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

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

الخلاصة

الخلفية العلمية : تعمل مضادات الأكسدة القابلة للذوبان في الماء(كفيتامينات أ و ج) كخط أول للدفاع ضد الجذور الحرة للأوكسجين الموجودة أساسا في البلازما. فحص المواد المضادة للأكسدة في المرضى الذين يعانون من مرض التدرن الرئوى . قد تساعد على تحديد أوجه القصور عند الإصابة الشديدة . ومع ذلك، معرفتنا فيما يخص مضادات ألاكسده وعلاقتها بمرضى التدرن الرئوى قليله، لا سيما في البلدان النامية .الهدف: تهدف هذه الدراسة إلى تقدير بعض الفيتامينات المضادة للأكسدة، فيتامين (أ) و (ج) في كل من الذكور والإناث من المرضى المصابين بلتدرن الرئوي .المرضى وطرق العمل: شملت الدراسة 26 مريضا (14 من الذكور و 12 من الإناث) المصابين بلتدرن الرئوي. تراوحت أعمارهم بين 29-72 سنة في الذكور و 22-65 سنة في الإناث. وفى مجموعة السيطرة الأصحاء أربع وعشرون (12 من الذكور و 12 من الإناث) كانت أعمارهم بين 23 – 67 سنة في الذكور و 23- 60 سنة في الإناث .

تم إجراء جمع الدم في العيادة الاستشارية للإمراض الصدرية والتنفسية في الحلة (وحدة التدرن) في بابل.حيث تم استخدام أنابيب اختبار عاديه لإعداد الأمصال للاختبارات !! !! ! ! !! !! ! لغرض تقييم مستوى المواد المضادة للأكسدة ، فيتامين (أ) وفيتامين (ج) في المرضى الذين يعانون من التدرن الرئوي.النتائج : هناك تغييرات يعتد بها إحصائيا للفيتامينات المضادة للأكسدة في مرضى التدرن الرئوي عند المقارنة مع مجموعة السيطرة الأصحاء. بينت النتائج وجود انخفاض معنوي عالي في قيمة الفيتامينات (أ) و (ج) لمصل الدم لدى المرضى المصابين بلتدرن الرئوي (للذكور ولإناث) مقارنة مع مجموعة السيطرة الأصحاء.

الاستنتاج:إن مستويات المواد المضادة للأكسدة غير الانزيميه مثل فيتامين (أ) وفيتامين (ج) في المصل قد استنفذت إلى حد كبير في مرضى التدرن الرئوي عند مقارنتها مع مجموعة السيطرة الأصحاء. مما يوحي بوجود ارتباط قوي بين نقص فيتامين (أ) وفيتامين (ج) وسوء التغذية في هؤلاء المرضى، فضلا عن وجود ارتباط بين العديد من الحالات كسوء التغذية والتراكيز المنخفضة من المواد المضادة للأكسدة التي قد تشير إلى تأثير انخفاض تناول الاغذيه وسوء امتصاصها في توليد الإجهاد التأكسدي لدى المرضى المصابين بلتدرن الرئوي.

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 [18]. 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 o.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 standard s 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[24]. Calculation : Thus

Absorbance of sample
---------- × Concentration of standard = µg /100ml vitamin A
Absorbance of standard

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 [25]:-

correspond absorbance of reagent blank – correspond absorbance of sample
---------- × 2 = mg / 100 ml ascorbic acid in sample
correspond absorbance of reagent blank – correspond absorbance of standard

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 ($P<0.05$)[26].

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) .

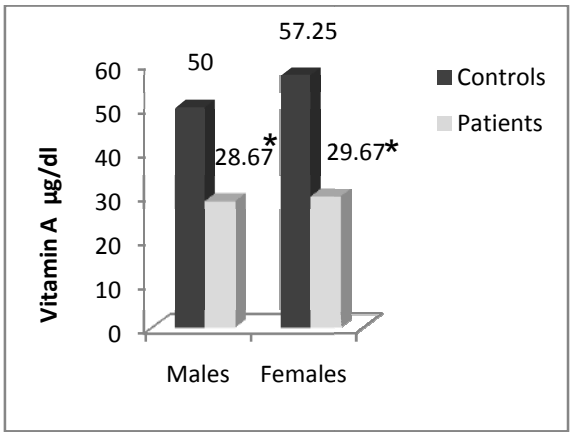

Figure (1) Changes in serum vitamin A in male and female patients with pulmonary tuberculosis * ($p<0.001$)

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 ± o.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 .

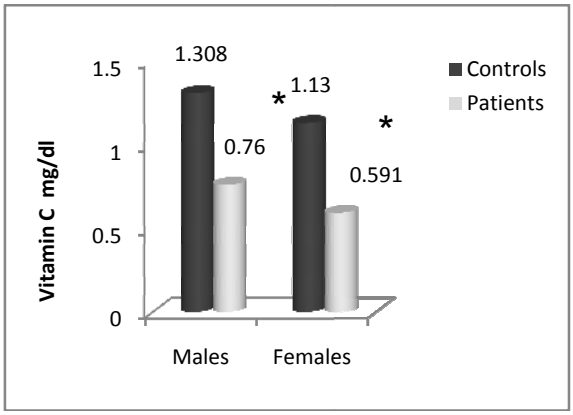

Figure (2) Changes in serum vitamin C in male and female patients with pulmonary tuberculosis.

* ($p<0.001$)

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 [27]. It is a chronic granulomatous infectious disease caused by Mycobacterium tuberculosis; the disease affects almost all the organs, lungs being primary[28,29]. 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 hypozincemeia as zinc is required for vitamin A metabolism **[8,4].** Vitamin A enhances white blood cell function, enhances resistance to infection, and helps maintain membrane defenses to infection[30] MOHOD *et al.,*[31] 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 [32] 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.[33,34] **.** As well as previous workers have reported significantly low levels of vitamin E, vitamin A, total antioxidant, Zn and beta-carotene in pulmonary tuberculosis [35]. In tuberculosis, the increase in reactive oxygen species may lead to an increased consumption with a corresponding reduction of antioxidants, thus causing oxidative damage[33, 34]. As well as reduced concentrations of vitamin A and of the antioxidant vitamins C and E were previously reported in patients with tuberculosis[35 ,36,37 ,38 ,39,40]. 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[41] .

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. of tuberculosis as compared to control.

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[42]**.** The present study has reflected similar observations by statistically significant decrease of vitamin C that recorded by other studies[11 ,4 ,32]. 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[31]. 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[43].

Akiibinu *eta.,*[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,*etal.,*[45]concludes that antioxidants (micro-nutrients) supplementation as an adjuvant therapy helps in reduction of oxidative stress and promotes recovery of patients**.** Moreover ,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[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

1-Kumar , V.; Abbas, A.K.; Fausto, N. and Richard N. (2007). Robbins basic pathology (8th ed.). Saunders Elsevier. pp. 516–522.

2-Moses, A.O.; Emmanuel, O.O.; Ganiyu, A.O.; Fidelis, A.A.and Dickson, A.O. (2008).Assessment of antioxidants and nutri-tional status of pulmonary tuberculosis patients in Ni-geria. Eur J Gen Med:5 (4): 208-211.

3-World Health Organization. (2008). Global Tuberculosis Control 2008 -Surveillance, Planning, Financing. WHO Report. Geneva :World Health Organization.

4-Vijayamalini, M. and Manoharan, S. (2004). Lipid peroxidation, vitamins C, E and reduced glutathione levels in patients with pulmonary tuberculosis. Cell Biochem Funct. ; 22(1):19-22.

5- Paton, N.I. ; Chua, Y.K.; Earnest, A. and Chee, C.B.E.(2004). Randomized controlled trial of nutritional supplementation in patients with newly diagnosed tuberculosis and wasting. Am J Clin Nutr; 80: 460–465.

6- Madebo, T.; Lindtjorn, B.; Aukrust, P. and Berge, R.K. (2003). Circulating antioxidants and lipid peroxidation products in untreated tuberculosis in Ethiopia. Am J Clin Nutr; 78:117-22.

7- Karyadi E, West CE, Schultink W, Nelwan RH, Gross R, Amin Z et al . (2002). A double-blind,placebo-controlled study of vitamin A and zinc supplementation in persons with tuberculosis in Indonesia: effect on clinical response and nutritional status. Am J Clin Nutr; 75:720-7.

8-Reddy, Y.N.; Murthy, S.V.; Krishna, D.R. and Prabhakar, M.C. (2009). Oxidative metabolic changes in pleural fluid of tuberculosis patients. Bangladesh J Pharmacol; 4:69-72.

9-Hashmi, M. A.; Ahsan1, B.; Shah, S. I. A. and Khan, M. I. U.(2012). Antioxidant Capacity and Lipid Peroxidation Product in Pulmonary Tuberculosis , Al Ameen J Med Sci 3 :3 1 3 -3 1 9.

10-Mohod, K. and Kumar, S. (2012). Oxidants and Antioxidants Levels in Pulmonary Tuberculosis Patients on ntitubercular Treatment. Biomedical Research ; 23 (3): 385-389.

11-Reddy, Y.N.; Murthy, S.V.; Krishna, D.R. and Prabhakar, M.C. (2004).Role of free radicals and antioxidants in tuberculosis patients. Indian J. Tuberc ; 51:213-218.

12-Chocano-Bedoya, P. and Ronnenberg, A. G. (2009). Vitamin D and tuberculosis,Nutri.Reviews67(5):289–293.

13-Srinivasan, S.; Pragasam, V.; Jenita, X.; Kalaiselvi, P., Muthu, V. and Varalakshmi, P. (2004). Oxidative stress in urogenital tuberculosis patients: a predisposing factor for renal stone formationamelioration by vitamin E supplementation. Clin Chim Acta; 350: 57-63.

14- Gao, F.; Koenitzer, J.R.; Tobolewski, J.M.; Jiang, D.; Liang, J.; Noble, P.W. et al. (2008). Extracellular Superoxide Dismutase Inhibits Inflammation by Preventing Oxidative Fragmentation of Hyaluronan. J BiolChem; 283: 6058-66.
15-Rojas, M.; Rugeles, M.T.; Gil, D.P. and Patino, P. (2002). Differential modulation of apoptosis and necrosis by antioxidants in immunosuppressed human lymphocytes. Toxicol Appl Pharmacol; 180 (2):67-73.
16-Starostenko, E.V.; Dolzhanskii, B.M.; Salpagarov, A.M. and Levchenko TN. (1991). The use of antioxidants in the complex therapy of patients with infiltrating pulmonary tuberculosis. Probl Tuberk; (1): 9- 11.
17-Chandra, R.K. (1988). Increased bacterial binding to respiratory epithelial cells in vitamin A deficiency. BMJ; 297 (6652): 834- 5.
18-Stephensen, C.B.; Alvarez, J.O.; Kohatsu, J.; Hardmeier, R.;Kennedy, J.r.and Gammon, R.B. (1994).Vitamin A is excreted in the urine during acute infection. Am J Clin Nutr; 60 (3): 388- 92.
19-Collier, L. (1998). Tuberculosis, In: Microbiology and Microbial infection. Around USA, Collier L, (eds) 9th ed WB. Saunders Company,; pp:124-1245.
20-Moriguchi, S. and Muraga, M. (2000).Vitamin E and immunity. *Vitam Horm*;59:305-36.
21- Lamsal, M.; Gautam, N.; Bhatta, N.; Toora, B.D.; Bhatta-charya, S.K. and Baral, N.(2007). Evaluation of lipid peroxidation product, nitrite and antioxidant levels in newly diag-nosed and two months follow-up patients with pulmo-nary tuberculosis. Southeast Asian J Trop Med Public Health. 38(4):695-703.
22- Asegawa, N.; Niimi, N. and Odani, F. (2002).Vitamin C is one of the lipolytic substances in green tea. Phytother. Res., 16: S91-S92. DOI:10.1002/ptr.843.
23-Lewis, S. M.; Bain, B. J. and Bates, I. (2006). Dacei & Lewis practical hematology. 10th ed., Churchil Livingstone Elsevier. Germany.
24-Bieri , J.G. (1971). In Sunderman ,F.W.,editor: Applied seminar onthe clinical pathology of the lipids, Philadelphia, Association of clinical Scientists.
25-Roe , J.H. (961). : Ann. N. Y. Acad. Sci. 92:277.
26-Daniel,W.W.(1999).Probability and t distribution biostatistics: A foundation for analysis in health science.7th ed. : 83-123.
27-WHO (2010). Global tuberculosis control - surveillance, planning,financing.In:http://www.who.int/tb/publications/global_report/2010/en/index.html.
28-Kaufmann, S.H. (2003). A short history of Robert Koch's fight against tuberculosis: those who do not remember the past are condemned to Repeat it.Tuberculosis,83:86-90.
29-Drancourt, M and Raoult, D. (2005). Palaeomicrobiology: current issues and perspectives. Natu. Revi. Microb., 3: 23-35.
30-Zeba, A.N.; Sorgho, H.and Rouamba ,N. (2008). "Major reduction of malaria morbidity with combined vitamin A and zinc supplementation in young children in Burkina Faso: a randomized double blind trial". Nutr. J., 7: 7.
31-Mohod, K. ; Gangane, N. and Kumar, S.(2008).Oxidants and antioxidants in lymph node tuberculosis , J MGIMS, , Vol 13, No (ii), 35 32-Dalvi, S. M., Patil, V. W. and Ramraje, N. N. (2012) . Carbonyl protein and antioxidant vitamins in pulmonary and extrapulmonary tuberculosis . J. Phys. Pharm . Adv . , 2(5): 210-215A.
33-Plit, M.; Theron, A.J.; Fickl, H.; Vani Rensburge, C.E.; Pendel, S. and Anderson, R. (1998). Influence of antimicrobial chemotherapy and smoking status on the plasma concentrations of Vitamin C, Vitamin E, betacarotene,acute phase reactants, iron and lipid peroxides in patients with pulmonary tuberculosis. Internation Journal of Tuberculosis and Lund diseases. 2 (7): 590-596.
34-Rwangabwoba ,J.M.; Fischman ,H. and Semba, R.D. (1998).Serum vitamin A level during tuberculosis and human immunodeficiency virus infection. J Int Tuberc Lung Dis; 2: 771 - 773.
35-Wiid ,I.S.; Medmen, T.; Hoal, E.G.; Benade, A.J. and Van Helden, P.D. (2004). Total antioxidants levels are low during active TB and rise with anti tuberculosis therapy. IUBMB Life, 56 (2):101-6.
36-Mohammadian, S. ; Baghdadchi , J.; Rahimi, A. O. ; Shahghasempour, S. ; Amiri M. V. ; Mohammadi, F.; Mirsaiedi, S. M. and Velayati A. A. (2005). Vitamin A Supplementary Effect on Immunologic Profiles in Tuberculosis Patients. Tanaffos , 4(14), 53-60.
37-Winkler, B.S.; Orselli, S.M. and Rex, T.S. (1994).The redox couple between glutathione and ascorbic acid: a chemical and physiological perspective. Free Radic Biol Med; 17:333-49.
38-Meister, A. (1994) .Glutathione-ascorbic acid antioxidant system in animals. J Biol Chem; 269:9397-9400.
39-Ramachandran, G.; Santha, T.; Garg, R.; Baskaran, D.; Iliayas, S.A.;Venkatesan, P.; et al: (2004) .Vitamin A levels in sputum-positive pulmonary tuberculosis patients in comparison with household contacts and healthy ‘normals’.Int J Tuberc Lung Dis, 8:1130-3.
40-A Pakasi,1.T.; Karyadi,1. E. ; Suratih, N.M.; Salean, M. ;Darmawidjaja, N. ; Bor, H.; Velden, K. V.; Dolmans, W. M. and van der Meer, J. W. M. (2010). Zinc and vitamin A supplementation fails to reduce sputum conversion time in severely malnourished pulmonary tuberculosis patients in Indonesia, Nutri. J. , 9:41.

41- Donald, B. and Harry L. (1999) .Vitamin, In: Tietz Textbook of clinical chemistry. Burtis CA, Ashwood EE, (eds) 3th ed, WB Saunders Company, Philadelphia,; pp: 1000-1007, 1110-1113.

42-Combs, G.F. (2008). The Vitamins: Fundamental Aspects in Nutrition and Health (3rd ed.). Burlington: Elsevier Academic Press. ISBN 9780121834937.

43-Nwanjo, H.U. and Oze G.O. (2007). Oxidative Imbalance and Non-Enzymic Antioxidant Status in Pulmonary Tuberculosis Infected Subjects: Carcinogenic Potential, Pakistan Journal of Nutrition 6 (6): 590-592,

44-Akiibinu, M.O; Arinola, O.G.; Ogunlewe, J.O. and Onih, E.A. (2007). Non-Enzymatic Antioxidants and Nutritional Profiles in Newly Diagnosed Pulmonary Tuberculosis Patients in Nigeria, Afr. J. Biomed. Res. 10: 223 – 228.

45-Pawar , B. D. ; Suryakar, A. N. and Khandelwal A.S. (2011). Effect of micronutrients supplementation on oxidative stress and antioxidant status in pulmonary tuberculosis. Biomedical Research 2011; 22 (4): 455-459.

46-Madhavi, M. ; Samudram, P. ; Kumar, A. H. and Victor, L.(2009). Effect of Antioxidant Vitamins C and E Supplementation on its Plasma Levels and on Lipid Profile in Pulmonary Tuberculosis Patients. Am. J. Infect. Dis., 5 (3): 263-272,