Immune modulatory and anti-oxidative effect of selenium against pulmonary tuberculosis

Muhammad Imran Hussain[1*], Waqas Ahmed[1], Muhammad Nasir[1], Muhammad Hassan Mushtaq[2], Ali Ahmad Sheikh[3], Arfat Yousaf Shaheen[3], Shahbaz Ali[4] and Sanaullah Iqbal[1]

[1]Department of Food Science and Human Nutrition, University of Veterinary and Animal Sciences, Lahore, Pakistan
[2]Department of Epidemiology and Public Health, University of Veterinary and Animal Sciences, Lahore, Pakistan
[3]University Diagnostic Lab, University of Veterinary and Animal Sciences, Lahore, Pakistan
[4]Department of Medicine, Rural Health Center, Farooq Abad, Sheikhupura, Lahore, Pakistan

Abstract: Prevalence of pulmonary tuberculosis (TB) in Pakistan is due to poor living conditions, malnutrition and low immunity. The present project was conducted to show the role of selenium complement to enhance the immune status against TB. Total of 80 human TB patients were divided into treatment (selenium and anti-tuberculosis drug) and control groups (anti-tuberculosis drug). Levels of selenium, immunoglobulin and leukocyte count were determined before and after treatment. Selenium showed significant increase in levels of immunoglobulin and leukocyte count in patients as compared to control group. The level of SOD, catalase, glutathione and total antioxidants were remarkably lowered among control type group as compared to treatment type group ($P<0.01$). However, the values of lipid peroxidation products malondialdehyde (MDA) were notably higher in control group than treatment group.

Keywords: Tuberculosis, malnutrition, selenium, TLC, SOD, MDA.

INTRODUCTION

Tuberculosis (TB) is transmissible pathogenic disease due to *Mycobacterium tuberculosis* (MTB). TB is considered as major disease burden globally and is prevailing from 3000-5000 years BC (Zink *et al*., 2003). A report of World Health Organization suggests that Tuberculosis is a comprehensive health crisis and is foremost factor of inevitable mortality in young adults (Kumar and Clark, 2001). Nearly 33% world population is under threat due to MTB. Traditionally it was believed that tuberculosis is the illness of developing countries but from past decade many cases of tuberculosis have also been reported in developing countries. Number of positive cases of TB in population of South East Asia is up to 44%. Pakistan is placed at 5th rank in top 22 graded countries (Saira *et al*., 2013). In TB subjects, there is an unbroken decrease in extent of macro- and micro-nutrients which inadequately depresses the muscular and immune systems (Saleem *et al*., 2012). Lack of different micronutrients is responsible for immunologic disorders in subjects leading to particular diseases **(**Field *et al*., 2002; Kant *et al*., 2015). It has been noticed that during the journey of the disease, use of micronutrients along with therapeutic agents also improves outcome of the disease (van Lettow *et al*., 2004). Studies showed that Selenium is an essential micronutrient and it enhances health standards and immunity during diseased period (Ensiyeh *et al*., 2006) (Mahan *et al*., 2004). Selenium is also an essential antioxidant and is necessary for enzyme glutathione peroxidase (GPx), it maintains immune processes thus help to clear mycobacterium. TB patients have reduced levels of antioxidants; rapid production of reactive oxygen species (Madebo *et al*., 2003) and there is high value of lipid per oxidation due to faulty activity of GPx. (Srinivasan *et al*., 2004). The present project was designed to show the anti-oxidative & immunomodulatory effects of selenium supplementation on the clinical, microbiological and roentgenological analysis of subjects with PTB.

MATERIALS AND METHODS

Ethical consideration

Registered interests were collected from every patient of this trial registered in TB clinic of Services Institute of Medical Sciences (SIMS), Lahore. Ethical and consent procedures were obtained by the ethical board of the UVAS, Lahore and Services Institute of Medical Sciences (SIMS), Lahore. The clinical tests of each study individuals were being discussed with the concerned practitioners at TB clinics.

Selection of patients

In order to observe the effect of Selenium supplementation as immune-modulator in patients with pulmonary tuberculosis, patients registered in SIMS, Lahore were selected. Patients with age groups from 20-40 and with clear signs of pulmonary tuberculosis, having positive acid-fast bacilli in their sputum samples, were included in the study. Patients with other forms of tuberculosis or being on treatment for more than two months were excluded from the study.

**Corresponding author:* e-mail: drimihassan99@hotmail.com

Study design

In this study a total of 80 patients (n=50 male, n=30 female) of pulmonary tuberculosis were selected during the period from February to October 2016. They were divided into treatment group (selenium and anti-tuberculosis drug) and control group (anti-tuberculosis drug) comprising forty patients per group. Both groups were treated with anti-tuberculosis regimen (Combination of Ethambutol @ 15mg/kg, Rifampicin @ 10mg/kg, Isoniazid @ 5mg/kg, and pyrazinamide @ 25mg/kg) as recommended by WHO (World Health Organization, 1999). Control group received anti-tuberculosis regimen and placebo (Starch capsule). Treatment received anti-tuberculosis regimen with selenium (100 mcg per day). Information regarding age, gender, location of residence, source of income/economic status, dietary status and habit of smoking/drugs abuse was also recorded. During the treatment period, subjects were advised for visit the hospital after 15 days to collect anti-TB drugs and selenium capsules (where applicable).

Clinical evaluations

In order to determine the response of the treatment on patients, chest X-rays were performed after two and six months of the therapy. The patients having cavitary lesion(s) in lungs, the decrease in radius of the cavity were noted.

Microbiological parameters

Microbiological examinations of sputum sample for the presence of MTB was done after every 15 days during two months of the treatment and were tested through ZN staining techniques (Monica, 2006). Results were recorded for conversion of acid-fast positive bacilli to acid-fast negative bacilli (*Mycobacterium tuberculosis*) indicating treatment response. Treatment was continued for 6-8 months as recommended by WHO (World Health Organization, 1999). Blood sample from the patients were collected through venipuncture and levels of IgG and IgM against *Mycobacterium tuberculosis* were determined using commercially available ELISA kit (IBL International, GmBH). While determination of CD4+ and CD8+ count in patient's blood was done through Flow Cytometery using Becton-Dickinson fluorescence activated cell sorter (FACS) Calibur by using CellQuest software as per instructions mentioned in the manual (Mehta *et al*., 2011).

Hematological parameters

Blood sample collected from patients were processed for hematological tests including ESR and TLC (Monica, 2006).

Biochemical parameters

Blood sample collected from the patients were also processed for biochemical tests to determine the level of Selenium. Level of selenium was measured through Atomic Absorption Spectrophotometer (Cuparigova and Stafilov, 2011). The concentration of lipid peroxidation products in the blood was evaluated using the thiobarbituric acid (TBA) procedure, this computes MDA reactive products by High Pressure Liquid Chromatography (HPLC) (Carbonneau *et al*., 1991). The enzyme superoxide dismutase (SOD) action was evaluated in RBCs by photo oxidation method (Misra and Fridowich, 1977). Catalase level was determined on the base of capability of catalase to oxidize H_2O_2 (Beers and Sizer, 1952). 2.25 ml of potassium phosphate (KH_2PO_4) buffer (65 mM, pH 7.8) was introduced to 0.1 ml of serum and incubated at 25^{o}C for 30 min. 650 ml of H_2O_2 (7.5 mM) were mixed to start the reaction. Absorbance change was noted at 240 nm for 3 min.1IU of catalase is the enzyme, which decomposes one mM of H_2O_2 per min at 25^{o}C.The level of Glutathione in the blood was determined by taking 0.5 ml of 5% TCA solution and was added to 0.5 ml of citrated blood to make ppt of protein and then centrifuged at 3000 RPM for 20 min. To 0.1 ml of the supernatant, 1 ml of sodium phosphate buffer (pH 8) and 0.5 ml of DTNB (39.6 mg in 100 ml of 1% sodium citrate solution to give a concentration of 1 mM) were added. The absorbance of the yellow color produced which was measured at 412 nm (Beulter *et al*., *1963*). The level of total anti-oxidant in blood was measured by the method of Blios (Blios, 1958) by a stable free radical, a,a-diphenyl-b-picrylhydrazyl (DPPH) (Sigma Aldrich, USA), at a concentration of 0.2 Mm in methanol.

STATISTICAL ANALYSIS

Comparison between groups (before and after treatment) was made using 2-tailed t-test. Impact of micronutrient supplementation on cell mediated immunity and antioxidant levels were evaluated through correlation. *P*-value < 0.05 were observed significant (Douglas, 2000).

RESULTS

Results showing history and clinical evaluation of the patients reflected that most of TB patients (97%) were having cough for the last three weeks, about 96% of the subjects were having high fever at night time and other symptoms observed were hemoptysis, malaise, tiredness, anorexia, chest pain and night sweating with the relative frequency at the rate of 9%, 48%, 45%, 65%, 59% and 48% respectively (table. 1).

Microbiological evaluation revealed that 20% of the individuals were positive with AFB load while 62% of the individuals were negative with AFB load. Among the positive AFB individuals scanty, one plus, two plus and three plus patients were 20%, 25%, 20% and 35% respectively (table 2).

Average ESR in the control and treatment group was approximately the same during study period (table 3).

Table 1: Symptoms of tuberculosis alongwith their frequency

Symptoms	Frequency
Productive cough	78 (97%)
Hemoptysis	07 (9%)
Malaise	39 (48%)
Tiredness	36 45(%)
Anorexia	52 (65%)
Chest pain	47 (59%)
Night sweating	39(48%)
Fever	77 (96%)
Presence of BCG Scar	80(100%)

Table 2: Sputum samples for AFB load

Specimen for AFB stain	Positive	Negative
Sputum (n= 70) 87.5%	20 (25%)	50 (62%)
	Scanty 04(20%)	
	One plus 05 (25%)	
	Two plus 04 (20%)	
	Three plus 07 (35%)	
Non-sputum (n=10) 12.5%	20 (25%)	50 (67%)

Table 3: Comparison of Mean value of ESR in groups at various durations of treatment

Group	Mean mm/hr.Start	Mean mm/hr. 2nd Month	Mean mm/hr. 4th Month	Mean mm/hr. 6th Month
Control	54.55	11.25	11.25	10.75
Treatment	54.65	12.25	12.25	10.8

Table 4: Comparison of Mean value of TLC in groups at various durations of treatment

Group	Mean cells / mm^3 start of the treatment	Mean cells / mm^3 2nd Month	Mean cells / mm^3 4th Month	Mean cells / mm^3 6th Month
Control	11570	9310	8900	8560
Treatment	12510	8925	8700	8285

Table 5: Level of Selenium in Blood before and after treatment

Level of selenium in blood before treatment			Level of selenium in blood after treatment		
Group	Mean	Standard Deviation	Group	Mean	Standard Deviation
Control	52.3	14.66	Control	52.8	15.56
Treatment	52.45	15.43	Treatment	160.65	31.83

Table 6: Level of IgG in the blood before and after treatment

Level of IgG in the blood before treatment			Level of IgG in the blood after treatment		
Group	Mean	Standard Deviation	Group	Mean	Standard Deviation
Control	596.8	117.72	Control	626.85	131.05
Treatment	587.5	113.35	Treatment	1060.3	93.52

Table 7: Level of IgM in the blood before and after treatment

Level of IgM Before Treatment			Level of IgM after Treatment		
Group	Mean	Standard Deviation	Group	Mean	Standard Deviation
Control	26.3	5.69	Control	28.7	7.30
Treatment	19.45	10.43	Treatment	157.85	11.49

P-value < 0.05

Table 8: CD4 cell count before and after treatment

CD4 Cell count before treatment			CD4 Cell count after treatment		
Group	Mean	Standard Deviation	Group	Mean	Standard Deviation
Control	399.15	43.77	Control	418.55	63.90
Treatment	391.35	44.73	Treatment	1071.8	55.55

Table 9: CD8 cell count before and after treatment

CD8 cell count before treatment			CD8 cell count after treatment		
Group	Mean	Standard Deviation	Group	Mean	Standard Deviation
Control	116.25	25.36	Control	124.1	28.40
Treatment	117.25	24.33	Treatment	593.2	43.33

P-value < 0.05

Table 10: Micronutrient levels in control and treatment groups before and after treatment

Parameters	Control Group	Treatment Group
Serum MDA	(nmol/ml)	
Pre-treatment	6.35±0.46	6.07±0.51
Post-treatment	5.22±0.61	4.53±0.24
Superoxide Dismutase (SOD)	(IU/ml)	
Pre-treatment	63.48±4.62	61.74±5.42
Post Treatment	66.26±3.08	73.15±5.24
Catalase	(IU/ml)	
Pre-treatment	52.84±3.86	54.36±6.16
Post-treatment	68.87±4.16	75.06±6.16
Glutathione	(mmol/ml)	
Pre-treatment	0.48±0.04	0.51±0.05
Post-treatment	0.56±0.05	0.64±0.04
Total Antioxidant Status	(nmol/ml)	
Pre-treatment	55.26±2.59	54.82±3.06
Post-treatment	77.61±6.34	88.48±10.16

In start the average of TLC in both groups was high but after treatment the treatment group has fewer total leucocytes count (TLC) than control group. The Means and standard deviations of TLC are depicted in (table. 4).

The level of selenium in treatment group has remarkably been increased in the blood after giving selenium to the patients. However, there was no increase in the level of selenium in blood of control group patients after treatment (table. 5).

The mean values of level of IgG and IgM in blood of treatment group have significantly increased than control group (table. 6 & 7).The mean value of level of T cells count that is $CD4^+$ and $CD8^+$ in serum of treatment group had significantly increased than control group (table. 8 & 9).

The level of lipid peroxidation products malondialdehyde (MDA) was remarkably higher in control group then treatment group; however, the level of SOD, catalase, glutathione and total antioxidants was significantly lowered in control group as compare to treatment group (table 10).

DISCUSSION

The results of current study showed that most of the patients including male and female were having low economic status and it seems that most of poor people suffer with TB as they have deprived immune status and they eat diet without proper minerals and vitamins. The common clinical presentation of pulmonary TB (PTB) include, coughing more than two weeks, fever, night sweats, hemoptysis and wasting (Campbell and Bah-Sow 2006).

The ESR and TLC of the TB patients indicated no sufficient outcome of Selenium supplementation against ESR and TLC value of the patients and it is independent of the treatment. It is not 100% necessary of all TB patients to have high value of ESR and the justification of

this reason is in line with the findings of Al Marri and Kirkpatrick 2000 in which 33% had a standard ESR (<10 mm/hour) 67% had increased ESR (> or =10 mm/hour) at diagnosis time & there was no sufficient correlation against age or size of tuberculin skin test reactivity and ESR values (Murray *et al*., 1990).

The mean value of level of selenium has significantly increased in the blood after giving selenium to the patients. However, there was no increase in the level of selenium in control group patients after treatment. The result of these findings clearly showed that due to increase level of selenium in the blood of patients, immunity would have been enhanced which showed better treatment outcomes. The findings of our trial have similarities with previous data (Ensiyeh *et al*., 2006)

The amount of IgG and IgM in the blood of study patients indicated that the supplementation of micronutrients such as selenium increased the standards of IgG and IgM in the blood of treatment group except control group. The increased level of IgG and IgM in the blood depicts more immunity due to which patients' body could resist side effects of the antibiotics. One past finding is in line with our results that zinc and vitamin A deficiency is related with a decreased production of IgA in blood due to deficiency of vitamin and micro minerals so we can say that supplementation of vitamin E and selenium enhances levels of antibodies in blood which in turn enhances immunity and improves treatment outcomes against TB (Macallan *et al*., 1998).

The increased level of T cell count that is $CD4^+$ and $CD8^+$ in the blood of study subjects indicated that the supplementation of selenium increased the immunity and due to which patients' body could tolerate side effects of the antibiotics leading to quick recovery. Our findings are similar with the results of previous study conducted to determine the effect of supplementation of micronutrient on treatment outcome, morbidity, cell counts, and mortality in pulmonary TB adults. The findings of the study indicated that micronutrients supplementation had significantly increased $CD3^+$, $CD4^+$ and $CD8^+$cell counts (Mathur, 2007).

The decreased level of antioxidant potential and increased level of lipid per oxidation product (MDA) in the serum of treatment group clearly shows that selenium reduces oxidative stress in TB patients thus helping towards fast recovery against Tuberculosis. Our results are similar with the findings of previous study conducted to access the ability of free radicals and antioxidant in tuberculosis subjects. The results of the trial showed that in TB patients there was high activity of free radicals and reduced level of antioxidants (Reddy *et al*., 2004).

Another study revealed that increased in free radical activity is related with increased in MDA level while the level of antioxidants decreases in TB patients. The low antioxidants level is related with oxidative stress in patients. But when Selenium is provided to the TB patients the level of oxidative stress is decreased (Holy *et al*., 2018).

CONCLUSION

In light of above finding it is concluded that selenium supplementation should be recommended along with anti-tuberculosis therapy for better treatment, improved immunity and as an antioxidant role of TB patients.

REFERENCES

Beers RF and Sizer IW (1952). A spectrophotometer method of measuring the breakdown of hydrogen peroxide by catalase. *J. Biol. Chem*., **195**: 133-140.

Beulter DV, Durm O and Kelly BM (1963). Improved method for the determination of blood glutathione. *J. Lab. Chem. Med.*, **61**(5): 882-888.

Blios MS (1958). Antioxidant determination by the use of stable free radical. *Nature.*, **26**:1199.

Campbell IA and Bah-Sow O (2006). Pulmonary tuberculosis: Diagnosis and treatment. *BMJ. Clin. Res*. **332**(7551): 1194-1197.

Carbonneau MA, Peuchant E, Sess D, Canioni P and Clerc M (1991). Free and bound MDA measured as thiobarbituric acid abduct by HPLC in serum and plasma. *Clinical Chemistry.*, **37**(8): 1423-1429.

Cuparigova F and Stafilov T (2011). Determination of selenium in human blood serum by electrothermal atomic absorption spectrometry. *Chem. Sci. J*., **2011**: CSJ-46.

Douglas CM (2000). Design and analysis of experiments. 5th Edi. Arizona State University, USA.

Ensiyeh S, Ostadrahimi AR and Mahboob SA *et al* (2006). Vitamin E-Selenium supplement and clinical responses of active pulmonary tuberculosis. *Tanaffos*., **5**(2): 49-55.

Field CJ, Johnson IR and Schley PD (2002). Nutrients and their role in host resistance to infection. *J. Leukoc. Biol*., **71**(1): 16-32.

Holy B, Ben-Chioma, Adline E and Esanye O (2018). Oxidative Stress Markers and Selenium Levels of Pulmonary Tuberculosis Patients in Some Dot Centers in Port Harcourt. *J. Adv. Med. Medic. Res.*, **27**(6): 1-11.

Hugo Abei (1963). Methods of enzymatic analysis: Bergmeyer Ed: Catalase, 4th Ed. Academic Press, New York, USA, pp.672-683.

Kant S, Gupta H and Ahluwalia S (2015). Significance of nutrition in pulmonary tuberculosis. *Crit. Rev. Food. Sci. Nutr*., **55**(7): 955-63.

Kumar P and Clark M (2001). Clinical Medicine. 4th ed., W.B. Saunders, Philadelphia, Pa., USA, pp.1-6.0

Macallan DC, McNurlan MA, Kurpad AV, de Souza G, Shetty PS, Calder AG and Griffin GE (1998).

Whole body protein metabolism in human pulmonary tuberculosis and undernutrition: Evidence for anabolic block in tuberculosis. *Clin. Sci.* (Lond)., **94**: 321-31.

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

Mahan LK and Escott-Stump S (2004). Krause's Food, Nutrition & Diet Therapy. 11th ed. W.B. Saunders, Philadelphia, Pa.: pp.152-154.

Mathur ML (2007). Role of vitamin A supplementation in the treatment of tuberculosis. *Natl. Med. J. India*., **20**(1): 16-21.

Mehta S, Mugusi FM and Bosch RJ *et al* (2011). A randomized trial of multivitamin supplementation in children with tuberculosis in Tanzania. *Nutr. J.*, **10**: 120.

Misra HP and Fridowich I (1977). Superoxide dismutase, a photochemical augmentation assay. *Arch. Biochem Biophys.*, **181**: 308-312.

Monica C (2006). District laboratory practice in tropical countries. Part 2. 2nd Ed. Cambridge, New York, Melbourne, Madrid, Cape Town, Singapore, Sao Paulo.

Murray CJ, Styblo K and Rouillon A (1990). Tuberculosis in developing countries: Burden, intervention and cost. *Bull. Int Uni against Tubercu Lung Dis.*, **65**(1): 6-24.

Reddy YN, SV Murthy and DR Krishna MC (2004). Prabhakar. *Indian J. Tuberc.*, **51**: 213-218.

Saira B and Devrajani BR and Atta-ur-Rahman A (2013). The prevalence of smear-positive pulmonary tuberculosis in Hyderabad Sindh. *Elixir Human Physio.,* **60**: 16447-16450.

Saleem K, Paracha PI, Habib F, Khan I, Anjum R and Badshah S (2012). Effect of dietary counselling on the nutritional status of tuberculosis patients. *Sarhad. J. Agric*., **28**(2): 303-307.

Shor-Posner G, Miguez MJ, Pineda LM, Rodriguez A, Ruiz P and Castillo G (2002). Impact of selenium status on the pathogenesis of mycobacterial disease in HIV-1- infected drug users during the era of highly active antiretroviral therapy. *J. Acquir. Immune Defic. Syndr.* **29**(2): 169-173.

Srinivasan S, Pragasam V, Jenita X, Kalaiselvi P and Muthu V *et al* (2004). Oxidative stress in urogenital tuberculosis patients: A predisposing factor for renal stone formation-amelioration by vitamin E supplementation. *Clin. Chim. Acta.*, **350**: 57-63.

Van Lettow M, Harries AD, Kumwenda JJ, Zijlstra EE, Clark TD and Taha TE (2004). Micronutrient malnutrition and wasting in adults with pulmonary tuberculosis with and without HIV co-infection in Malawi. *BMC. Infect. Dis*., **4**(1): 61.

World Health Organization (1999). Global Tuberculosis Program. Global Tuberculosis Control. WHO Report. Geneva, Switzerland, p.181.

Zink AR, Sola C and Reischl U *et al* (2003). Characterization of Mycobacterium tuberculosis complex DNAs from Egyptian mummies by spoligotyping. *J. Clin. Microbiol.*, **41**: 359-367.