Original Research Article

Study of vitamins in pulmonary Tuberculosis

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ABSTRACT

Background: In recent years, progress in Tuberculosis (TB) control and eradication has been threatened by the emergence of drug resistant strains of Mycobacterium tuberculosis (MTB). Several studies describe micronutrient malnutrition in TB. This study focuses on the effect of drug resistance and disease progression on levels of various vitamins.

Methods: The case control study comprised of 50 normal healthy human volunteers (Control), 50 newly diagnosed TB patients (CAT I) and 50 TB patients showing multi drug resistance (MDR). Recruited subjects were of both genders in age group of 18-60 years and from different socioeconomic status. Blood serum samples analysed for levels of Vitamin A, Vitamin D, Vitamin E, Vitamin C, Vitamin B12 and Folic acid by using Spectrophotometer, ELISA and Chemiluminescence instruments. Statistical evaluation was done for correlation among variables.

Results: The levels of vitamins in TB patients were significantly reduced when compared with controls. Also, MDR-TB patients showed severe malnourished state in comparison to those who were newly diagnosed for the disease.

Conclusions: There is a need to provide vitamin supplements in proper sufficient dosage with anti-TB drugs during treatment, which will help fight against the disease and ensure rapid recovery of the patients.

Keywords: Folic acid, Tuberculosis, Vitamin

INTRODUCTION

Tuberculosis (TB) continues to be a major public health challenge globally and now ranks alongside HIV as a leading cause of death worldwide. The World Health Organization reported 10.4 million incident tuberculosis cases and 1.8 million associated deaths in 2015, the vast majority occurring in low- and middle-income countries. In recent years, progress in TB control and eradication

has been threatened by the emergence of drug resistant strains of Mycobacterium tuberculosis (MTB). Multidrug-resistant tuberculosis (MDR-TB) is defined as a disease caused by MTB strain resistant to both rifampicin and isoniazid, which are frontline antituberculosis drugs presently used in chemotherapy. Multiple lines of evidence suggest that the association between socioeconomic status and tuberculosis may be
mediated through nutritional status. Vitamins form an important part of the dietary supplements of the human diet. They have to be taken exogenously either because humans cannot synthesize vitamins or because, if produced, the concentration is not sufficient. Several studies describe micronutrient malnutrition in pulmonary TB and have suggested that patients with TB are at high risk of deficiency of vitamins A, C, D and E as well as zinc.

Vitamin A (Vit A) has been shown to inhibit multiplication of MTB in macrophages in vitro and plays a vital role in lymphocyte proliferation and maintaining the function of epithelial tissue. Vitamin A deficiency is a common feature of pulmonary tuberculosis which increases bacterial adherence to respiratory epithelial cells. Vit A is excreted in the urine in patients with fever, and this has since been confirmed in subjects with acute infections, including pneumonia, thus increasing the requirement for vit A by its increased rate of excretion and metabolism. Immunoprotective role of vit A by modifying immune responses against human tuberculosis has been shown in many studies. 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.

Antioxidants in different forms scavenge free radicals and suppress the actions of ROS, protecting the host from tissue inflammation. Vitamins C (Vit C) i.e. ascorbic acid and Vitamin E (Vit E) i.e. alpha tocopherol, act as potent and probably the most important hydrophilic and lipophilic antioxidants respectively. Vit C is an essential dietary nutrient in humans that is involved in a wide range of biological processes including collagen biosynthesis, as a cofactor of several enzymes, facilitating iron transport and as a physiological antioxidant. Vit C scavenges superoxide radical, hydrogen peroxide and thiol radicals and is a potent quencher of singlet oxygen while Vitamin E converts superoxide radical, hydroxyl and lipid peroxy radicals to less reactive forms. Vit E acts as a mobilizable antioxidant being released from tissue stores and diverted to lungs of TB patients during oxidative stress and radical mediated pulmonary fibrosis. This leads to the formation of tocopherol radical which is converted to tocopherol by ascorbic acid, thereby conferring Vit E sparing ability on Vit C.

The classic function of Vitamin D (Vit D) is to maintain hemostatic calcium and phosphate metabolism which helps maintain bone mineralization through osteoblast regulation. Non-classical functions of vitamin D include suppressing cancer cell growth, regulating cell apoptosis by controlling cell hyperplasia, and modulating the immune system. Calcitriol (1,25-dihydroxycholecalciferol), the major active form of Vit D, has shown in vitro activity against MTB. Calcitriol induces the antimycobacterial peptide cathelicidin, which is involved as the first line of defense in the prevention of infections caused by mycobacteria. Calcitriol modulates the host response to mycobacterial infection by induction of reactive nitrogen, and oxygen intermediates suppression of matrix metalloproteinase enzymes implicated in the pathogenesis of pulmonary cavitation. Vitamin D also has immunomodulatory effects on various cells of the immune system, such as inflammatory dendritic cells, T cells, B cells, plasma cells, macrophages, and antigen-presenting cells (APC), as well as inhibitory effects on the secretion of interleukins.

Vitamin B12 refers to any of the biologically active forms related to cyanocobalamin. Vitamin B12-dependent enzymes function in core biochemical pathways in Mycobacterium tuberculosis. Though the direct association between tuberculosis and vitamin-B deficiency is not known, but vitamin-B supplementation is well recommended in order to avert several neurological complications in tuberculosis patients.

METHODS

The Case Control study comprised of 50 normal healthy human volunteers (Control), 50 newly diagnosed TB patients (CAT I) and 50 TB patients treated with dots showing multidrug-resistance (MDR). Recruited subjects were of both genders in age group of 18-60 years and from different socioeconomic status. Patients admitted and those visiting Out Patient Department at Sir J.J. group of Hospitals, Mumbai were included in study. Subjects not willing to participate in the study and HIV positive were excluded. Blood serum samples were collected, stored at -80°C and analysed for Vitamin A, Vitamin E, Vitamin C, were estimated by chemical method using Spectrophotometer JASCO V670, Vitamin D by ELISA ERBA while Vitamin B12 and Folic acid by Chemiluminescence Immulite 1000. Ethical Clearance approval was taken from the institutional ethics committee of Grant Government Medical College and Sir J. J. Group of Hospitals, Mumbai and informed consents along with details of patients were taken prior to the study. Statistical evaluation was done by ANOVA test using Minitab 17 software. The other variables were statistically analysed with Pearson correlation. Statistical significance was accepted at P <0.05 and data were interpreted using 95% confidence interval.

RESULTS

All the three groups of the study include equal number of males and females (n=25). Also, the age distribution of the subjects in these groups is represented in Mean±Standard Deviation as shown in Table 1.

Table 2 shows that vitamin A, D, E, C, B12 and folic acid are significantly low in MDR TB group as compared with Category I and control groups.

In comparison to control group, the levels of fat soluble vitamins A, D and E in Cat I group show a decrease of
0.85, 0.52 and 0.49 folds respectively, while the levels of water soluble vitamins C, B12 and folic acid in Cat I group show a decrease of 0.72, 0.67 and 0.83 folds respectively. In comparison of MDR group with control, the MDR group levels of fat-soluble vitamins A, D and E show a significant 0.56, 0.06 and 0.29 fold decrease respectively. Simultaneously, levels of water-soluble vitamins C, B12 and folic acid show a significant decrease of 0.49, 0.43 and 0.92 folds respectively.

**Table 1: Age and Sex Wise Distribution in Control and Pulmonary Tuberculosis.**

| Group                               | Age (Mean±SD) | Sex |
|-------------------------------------|---------------|-----|
| Control (n=50)                      | 37.06±10.01   | 25  |
| Pulmonary Tuberculosis              |               |     |
| Category I (n=50)                   | 32.3±9.34     | 25  |
| Multi drug resistant (n=50)         | 33.66±11.05   | 25  |

**Table 2: Levels of Vitamin A, D, E, C, B12 and Folic acid in Control and Pulmonary TB.**

| Group               | Vit A µg/L | Vit D mg/ml | Vit E µg/L | Vit C µg/L | Vit B12 pg/ml | Folic acid ng/ml |
|---------------------|------------|-------------|------------|------------|----------------|-----------------|
| Control (n=50)      | 1.24±0.073 | 47.33±1.74  | 12.37±0.19 | 11.48±1.73 | 555.04±141.54  | 12.11±3.42      |
| CAT I (n=50)        | 1.06±0.06  | 24.51±2.54  | 6.15±0.19  | 8.3±0.46   | 370.76±119.12  | 10.14±3.06      |
| MDR (n=50)          | 0.70±0.03  | 2.66±1.15   | 3.70±0.52  | 5.71±0.42  | 239.48±50.79   | 11.24±14.38     |

The observations clearly interpret the severe deficiency of vitamins in MDR group than in Category I (Table 2). Levels of both water soluble and fat-soluble vitamins show positive correlations with each other which are shown in Table No. 3A, 3B, 3C, 3D, 3E and 3F. Table 3A,3B,3C,3D,3E In Table No. 3A, we see a significant positive correlation of Vitamin A in controls with Vitamin D and B12 in the MDR group. Table 3A, Graph 1A and 1B Also, vitamin D in control shows a highly significant correlation with Vitamin D and A in MDR group as shown in Table 3B. Table 3B, Graph 2A and 2B.

**Table 3A: Correlations of Vitamin A with A, D, E, C, B12 and Folic acid in Control and Pulmonary TB groups.**

| Sr. No. | r-value | p-value | r-value | p-value | r-value | p-value |
|---------|---------|---------|---------|---------|---------|---------|
|         | Control / Category I | Control / MDR | Category I / MDR |
| 1       | Vit A / Vit A | 0.170 | 0.237 | 0.273 | 0.055 | 0.260 | 0.068 |
| 2       | Vit A / Vit D | 0.417 | 0.003 | 0.459 | 0.001 | 0.270 | 0.058 |
| 3       | Vit A / Vit E | 0.214 | 0.136 | 0.150 | 0.299 | 0.001 | 0.996 |
| 4       | Vit A / Vit C | 0.062 | 0.671 | 0.163 | 0.258 | 0.002 | 0.988 |
| 5       | Vit A / Vit B12 | -0.077 | 0.597 | 0.393 | 0.005 | -0.026 | 0.858 |
| 6       | Vit A / Folic acid | 0.082 | 0.573 | -0.159 | 0.269 | -0.184 | 0.200 |

*p <0.05 – Significant **p <0.01- Highly significant

In Table No. 3A, we see a significant positive correlation of Vitamin A in controls with Vitamin D and B12 in the MDR group. Table 3A, Graph 1A and 1B Also, vitamin D in control shows a highly significant correlation with Vitamin D and A in MDR group as shown in Table 3B. Table 3B, Graph 2A and 2B.

**Table 3B: Correlations of Vitamin C with A, D, E, C, B12 and Folic acid in Control and Pulmonary TB groups.**

In contrast to these two fat soluble vitamins, vitamin E levels are insignificantly correlated as displayed in Table No. 3C. Table 3C When we observe the correlations of water soluble vitamins in Table No. 3D, 3E and 3F, we note that Vitamin C in controls show significant correlation with levels of Vitamin C and B12 in MDR. Graph 3A and 3B While, the vitamin B12 and folic acid levels control group are significantly correlated with vitamin A and D levels in MDR group. Table 3D, 3E, 3F, Graph 4A, 4B, 5A and 5B. The distribution of subjects according to their socioeconomic status as shown in Table 4 states that the disease prevalence is higher in low income group which specifies the poor diet (nutrition) conditions (Table 4).

**Table 3C: Correlations of Vitamin E with A, D, E, C, B12 and Folic acid in Control and Pulmonary TB groups.**

| Sr. No. | r-value | p-value | r-value | p-value | r-value | p-value |
|---------|---------|---------|---------|---------|---------|---------|
|         | Control / Category I | Control / MDR | Category I / MDR |
| 1       | Vit A / Vit A | 0.170 | 0.237 | 0.273 | 0.055 | 0.260 | 0.068 |
| 2       | Vit A / Vit D | 0.417 | 0.003 | 0.459 | 0.001 | 0.270 | 0.058 |
| 3       | Vit A / Vit E | 0.214 | 0.136 | 0.150 | 0.299 | 0.001 | 0.996 |
| 4       | Vit A / Vit C | 0.062 | 0.671 | 0.163 | 0.258 | 0.002 | 0.988 |
| 5       | Vit A / Vit B12 | -0.077 | 0.597 | 0.393 | 0.005 | -0.026 | 0.858 |
| 6       | Vit A / Folic acid | 0.082 | 0.573 | -0.159 | 0.269 | -0.184 | 0.200 |

*p <0.05 – Significant **p <0.01- Highly significant

In Table No. 3A, we see a significant positive correlation of Vitamin A in controls with Vitamin D and B12 in the MDR group. Table 3A, Graph 1A and 1B Also, vitamin D in control shows a highly significant correlation with Vitamin D and A in MDR group as shown in Table 3B. Table 3B, Graph 2A and 2B.
**Table 3B: Correlations of Vitamin D with A, D, E, C, B\(_{12}\) and Folic acid in Control and Pulmonary TB groups.**

| Sr. No. | r-value | p-Value | r-value | p-Value | r-value | p-Value |
|---------|---------|---------|---------|---------|---------|---------|
|         | Control / Category I | Control / MDR | Category I / MDR |
| 1       | Vit D/Vit A | Vit D/Vit A | Vit D/Vit A |
|         | 0.241     | 0.092    | 0.568    | 0.000    | 0.592    | 0.000    |
| 2       | Vit D/Vit D | Vit D/Vit D | Vit D/Vit D |
|         | 0.964     | 0.000    | 0.983    | 0.000    | 0.970    | 0.000    |
| 3       | Vit D/Vit E | Vit D/Vit E | Vit D/Vit E |
|         | 0.074     | 0.609    | -0.000   | 0.998    | 0.062    | 0.668    |
| 4       | Vit D/Vit C | Vit D/Vit C | Vit D/Vit C |
|         | 0.218     | 0.129    | 0.200    | 0.164    | 0.177    | 0.219    |
| 5       | Vit D/Vit B\(_{12}\) | Vit D/Vit B\(_{12}\) | Vit D/Vit B\(_{12}\) |
|         | -0.518    | 0.000    | 0.290    | 0.041    | 0.334    | 0.018    |
| 6       | Vit D/Folic acid | Vit D/Folic acid | Vit D/Folic acid |
|         | 0.402     | 0.004    | -0.167   | 0.248    | -0.178   | 0.217    |

*\(p < 0.05\) – Significant ** \(p < 0.01\) - Highly significant

**Table 3C: Correlations of Vitamin E with A, D, E, C, B\(_{12}\) and Folic acid in Control and Pulmonary TB groups.**

| Sr. No. | r-value | p-Value | r-value | p-Value | r-value | p-Value |
|---------|---------|---------|---------|---------|---------|---------|
|         | Control / Category I | Control / MDR | Category I / MDR |
| 1       | Vit E/Vit A | Vit E/Vit A | Vit E/Vit A |
|         | -0.031    | 0.829    | -0.019   | 0.898    | 0.071    | 0.626    |
| 2       | Vit E/Vit D | Vit E/Vit D | Vit E/Vit D |
|         | -0.255    | 0.074    | -0.270   | 0.058    | 0.088    | 0.543    |
| 3       | Vit E/Vit E | Vit E/Vit E | Vit E/Vit E |
|         | -0.117    | 0.419    | 0.025    | 0.862    | 0.128    | 0.375    |
| 4       | Vit E/Vit C | Vit E/Vit C | Vit E/Vit C |
|         | -0.075    | 0.606    | 0.110    | 0.447    | -0.116   | 0.422    |
| 5       | Vit E/Vit B\(_{12}\) | Vit E/Vit B\(_{12}\) | Vit E/Vit B\(_{12}\) |
|         | 0.304     | 0.032    | -0.075   | 0.605    | -0.103   | 0.477    |
| 6       | Vit E/Folic acid | Vit E/Folic acid | Vit E/Folic acid |
|         | 0.094     | 0.518    | -0.206   | 0.152    | -0.110   | 0.448    |

*\(p < 0.05\) – Significant ** \(p < 0.01\) - Highly significant

**Table 3D: Correlations of Vitamin C with A, D, E, C, B\(_{12}\) and Folic acid in Control and Pulmonary TB groups.**

| Sr. No. | r-value | P-Value | r-value | P-Value | r-value | P-Value |
|---------|---------|---------|---------|---------|---------|---------|
|         | Control / Category I | Control / MDR | Category I / MDR |
| 1       | Vit C/Vit A | Vit C/Vit A | Vit C/Vit A |
|         | -0.006    | 0.967    | 0.061    | 0.672    | -0.180   | 0.210    |
| 2       | Vit C/Vit D | Vit C/Vit D | Vit C/Vit D |
|         | 0.095     | 0.513    | 0.140    | 0.331    | 0.220    | 0.124    |
| 3       | Vit C/Vit E | Vit C/Vit E | Vit C/Vit E |
|         | -0.113    | 0.434    | 0.038    | 0.796    | -0.141   | 0.330    |
| 4       | Vit C/Vit C | Vit C/Vit C | Vit C/Vit C |
|         | -0.115    | 0.428    | 0.490    | 0.000    | 0.021    | 0.885    |
| 5       | Vit C/Vit B\(_{12}\) | Vit C/Vit B\(_{12}\) | Vit C/Vit B\(_{12}\) |
|         | 0.052     | 0.718    | 0.340    | 0.016    | -0.012   | 0.935    |
| 6       | Vit C/Folic acid | Vit C/Folic acid | Vit C/Folic acid |
|         | -0.136    | 0.345    | -0.210   | 0.142    | 0.021    | 0.885    |

*\(p < 0.05\) – Significant ** \(p < 0.01\) - Highly significant
Table 3E: Correlations of Vitamin B<sub>12</sub> with A, D, E, C, B<sub>12</sub> and Folic acid in Control and Pulmonary TB groups.

| Sr. No. | Control / Category I | Control / MDR | Category I / MDR |
|---------|----------------------|---------------|------------------|
| 1       | Vit B<sub>12</sub>/Vit A | Vit B<sub>12</sub>/Vit A | Vit B<sub>12</sub>/Vit A |
|         | -0.129               | -0.497        | -0.316           |
| 2       | Vit B<sub>12</sub>/Vit D | Vit B<sub>12</sub>/Vit D | Vit B<sub>12</sub>/Vit D |
|         | -0.713               | -0.693        | -0.456           |
| 3       | Vit B<sub>12</sub>/Vit E | Vit B<sub>12</sub>/Vit E | Vit B<sub>12</sub>/Vit E |
|         | -0.127               | -0.032        | -0.109           |
| 4       | Vit B<sub>12</sub>/Vit C | Vit B<sub>12</sub>/Vit C | Vit B<sub>12</sub>/Vit C |
|         | 0.077                | 0.047         | 0.744            |
| 5       | Vit B<sub>12</sub>/B<sub>12</sub> | Vit B<sub>12</sub>/B<sub>12</sub> | Vit B<sub>12</sub>/B<sub>12</sub> |
|         | 0.608                | 0.032         | 0.823            |
| 6       | Vit B<sub>12</sub>/Folic acid | Vit B<sub>12</sub>/Folic acid | Vit B<sub>12</sub>/Folic acid |
|         | -0.317               | 0.025         | 0.973            |

*p <0.05 - Significant  ** p <0.01 - Highly significant

Table 3F: Correlations of Folic acid with A, D, E, C, B<sub>12</sub> and Folic acid in Control and Pulmonary TB groups.

| Sr. No. | Control / Category I | Control / MDR | Category I / MDR |
|---------|----------------------|---------------|------------------|
| 1       | Folic acid/Vit A     | Folic acid/Vit A | Folic acid/Vit A |
|         | 0.541                | 0.563         | 0.600            |
| 2       | Folic acid/Vit D     | Folic acid/Vit D | Folic acid/Vit D |
|         | 0.357                | 0.295         | 0.386            |
| 3       | Folic acid/Vit E     | Folic acid/Vit E | Folic acid/Vit E |
|         | 0.054                | 0.023         | 0.026            |
| 4       | Folic acid/Vit C     | Folic acid/Vit C | Folic acid/Vit C |
|         | -0.189               | -0.023        | -0.044           |
| 5       | Folic acid/B<sub>12</sub> | Folic acid/B<sub>12</sub> | Folic acid/B<sub>12</sub> |
|         | -0.317               | -0.115        | -0.046           |
| 6       | Folic acid/Folic acid | Folic acid/Folic acid | Folic acid/Folic acid |
|         | 0.959                | 0.195         | -0.266           |

*p <0.05-Significant  ** p <0.01-Highly significant

Figure 1A: Correlation of Vitamin A in control group with Vitamin D in MDR group.

Figure 1B: Correlation of Vitamin A in control group with Vitamin B<sub>12</sub> in MDR group.
Figure 2A: Correlation of Vitamin D in control group with Vitamin D in MDR group.

Figure 2B: Correlation of Vitamin D in control group with Vitamin A in MDR group.

Figure 3A: Correlation of Vitamin C in control group with Vitamin C in MDR group.

Figure 3B: Correlation of Vitamin C in control group with Vitamin B12 in MDR group.

Figure 4A: Correlation of Vitamin B12 in control group with Vitamin A in MDR group.

Figure 4B: Correlation of Vitamin B12 in control group with Vitamin D in MDR group.
DISCUSSION

Vitamins are known to be associated with immunity and nutrition. Moreover, vitamin deficiency can affect host immunity to various infectious diseases, including tuberculosis. Vitamin deficiencies are common in patients with tuberculosis.11,12 Omowunmi Aibana et al, have found that vitamin A levels strongly predict progression to tuberculosis disease. This relationship remained strong after adjustment for socioeconomic status, BMI, and other comorbid conditions that might be associated with increased TB risk and lower vitamin A levels.3 In our study, similar results have been seen in Table 2 showing lower serum levels of vitamins in TB patients when compared to healthy controls. Also, Table 4 shows that the prevalence of TB is seen in subjects belonging to the low socioeconomic status, which clearly indicates the state of malnutrition linked with disease progression.

V.F. Edem et al, have reported in their previous study that micronutrient malnutrition is observed in patients with drug-sensitive TB at diagnosis and throughout the period of anti-TB chemotherapy. Because micronutrient deficiency has been reported to impair resistance to infection, lead to active TB disease and poor outcome of anti-TB chemotherapy.2 Thus in our study we have included the group of TB patients undergoing drug resistance and the levels of vitamin deficiencies in them. This finding is similar to our study in which MDR-TB patients on standard anti-TB treatment without micronutrient supplementation show reduced vitamin levels as shown in Table 2. Active tuberculosis disease can lead to profound weight loss and metabolic disturbances, and the micronutrient deficiencies observed in patients often improve or resolve with successful tuberculosis treatment.3

In our study, we have studied various correlations among the vitamin A, C, D, E, B_{12} and folic acid levels at the preliminary and drug resistance stages of TB patients in Tables 3A, 3B, 3C, 3D, 3E and 3F. The levels are also compared with the normal levels of vitamins found in the healthy control group. It was observed that alone anti-TB treatment was not enough to improve the health status of TB patients with respect to the nutrient and vitamin status which had detrimental effects on the immunity to fight against disease. Thus, supplementation of vitamins along with anti-TB treatment would be an option to resolve the situation.

However, V.F. Edem et al, also found that following the commencement of anti-TB treatment combined with micronutrient supplementation, reduced plasma concentrations of iron and vitamins A, C and E in MDR-TB patients at two months of chemotherapy compared with controls were observed.2

While, Vesna Skodric-Trifunovic et al, have shown that despite supplementation of TB chemotherapy with vitamins, the levels of vitamins C and E were not raised as expected, therefore, there is need to determine effective dosage of vitamins for supplementation in MDR-TB patients.8

CONCLUSION

Tuberculosis is a disease which shows detrimental effect on immunity and metabolism of the body. In order to fight against the bacteria, we need to keep check of the nutritional status of the patient for fat soluble and water soluble vitamins along with the anti-TB treatment. Thus, vitamin supplements in proper sufficient dosage with anti-TB drugs must be provided which will help fight against the disease and ensure rapid recovery of the patient.
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Conflict of interest: None declared

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