

Tanaffos (2006) 5(2), 49-55

©2006 NRITLD, National Research Institute of Tuberculosis and Lung Disease, Iran

Vitamin E-Selenium Supplement and Clinical Responses of Active Pulmonary Tuberculosis

Ensiyeh Seyedrezazadeh ¹, Ali Reza Ostadrahimi ¹, Soltan Ali Mahboob ¹, Yaghoob Assadi ², Khalil Ansarin³, Parvin Shakoori ³, Masoud Pourmoghaddam ³

¹ Faculty of Health and Nutrition, Tabriz University of Medical Science, ² Faculty of Chemistry, Department of Analytical Chemistry, University of Science and Technology, Tehran, ³ Tuberculosis and Lung Diseases Research Center, Tabriz University of Medical Science and Health Services, TABRIZ-IRAN.

ABSTRACT

Background: It has been suggested that some micronutrients have antioxidant and immunomodulating effects on the treatment of mycobacterial disease. In this study, we investigated the effect of vitamin E and selenium supplementation on clinical responses in tuberculosis patients.

Materials and Methods: Thirty-five patients with pulmonary tuberculosis diagnosed on the basis of a positive sputum smear for acid fast bacilli or culture for *Mycobacterium tuberculosis* were selected. Serial sputum examinations were performed before the diagnosis and at the end of every 15 days, during two months of therapy; chest X-ray of all patients were also evaluated. In a setting of double-blind, placebo-controlled trial, the patients were divided into two groups. Group I (n=17) received combination of vitamin E and selenium which composed of 140 mg of α -TE and 200 μ g selenium per day, and group II received placebo. All patients in both groups received the same antituberculosis standard therapy. Clinical examination and assessment of micronutrient levels were carried out before and after 2 months of intervention.

Results: In group I, elimination of tubercle bacilli from sputum occurred earlier than in group II (6 weeks versus 8 weeks, respectively; $p=0.001$). At the end of the 2nd and 6th month of therapy, the median reduction in cavity surface area on chest X-ray in group I was significantly more than group II (2nd month: 1.5(0.0-4.5 versus 9.0(4.0-18.0); $p=0.03$, and 6th month: 0.0(0.0-2.3) versus 6.3(1.0-15.8); $p<0.05$, respectively).

Conclusion: Vitamin E plus selenium supplementation may improve the microbiological and radiological outcomes of the treatment in patients with pulmonary tuberculosis. (*Tanaffos* 2006; 5(2): 49-55)

Key words: Tuberculosis, Supplementation, Vitamin E, Selenium

INTRODUCTION

Tuberculosis was announced by WHO as a global health emergency in 1999. It is still a worldwide health problem and one of the leading causes of death in young adults (1-3). Currently, micronutrient

deficiencies are thought to be the most common cause of secondary immunodeficiency and the most prominent risk factor for infection-related morbidity, which potentially affect individuals susceptibility to tuberculosis and may alter the natural course of this disease (4). Vitamin E-mediated protection of lipid membranes may decrease the requirement for

Correspondence to: Seyedrezazadeh E

Address: Faculty of Health and Nutrition, Tabriz University of Medical Sciences and Health Services, Tabriz-Iran

Email address: esrz80@yahoo.com

glutathione peroxidase by reducing free radicals at the membrane, and thereby preventing leakage of the free radicals into the cytosol and maintaining intracellular killing capacity of the cell (5). On the other hand, it has been proved that acute infections alter tissue metabolism, increasing the demand of various nutrients for maintaining local tissue homeostasis. Therefore, their supplementation may potentially improve the course and outcome of active pulmonary tuberculosis. In this line of thoughts, a few trials have been conducted so far to investigate the possible impact of micronutrients supplementation on the course and outcome of tuberculosis (6-8). It has been shown that a serum vitamin E level is lower in patients with pulmonary tuberculosis (9, 10). In biological media, vitamin E acts in concert with the selenoprotein, glutathione peroxidase (GPX), to prevent free radical formation and halts the process of highly damaging free radical chain reactions from proceeding once it begins (5, 8). Selenium that is an essential component of several major metabolic pathways including thyroid hormone metabolism, antioxidant defense systems and immune function (11), has been found to exert a crucial role in clearance and persistence of immunity against *Mycobacterium* (12). Soil in many parts of the world is deficient of selenium (Se) and feedstuffs grown on these areas lack adequate amount of dietary Selenium (13).

Although vitamin E and selenium have a combined anti-oxidative and Immunomodulatory function, it is not known whether vitamin E and selenium supplements given together with antituberculosis drugs would increase the efficacy of the antituberculosis treatment or not. Thus, we decided to conduct this double-blind, placebo-controlled trial to investigate the effects of vitamin E and selenium supplementation on the clinical, microbiological and rontgenological outcomes of patients with pulmonary tuberculosis.

MATERIALS AND METHODS

This study was approved by local institutional board of reviewers and central research council of the university. From April 2003 through May 2004, 42 patients with pulmonary tuberculosis were enrolled from the outpatient clinic of the Tuberculosis and Lung Disease Research Center of Tabriz University of Medical Sciences, Tabriz-Iran. This study was designed as a double-blind, placebo-controlled trial. All patients were confirmed to have active pulmonary tuberculosis by compatible clinical findings and at least two positive sputum smears for acid fast bacilli and cultures for *Mycobacterium tuberculosis*. The exclusion criteria were previous antituberculosis treatment; concurrent use of supplements containing selenium and vitamin E; illicit drug addiction (e.g. heroin and hashish); and signs of severe adverse effects of antituberculosis regimen during the treatment. All subjects who were noncompliant with drug treatment, even on occasion, were also excluded from the study.

A total of 35 subjects with newly-diagnosed pulmonary tuberculosis were recruited and randomized into two groups. One group (n=17) was defined as micronutrient group, received antituberculosis regimen plus vitamin E and selenium supplementation, while the other (n=18) received antituberculosis agents and placebo as control. Micronutrients were administered as capsules, each containing 140 mg vitamin E and 200 µg selenium in mixture with starch, whilst the placebo consisted of starch only. All capsules were formulated by a pharmacologist and were similar in size and red in color.

During the course of the study, the patients were asked to come to the clinic every alternate week in order to receive their anti-TB drugs and red (micronutrient or placebo) capsules. To ascertain the patients' compliance, they were also requested to bring their last drug package to be checked by the

health staff. At least one family member was requested to monitor the patient's drug consumption which was in accord with directly observed therapy short-course (DOTS) strategy (14).

Clinical evaluations

At the time of initial diagnosis, a baseline clinical examination was performed by an expert pulmonologist, including assessment of the presence of B.C.G scar, response to tuberculin skin test, erythrocyte sedimentation rate (ESR) and chest x-ray (CXR). Subsequently, in order to assess the patients' response to therapy, CXR was performed at the end of the second month after initiation of therapy and 4 months thereafter at the end of the treatment protocol. In patients with cavitory lesion(s) on CXR, total area of the cavity was calculated from the radius of visible cavities. Sputum smears, 3 specimens per occasion, were obtained every 15 days during the two months of the therapy to look for sputum conversion from positive to negative acid-fast bacilli. At least 3 consecutive negative sputum smears were obtained before considering a case as a negative conversion. The conversion time was considered to last till the first of these three negative specimens.

Vitamin E and selenium quantification

Blood samples were collected with or without EDTA admixture to measure plasma vitamin E and serum level of selenium; respectively. Plasma and serum were separated by centrifugation within an hour of blood withdrawal and stored at -20°C and -75°C until analysis.

Plasma vitamin E concentration was measured by high pressure liquid chromatography (HPLC, cecil1100). After deproteinizing, 0.5 ml of plasma with 1 ml of ethanol and centrifuging at low speed for 5 minutes, vitamin E was extracted by addition of hexane, 2 ml, and then its evaporation to dryness under a nitrogen stream. Finally, the residues were redissolved in methanol and injected into C18 NovaPak column (L, 125nm; ID, 3mm; dP, 3 μm) for

quantification of plasma vitamin E (15). Serum level of selenium was assessed by Graphite furnace atomic absorption spectrometry (Schimadzu; 6300) (16).

Statistical analysis

Data were expressed as median, interquartile ranges, minimum and maximum. All statistical analyses were performed using SPSS software. Comparisons within a group (before and after the treatment) and between the groups were made using Wilcoxon and Mann-Whitney rank tests. P-value less than 0.05 was considered statistically significant.

RESULTS

Thirty-five patients completed the study after exclusion of 7 subjects according to the criteria described previously.

The reasons for their exclusion from the study were previous anti-TB treatment in 5 cases, illicit drug addiction in one case, and drug resistance in one case. Resistance to Anti-TB medication in susceptibility testing was found in none of the patients' specimens. Referring to socio-economical status, most patients were housewives consisting 50% and 41.2% of micronutrients and placebo groups, respectively. 16.7% and 35.4% of patients belonged to the market subgroups of micronutrients and placebo groups, respectively. The remaining were workers, carpet-weavers, clerks or farmers. Baseline (pretreatment) comparisons of age, BMI, erythrocyte sedimentation rate (ESR), tuberculin skin test, size and frequency of BCG scar did not reveal significant differences between the groups (Table 1). Although the women were slightly older than men; this difference was not statistically significant. Clinical symptoms were similar in both groups before anti-TB treatment. In both groups, the BMI showed constant increment during two months of treatment and there were significant decreases in ESR, but the difference between the groups was not significant (data not shown). In micronutrients group

sputum conversion (elimination of acid-fast bacilli from sputum) occurred earlier in comparison with the control group (6 weeks compared to 8 weeks; $p=0.001$). The percentage of patients with positive sputum smear in control group (33.3 at the end of 2 months of drug therapy) was twice that of the micronutrients group (33.3% versus 17.6% as shown in Table 2).

Table1. Baseline indicators of disease status in study patients

Indicators	Micronutrients group	Placebo group
Number	(n=11 F, 6 M)	(n=12 F, 6 M)
Age (y)		
Men	53.0 (25-58)*	56.0 (20-58)
Women	50.0 (18-63)	59.5 (15-65)
BMI (kg/m ²)		
Men	19.6 (16.0-27.1)	21.0 (16.8-23.1)
Women	19.5 (16.6-25.8)	22.0 (17.0-26.7)
ESR (mm/h)		
ESR hr1	51.5 (17-100)	82.0 (28-120)
ESR hr2	89.0 (30-135)	109.0 (54-128)
BCG scar (n)	7	7
Skin test (PPD) (mm)	3.5 (0.0-20)	0.0 (0.0-20)

* Values are given as median, interquartile ranges, minimum and maximum.

Table2. Percentage and frequency of sputum smears in every 15 days interval in the studied patients

Day	Micronutrient group				Placebo group			
	(n=17)				(n= 18)			
	Sputum +		Sputum -		Sputum +		Sputum -	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
15	14	82.4	3	17.6	15	83.3	3	16.7
30	11z	64.7	6	35.3	13	72.2	5	27.8
45	3	17.6	14	82.4	7	41.2	11	64.7
60	3	17.6	14	82.4	6	33.3	12	66.7

No significant differences were detected in the baseline clinical measurements of vitamin E and selenium concentrations of the two groups. Median level of plasma vitamin E was increased from 24.7(0.0-87.0) to 28.2(10.5-86.5) $\mu\text{mol/l}$ in

micronutrients group ($p<0.02$), while decreased from 20.2(5.1-49) to 19.3(5.1-48.6) $\mu\text{mol/l}$ in the control group ($p>0.05$) (Figure.1).

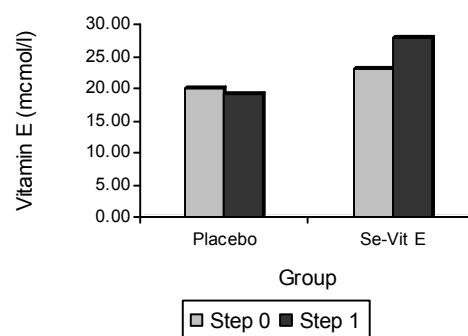


Figure 1. Plasma concentrations of vitamin E in the placebo (n=18) and micronutrients groups (n=17). Values are given as median. In micronutrients group it was significantly different from baseline: $P<0.03$.

Also median level of serum selenium was marginally deficient (normal range: 1.2-2.09 $\mu\text{mol/l}$) in both groups at the time of diagnosis and before the treatment was started; 1.0(0.34-2.5) $\mu\text{mol/l}$ in micronutrients group versus 0.93(0.1-1.9) $\mu\text{mol/l}$ in controls. After two months of micronutrient supplementation, serum selenium concentration increased, but this increment was not statistically significant. However, without this supplementation its serum concentration further declined in placebo group (Figure 2).

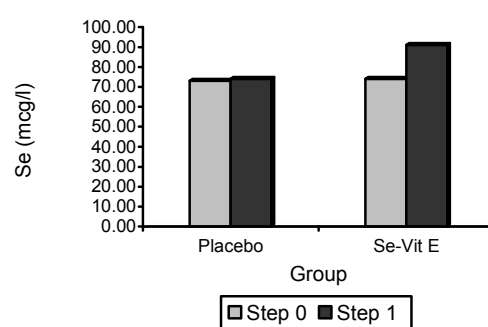


Figure 2. Serum concentrations of selenium in placebo (n=18) and micronutrients groups (n=17). Values are given as median.

There was consistent radiographic improvement from baseline to 2 to 6 months after treatment in both

groups as indicated by the reduction in total area of the lung cavities. However, this trend was more pronounced in the micronutrients group ($P < 0.05$) (Table 3).

Table 3. Radiological data in studied TB patients at 0, 2 and 6 months of anti- TB treatment

Radiologic sign and time of assessment	Micronutrient group (n=17)	Placebo group (n= 18)
No. of cavities (n)		
0 month	3	5
2 month	2	4
6 month	1	3
Cavity surface area (cm²)		
0 month	4.0 (1.0-10.0)	8.0 (4.0-9.0)
2 month	1.5 (0.0- 4.5)	9.0 (4.0- 18.0) ¹
6 month	0.0 (0.0- 2.25)	6.3 (1.0- 15.8) ²
Mean lesion area (cm²)		
0 month	73.8 (10.5- 379)	80.0 (45.0- 249)
2 month	48.8 (5.0- 293)	50.0 (0.0- 247)
6 month	18.0 (0.0- 177)	23.8 (0.0-113)

^{1, 2} Significantly different between the two groups in 2 and 6 mo: $P=0.03$ and $P<0.05$, respectively.

Values are given as median, interquartile ranges, minimum and maximum.

DISCUSSION

To the best of our knowledge, this is the first study demonstrating the effect of concurrent supplementation with vitamin E and selenium on the treatment of pulmonary tuberculosis. The results showed that vitamin E and selenium supplementation hastened the recovery of patients with active pulmonary tuberculosis and this effect was more remarkable after two months of treatment. This supplementation increased the probability of early negative conversion of sputum smear in patients with pulmonary tuberculosis. Micronutrient group demonstrated a significant and sharp improvement in sputum conversion at the end of the first month of antituberculosis treatment compared to the control

group, while the plasma level of vitamin E was increased. Total area of the lung cavitory lesions was decreased to smaller sizes compared to control group. The major benefit of earlier sputum conversion and elimination of acid-fast bacilli may be seen at the community level, as reducing the risk of tuberculosis transmission (17).

Tissue concentration of selenium is directly related to its dietary intake that is of great regional variation depending on the locality in which foods are produced (13). Also the questionnaires demonstrated low intake of selenium and vitamin E-rich dietary sources.

Selenium functions primarily in the form of selenoproteins. At least 30 selenoproteins have been identified including glutathione peroxidase (GPX), selenoprotein P, and thioredoxin reductase that are the major components of the body antioxidant system (18,19). Thioredoxin reductase provides reducing power for several biochemical processes and is responsible for degrading peroxides and hydroperoxides outside cell membranes (20). It has been shown that selenium deficiency increases the risk of developing mycobacterial diseases in HIV-infected individuals by 13-folds (12).

Vitamin E and the selenoprotein, GP, function at two different locations within the cell; glutathione peroxidase in cytosol of the cell and vitamin E within lipid membranes. Although no synergy between vitamin E and selenium has been detected in experimental animal studies (21). Vitamin E and GPX have reciprocal sparing effects on their requirements relative to the intracellular killing of bacteria (13). Vitamin E-mediated protection of lipid membranes may spare the requirement for GPX, by reducing free radicals at the membrane and thereby preventing leakage of free radicals into the cytosol and maintaining intracellular killing capacity of the cell. Conversely, glutathione peroxidase activity in the cytosol may spare the requirement for vitamin E

in the membranes (5). Furthermore, vitamin E and selenium co-administration significantly reduces the tissues levels of malondialdehyde and protein carbonyl (22).

There are several possible explanations for the observed benefit of vitamin E-selenium supplementation on the recovery process of pulmonary tuberculosis patients. First, vitamin E and selenium may improve immune functions through several different pathways. Their deficiencies alter complement system, phagocytic functions, cytokine production, antibody affinity and finally cell-mediated immune responses (23). Vitamin E supplementation has been shown to improve T-cell mediated immune functions and thus delayed-type hypersensitivity skin response in the elderly through increased production of interleukin (IL-2), leading to enhanced proliferation of T cells, and reduced production of prostaglandin E₂, a T-cell suppressive factor (24). Second, vitamin E and selenium are essential antioxidant agents that protect cells from deleterious effects of free radicals generated during disease conditions or natural body metabolisms. Vitamin E is the most important lipid-soluble antioxidant and an integral component of all lipid membranes that serves to protect lipid membranes from attack by ROS (25-28). Combined deficiency of vitamin E and selenium markedly increases oxidative load of immune system and adversely affects its function. Furthermore, α -tocopherol is directly utilized by pulmonary tissue and may act as a free radical scavenger to block the generation of reactive oxygen substrates.

In conclusion, it seems that vitamin E and selenium supplementation in diet has an additional favorable effect on the outcome of tuberculosis therapy that hastens the microbiological conversion from positive to negative and improves clinical and radiological resolution of pulmonary tuberculosis. In our research center due to the limitation in case

selection of this study, further studies in larger trials will be performed.

ACKNOWLEDGMENTS

This study was supported by Tuberculosis and Lung Disease Research Center, Tabriz, Iran.

REFERENCES

1. Kumar P, Clark M. Clinical Medicine. 4th ed, UK, W.B. Saunders, Philadelphia, , 2001, p. 1-6 and 40.
2. World Health organization. Global tuberculosis controls WHO Report 1999. Geneva: World Health organization, 1999.
3. Miller LG, Asch SM, Yu EI, Knowles L, Gelberg L, Davidson P. A population-based survey of tuberculosis symptoms: how atypical are atypical presentations? *Clin Infect Dis* 2000; 30 (2): 293- 9.
4. Chandra RK. Micronutrients and immune functions. An overview. *Ann N Y Acad Sci* 1990; 587: 9- 16.
5. Smith KL, Hogan JS, Weiss WP. Dietary vitamin E and selenium affect mastitis and milk quality. *J Anim Sci* 1997; 75 (6): 1659- 65.
6. van Lettow M, Harries AD, Kumwenda JJ, Zijlstra EE, Clark TD, Taha TE, et al. Micronutrient malnutrition and wasting in adults with pulmonary tuberculosis with and without HIV co-infection in Malawi. *BMC Infect Dis* 2004; 4 (1): 61.
7. Field CJ, Johnson IR, Schley PD. Nutrients and their role in host resistance to infection. *J Leukoc Biol* 2002; 71 (1): 16- 32.
8. Hernanz-Schulman M. Hyperechoic renal medullary pyramids in infants and children. *Radiology* 1991; 181 (1): 9- 11.
9. Plit ML, Theron AJ, Fickl H, van Rensburg CE, Pendel S, Anderson R. Influence of antimicrobial chemotherapy and smoking status on the plasma concentrations of vitamin C, vitamin E, beta-carotene, acute phase reactants, iron and lipid peroxides in patients with pulmonary tuberculosis. *Int J Tuberc Lung Dis* 1998; 2 (7): 590- 6.

10. Vijayamalini M, Manoharan S. Lipid peroxidation, vitamins C, E and reduced glutathione levels in patients with pulmonary tuberculosis. *Cell Biochem Funct* 2004; 22 (1): 19- 22.
11. Brown KM, Arthur JR. Selenium, selenoproteins and human health: a review. *Public Health Nutr* 2001; 4 (2B): 593- 9.
12. Shor-Posner G, Miguez MJ, Pineda LM, Rodriguez A, Ruiz P, Castillo G, et al. 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* 2002; 29 (2): 169- 73.
13. Mahan LK, Escott-Stump S. Krause's Food, Nutrition & Diet Therapy. 11th ed. W.B. Saunders, Philadelphia, 2004: 152-4.
14. World Health Organization. What is DOTS? A guide to understanding the WHO-recommended TB control strategy known as DOTS. Geneva: WHO, 1999.
15. Fidanza F. Nutrition status assessment: A manual for population studies. 1st ed, Chapman and Hall, London, 1991; 191:209, 309-15.
16. Gardiner PH, Littlejohn D, Halls DJ, Fell GS. Direct determination of selenium in human blood serum and plasma by electrothermal atomic absorption spectrometry. *J Trace Elem Med Biol* 1995; 9 (2): 74-81.
17. Appropriate Health resources and Technologies Action group (AHRTAG). Tuberculosis and children: the missing diagnosis. Nottingham. United Kingdom: Russell Press, 1996.
18. Holben DH, Smith AM. The diverse role of selenium within selenoproteins: a review. *J Am Diet Assoc* 1999; 99 (7): 836- 43.
19. Beckett GJ, Arthur JR. Selenium and endocrine systems. *J Endocrinol* 2005; 184 (3): 455- 65.
20. Patrick L. Selenium biochemistry and cancer: a review of the literature. *Altern Med Rev* 2004; 9 (3): 239- 58.
21. Castellini C, Lattaioli P, Bosco AD, Beghelli D. Effect of supranutritional level of dietary alpha-tocopheryl acetate and selenium on rabbit semen. *Theriogenology* 2002; 58 (9): 1723- 32.
22. Chandra RK. Nutrition and the immune system from birth to old age. *Eur J Clin Nutr* 2002; 56 Suppl 3: S73- 6.
23. Gay R, Meydani N. The effect of vitamin E, vitamin B₆, and vitamin B₁₂ on immune function. *Nutr Clin care* 2001; 4(4): 188.
24. Meydani SN, Han SN, Wu D. Vitamin E and immune response in the aged: molecular mechanisms and clinical implications. *Immunol Rev* 2005; 205: 269- 84.
25. Madebo T, Lindtjorn B, Aukrust P, Berge RK. Circulating antioxidants and lipid peroxidation products in untreated tuberculosis patients in Ethiopia. *Am J Clin Nutr* 2003; 78 (1): 117- 22.
26. Plit ML, Theron AJ, Fickl H, van Rensburg CE, Pendel S, Anderson R. Influence of antimicrobial chemotherapy and smoking status on the plasma concentrations of vitamin C, vitamin E, beta-carotene, acute phase reactants, iron and lipid peroxides in patients with pulmonary tuberculosis. *Int J Tuberc Lung Dis* 1998; 2 (7): 590- 6.
27. Jack CI, Jackson MJ, Hind CR. Circulating markers of free radical activity in patients with pulmonary tuberculosis. *Tuberc Lung Dis* 1994; 75 (2): 132- 7.
28. Deveci F, Ilhan N. Plasma malondialdehyde and serum trace element concentrations in patients with active pulmonary tuberculosis. *Biol Trace Elem Res* 2003; 95 (1): 29- 38.