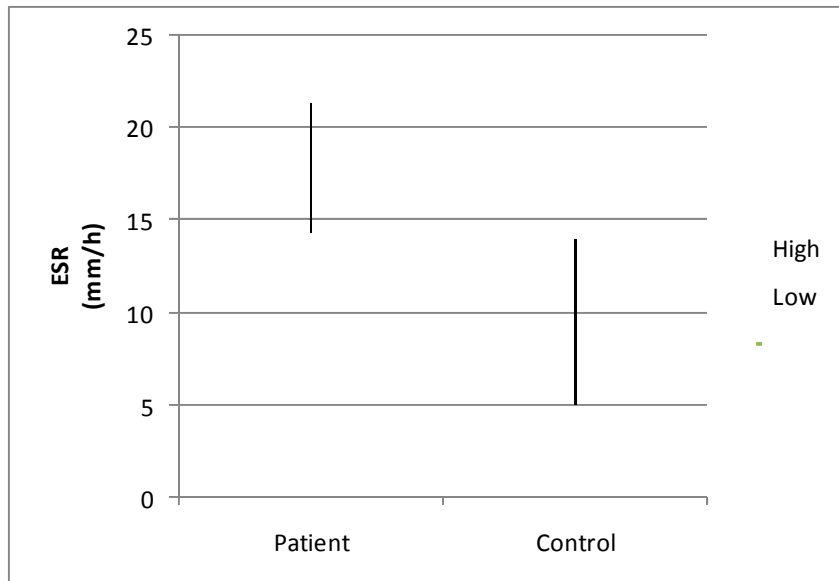
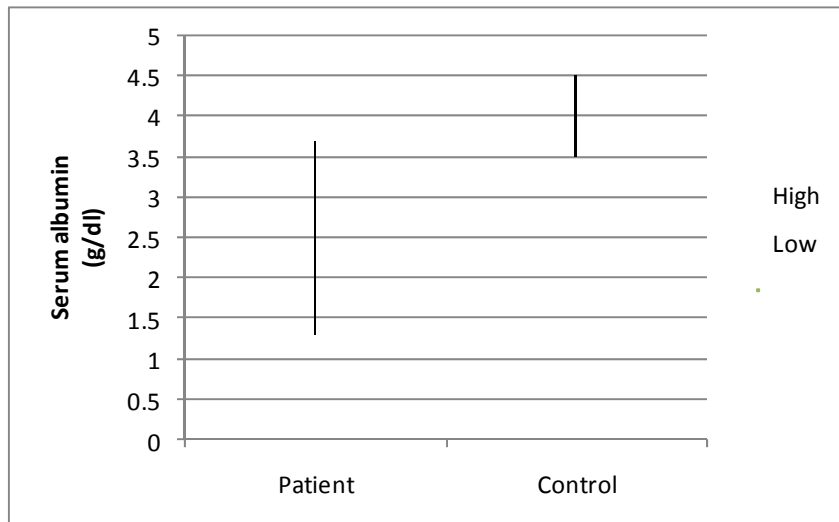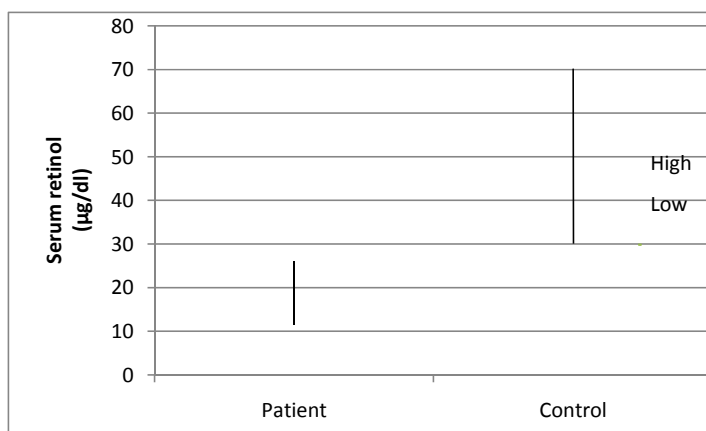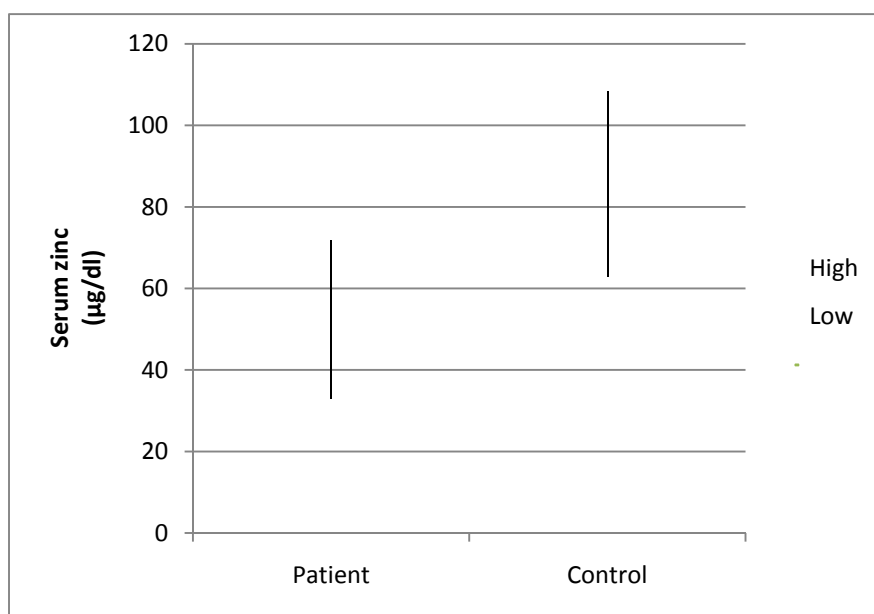| Biochemical variables	Pulmonary tuberculosis patients	Controls
Haemoglobin (g/dl)	10.8±2.7	14.75±1.75
WBC count (cells/mm^3)	10100±2450	7550±2250
ESR (mm/h)	17.8±3.5	9.5±4.5
Serum albumin(g/dl)	2.5±1.2	4.0±0.5
Serum retinol (µg/dl)	18.75±7.3	50.15±20.05
Serum zinc (µg/dl)	52.5±19.5	85.6±22.8
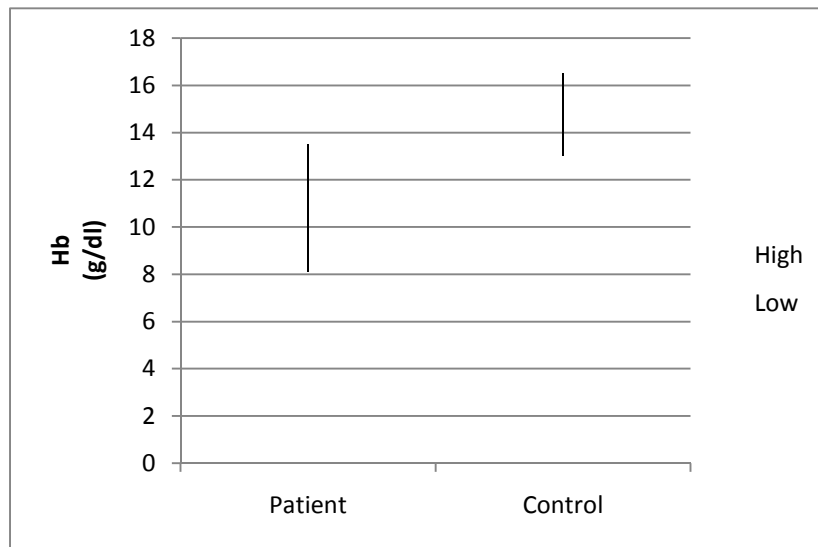
(A)
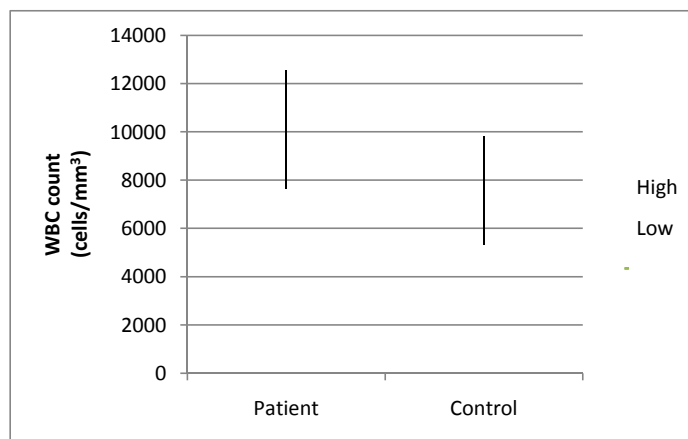
(B)

(C)

(D)

(E)

(F)

Figure 1. Distribution of biochemical variables in active tuberculosis patients and healthy controls. (A) Blood haemoglobin concentration; (B) WBC count; (C) ESR; (D) Serum albumin; (E) Serum retinol; (F) Serum zinc range; for all other measurements, the stock represent mean ± SD. Significance of differences was tested with the independent sample t test.

Discussion

In this study we demonstrated that, concentrations of selected micronutrients tested in TB patients were significantly lower than in controls. Low concentrations of haemoglobin and serum retinol and zinc in malnourished patients were more pronounced than in healthy controls and well-nourished patients. Furthermore, the prevalence of low concentrations of vitamin A and zinc was higher in patients than in controls. Low concentrations of serum retinol can be due to a number of factors, including reduced intake or reduced absorption of fat. In addition, the infection itself can compromise vitamin A status in a number of ways. It can increase urinary excretion of vitamin A as has been shown in patients with fever, e.g., due to pneumonia and shigellosis [11, 12]. Finally, low serum retinol levels can also result from increased utilization of retinol by tissues [13]. It is likely that a combination of mechanisms is operative in TB patients.

TB patients had significantly lower weight and serum albumin concentration than healthy controls. As a result, serum albumin concentration in malnourished patients was lower than that in well-nourished healthy controls, well-nourished patients and malnourished healthy controls. The poorer nutritional status of patients with pulmonary TB may be due to anorexia [14], impaired absorption of nutrients or increased catabolism. On the other hand, patients and controls may have similar food habits and food intakes because their socioeconomic background and living conditions are similar. Thus, infectious disease such as TB may led to impaired absorption and increased rates of metabolism [15, 16]. The disease induced production of cytokines such as interleukin-6 and tumour necrosis factor-a may induce fever, hepatic synthesis of acute phase reactant proteins, inhibit production of serum albumin and cause dramatic shifts in plasma concentration of certain essential micronutrients [17].

Conclusion

This study shows that the nutritional status of patients with active pulmonary TB was poor compared with healthy controls. The prevalence of low concentrations of serum retinol and zinc was significantly higher in patients than in controls. The low concentrations of haemoglobin and serum retinol and zinc were more pronounced in malnourished TB patients. Further studies are required to es-

tablish the role of these low concentrations in host defence against TB.

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