Evaluation of antioxidant vitamins (A and C) in patients with pulmonary tuberculosis

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(Received 6/3/2013, Accepted 9/6/2013)

Abstract
Background: The antioxidant vitamins such as vitamin A and vitamin C functions as the first line antioxidant defense against free oxygen radicals present primarily in the plasma. Examination of antioxidants in patients with tuberculosis may identify deficiencies that predispose to severe oxidant injury. However, our knowledge of the antioxidant profile and its relation to tuberculosis patients is scarce, particularly in developing countries.

Aim: This study aims to estimate some vitamins antioxidant, Vitamin A and C in both male and female patients with pulmonary tuberculosis.

Patients and Methods: The present study included 26 patients (14 males and 12 females) affected with pulmonary tuberculosis. Their ages from 29-72 years in male patients and in female patients from 22-65 years. Twenty four healthy controls(12 males and 12 females) their aged from 23 – 67 years in male and in female from 23 - 60 were also included in this study . Study was under taken to evaluate the level of antioxidants like vitamin A and vitamin C in patients with pulmonary tuberculosis.

Results: There is a statistically significant changes in vitamins antioxidant in pulmonary tuberculosis patients in compared with healthy control. These results of serum vitamins A and C are significantly ( p < 0.001 ) decrease in both male and female pulmonary tuberculosis patients in comparison with healthy control.

Conclusion: The levels of non-enzymic antioxidants such as Vitamin A, and vitamin C in serum were significantly depleted in the pulmonary tuberculosis infected subjects when compared with control. There is a strong association between vitamin
A and vitamin C deficiency and malnutrition in these patients, as well as a correlation between several indexes of malnutrition and low concentrations of antioxidants may suggest the involvement of low food intake and nutrient malabsorption in the generation of oxidative stress in pulmonary tuberculosis patients.

**Introduction**

Tuberculosis or TB is an infectious disease caused by acid-fast bacillus, Mycobacterium tuberculosis (MTB) in humans\(^1\). Tuberculosis most commonly affects the lungs, where it is called pulmonary tuberculosis (PTB). In lungs, it forms a localized infection after inhalation of bacilli\(^2\). According to the World Health Organization (WHO), there were 9.2 million incident cases and 1.7 million deaths from tuberculosis (TB) worldwide in 2006. A problem that has already been reported in over 100 countries in the world\(^3\). Tuberculous bacilli induce oxidative substances such as reactive oxygen species (ROS) derived from free radicals, which in turn can promote tissue injury and inflammation. ROS are highly toxic to all types of cells, but especially to lipids (fat cells) causing peroxidation. This results in damage to cell membrane which promotes lung fibrosis and dysfunction in pulmonary TB\(^4\). Pulmonary tuberculosis, an infectious disease is one of the world’s leading causes of death\(^5\). TB patients have been reported to have decreased concentrations of antioxidants, enhanced generation of ROS\(^6\). In developing countries, infection and malnutrition form a vicious cycle,\(^7\) hence TB is often said to be the disease of the malnourished. Lipid peroxidation, a general mechanism of tissue damage by free radicals, is known to be responsible for cell damage and may induce many pathological events\(^8\). There is limited knowledge available for antioxidant status. Oxidative stress and resultant lipid peroxidation may lead to increased levels of ROS, which may be responsible for pulmonary damage and lung fibrosis during tuberculosis\(^9\). TB patients are unable to produce sufficient amount of antioxidants to cope up with the increased oxidative stress in them. Antioxidants supplementation along with antitubercular therapy may prove beneficial and may help in fast recovery in the management of these cases\(^10\). It was observed that the free radicals activity was increased and total antioxidant status (enzymatic and non-enzymatic) was low in all TB cases, irrespective of treatment status, indicating that there is an oxidative stress. The antioxidants were nearly completely utilized to scavenge the superoxide free radicals\(^11\). Although the immune system responds quickly to the presence of *M. tuberculosis* bacilli, this pathogen has developed several ways to avoid being killed by macrophages and, instead, to reproduce inside them\(^12\). TB patients have been reported to have increased levels of lipid peroxidation product as a consequence of impaired activity of scavenging enzymes\(^13\). Superoxide, a free radical is produced during cellular metabolic reactions. An antioxidant enzyme, changes superoxide anion into hydrogen peroxide and oxygen\(^14\). Investigations show that vitamin supplementation can improve cell-mediated immunity efficacy which is presented by T cell and lymphocyte proliferations\(^15\). Vitamin A deficiency in TB adult patients has been detected\(^16\). Vitamin A Insufficiency increases bacterial penetration into respiratory epithelial cells\(^17\). In addition, the need for vitamin A is increased during infection due to increased metabolism\(^15\). Also, vitamin A and zinc supplementations improve nutritional state and therapeutic effects of anti-TB drugs. It has been recently known that retinoic acid can prevent
mycobacterial proliferation in macrophages\(^{(19)}\). As a whole, the body has a complex defence system including vitamins and enzymes which have protective effects on free radicals directly or indirectly\(^{(20)}\). In patients diagnosed with pulmonary tuberculosis, lower antioxidant levels of vitamins C and E and higher lipid peroxidation product malondialdehyde with activated immune response as indicated by nitrite levels show marked oxidative stress in patients compared to control subjects\(^{(21)}\). Vitamin C’s antioxidant protection facilitates the faster uptake of triglyceride from the plasma and promotes its removal from circulation thereby decreasing the serum triglyceride concentration\(^{(22)}\).

The aim of the study
This study aims to evaluation of concentrations of circulating antioxidants in pulmonary tuberculosis because this will help the medical staff for proper management with less morbidity and mortality. So this study is designed to determine the serum vitamin A and serum vitamin C in both male and female patients.

Patients and Methods
The patients:-The present study included 26 patients (14 males and 12 females) affected with pulmonary tuberculosis, were assessed by specialist doctor on the basis of history, clinical examination, chest radiography and, direct smear sputum examination. This study was carried out over 4 months in the Al-Hilla Consultation Clinic for Chest and Pulmonary Diseases, Al-Hilla Primary Health Care District (TB Unit). The patients group aged from 29-72 years in male patients (45.35 ± 14.81 years) and in female patients from 22-65 years (38.16 ± 14.06 years). Patients with complications such as renal, endocrine or hepatic diseases, diabetes mellitus, obesity, viral and other bacterial infections extra pulmonary TB, non tuberculous pulmonary infection, pregnancy, were excluded from the study. Twenty four healthy controls (12 males and 12 females) their aged from 23 – 67 years in male (43.58 ± 15.54 years) and in female from 23 - 60 (37.41±11.7 years) were also included in this study.

The methods
Blood collection:-The collection of blood was performed in the Al-Hilla Consultation Clinic for Chest and Pulmonary Diseases, Al-Hilla Primary Health Care District (TB Unit) in Babylon. Collection was always performed at 9 a.m. by using venipuncture needles. The plain tubes, for blood to be used for preparing sera for subsequent biochemical tests. The blood is allowed to clot for 45 minutes, the clot shrinks and serum can be obtained by centrifugation and precautions were taken to avoid hemolysis. The serum samples were isolated in sterile test tubes using micropipette with sterile disposable tips. Each sample was labeled and given a serial number together with the patient name, the serum samples were frozen at 20°C for Biochemical analysis\(^{(23)}\).

Biochemical studies:-
1-Determination of serum vitamin A:- Vitamin A is determined by the reaction with trifluoroacetic acid (TFA) to give a transient blue color. Place 4 milliliter (ml) petroleum ether into a colorimeter and evaporate to dryness in a water bath at about 60°C, preferably with the aid of a stream of nitrogen or carbon dioxide. Dissolve the residue in exactly 0.5 ml
chloroform. Adjust the colorimeter to zero absorbance with a chloroform blank at 615 nanometer (nm). Add 3 ml TFA reagent quickly to the sample and read at once in the colorimeter. The color develops rapidly, reaching a maximum in 5-10 second, and then fades. Record the maximum reading. Make similar reading with a series of vitamin A standards by adding to a series of cuvets 0.1, 0.2, 0.3 and 0.4 ml of the diluted vitamin A standard (4 µg / ml) and diluting to 0.5 ml with chloroform. These standards contain 0.4 – 1.6 µg vitamin A and are treated in the same manner as the extract equivalent to 2 ml of serum. Thus they are equivalent to 20, 40, 60 and 80 µg /100ml vitamin A in the serum. Calculation: Thus

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\text{Absorbance of sample} = \frac{\text{Absorbance of standard}}{} \times \text{Concentration of standard} = \mu g / 100 ml \text{ vitamin A}
\]

2-Determination of serum vitamin C:- The dye, 2,6-dichlorophenol-indophenol is reduced to a colorless form by ascorbic acid. When excess dye is added to a solution containing ascorbic acid, the decrease in color, determined in a photometer, is a measure of the amount of ascorbic acid present. Since ascorbic acid is not stable in blood, plasma (oxalate is satisfactory as anticoagulant) is preferable to serum, since the former can be separated from the cells more rapidly. Ascorbic acid is stable in plasma for only about 30 minute at room temperature, so the analysis should be performed as soon as possible after the sample is obtained. Sulfhydryl compounds will interfere with the determination. The interference is only slight for plasma and may be neglected. Proteins are precipitated by metaphosphoric acid, which aids in the stabilization of the ascorbic acid. The protein-free filtrates are stable for several hours. Add metaphosphoric acid to plasma, mix well and centrifuge to obtain a clear supernatant. In separate cuvets set up the following: reagent blank; standard and the sample tubes. To each above tube add the working dye solution, mix and read after 30 second against a water blank at 520 nm. To each tube, then add a few crystals of ascorbic acid to completely decolorize and read again. For each tube, subtract the absorbance obtained after the addition of the excess ascorbic acid from that obtained initially. These differences are then recorded as the corrected absorbances for the reagent blank, standard and sample. And calculation as the following:

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\text{correspond absorbance of reagent blank} - \text{correspond absorbance of sample}
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\text{correspond absorbance of reagent blank} - \text{correspond absorbance of standard}
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\times 2 = mg / 100 ml \text{ ascorbic acid in sample}
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Statistical analysis: All values were expressed as means ± standard deviation (SD). The data were analyzed by using computerized SPSS program. Independent t-test was used to estimate differences between groups. The differences were considered significant when the probability (P) was less than 0.05 (P<0.05).
Results

1- Serum vitamin A:- The mean and standard deviation (SD) of serum vitamin A for males of pulmonary tuberculosis patients and healthy control are: 28.67 ± 6.44; 50.0 ± 10.89 microgram/deciliter (µ/dl) respectively. These results are significantly (p < 0.001) decrease in comparison with healthy control. The mean and standard deviation of serum vitamin A for female pulmonary tuberculosis patients and healthy control are 29.67 ± 7.86; 57.25 ± 9.26 µ/dl respectively. These results also show significant (p < 0.001) decrease in comparison with healthy controls (figure 1).

![Figure (1)](image1)

Figure (1) Changes in serum vitamin A in male and female patients with pulmonary tuberculosis * (p<0.001)

2-Serum vitamin C:- Figure 2 shows the results of serum vitamin C of pulmonary tuberculosis patients and healthy control. The mean and standard deviation of serum vitamin C for males of pulmonary tuberculosis patients and healthy control are: 0.76 ± 0.2; 1.308 ± 0.38 milligram/deciliter (mg/dl) respectively. These results are significantly (p < 0.001) decrease in comparison with healthy control. The mean and standard deviation of serum vitamin C for female pulmonary tuberculosis patients and healthy control are 0.591 ± 0.25; 1.13 ± 0.28 mg/dl respectively. These results also show significant (p < 0.001) decrease in comparison with healthy controls.

![Figure (2)](image2)

Figure (2) Changes in serum vitamin C in male and female patients with pulmonary tuberculosis.

*(p<0.001)
**Discussion**

Tuberculosis remains one of the top killers among infectious diseases. It is the most feared diseases in the world and spread from person to person via aerosols. It is a chronic granulomatous infectious disease caused by Mycobacterium tuberculosis; the disease affects almost all the organs, lungs being primary. In present study observed in figure 1 significant lowering of serum vitamin A levels in both male and female patients with pulmonary tuberculosis in comparison with healthy control. Vitamin A transport proteins such as pre-albumin and retinol binding protein. Vitamin A is required for normal epithelial structure and function and can cause stimulation of the immune response. Whether hypovitaminosis A play any role in the defective cellular immune response. The decrease concentration of serum vitamin A may result from the hypozincemia as zinc is required for vitamin A metabolism. Vitamin A enhances white blood cell function, enhances resistance to infection, and helps maintain membrane defenses to infection. MOHOD et al. showed there is a significant correlation between high oxidants concentration and low concentration of antioxidants, suggest increased utilization by ROS as an important contributing factor to the lower concentration of antioxidants in TB patients. These results are in agreement with other study who found there is significant reduction in vitamins A and C. Vitamin A is required for normal epithelial structure and function and can cause stimulation of the immune response. Reduced concentrations of vitamin A and antioxidant vitamins C and E have been already reported in patients with tuberculosis. As well as previous workers have reported significantly low levels of vitamin E, vitamin A, total antioxidant, Zn and beta-carotene in pulmonary tuberculosis. In tuberculosis, the increase in reactive oxygen species may lead to an increased consumption with a corresponding reduction of antioxidants, thus causing oxidative damage. As well as reduced concentrations of vitamin A and of the antioxidant vitamins C and E were previously reported in patients with tuberculosis. Serum vitamin A levels lower than 30 μg/dl, 30-80 μg/dl and higher than 80 μg/dl were considered as low, normal and high, respectively.

As shown in figure 2 the serum vitamin C in both male and female patients with pulmonary tuberculosis and control subjects demonstrate that there is a significant reduction in vitamin C. Vitamin C is an immune system booster par excellence. White blood cells use vitamin C to combat infections, and in the face of inflammation or microbial challenge, levels of Vitamin C are depleted. Vitamin C can enhance the body's resistance to an assortment of diseases, including infectious disorders and many types of cancer. The present study has reflected similar observations by statistically significant decrease of vitamin C that recorded by other studies. They pointed out reduced ascorbic acid is the biological form of vitamin C. The water soluble antioxidant functions as the first line antioxidant defense against free oxygen radicals present primarily in the plasma. It functions as an antioxidant by donating two hydrogen atoms, a process by which the reduced form is converted to the oxidized state. In situation where ascorbic acid is consumed in quenching the free radicals it is the reduced form which is decreased. As well as vitamin C were significantly decreased in tuberculosis patients compared to controls. Who findings of a significant correlation between high oxidants concentration and low concentration of antioxidants, suggest increased utilization by ROS as an
important contributing factor to the lower concentration of antioxidants in TB patients. Also the levels of non-enzymic antioxidants such as Vitamin C, in plasma were significantly depleted in the pulmonary tuberculosis infected subjects when compared with control. This shows that pulmonary tuberculosis could probably be associated with excess ROS production\(^\text{43}\).

Akiibinu et al.,\(^\text{44}\) found low levels of total antioxidant, vitamins C was observed in pulmonary tuberculosis. This might be due to malnutrition or exhaustion in attempt to neutralize heavy load of free radical in these patients. Pawar, et al.,\(^\text{45}\) concludes that antioxidants (micro-nutrients) supplementation as an adjuvant therapy helps in reduction of oxidative stress and promotes recovery of patients. Moreover,\(^\text{46}\) other study showed that vitamin C supplementation influenced the lipid profile by increasing the serum high density lipoprotein-cholesterol (HDL-C), improving the total cholesterol levels and decreasing the low density lipoprotein-cholesterol (LDL-C) concentration in the antioxidant vitamin supplemented groups. In addition to the mean plasma ascorbic acid levels were significantly lower in all TB patients when compared to controls\(^\text{46}\).

**Conclusion**

In present study observed significant lowering of serum vitamins A and vitamin C levels in both male and female patients with pulmonary tuberculosis in comparison with healthy control. It seems that tissue inflammation, oxidative stress and continuous production of free radicals in pulmonary tuberculosis patients may cause lower levels of antioxidants.

**References**