ORIGINAL ARTICLE

Serum Levels of Alpha-Tocopherol, Vitamin C, Beta-Carotene, and Retinol in Malignant Pleural Mesothelioma

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Abstract

The aim of this study was to investigate the possible relationship between antioxidant vitamin levels and malignant pleural mesothelioma (MPM). For this purpose, we measured the serum levels of 4 antioxidant vitamins, β-carotene, α-tocopherol, retinol, and ascorbic acid, in patients with environmentally induced MPM and in healthy controls from one tremolite village (Kureysler), the biggest erionite village (Tuzkoy) and Ankara. A total of 160 subjects were enrolled in the study, 42 (26.3%) diagnosed with MPM and 118 (73.7%) healthy subjects. A comparison was made between the MPM group and three control groups of which two were exposed and one was unexposed to mineral fibers. The study population consisted of 82 males (51%) and 78 females (49%) with a mean of age of 44.8±14 years (range; 20-65 years). Lowest levels of β-carotene, ascorbic acid, and α-tocopherol were found in MPM patients (MPM vs control groups combined, p<0.0001 for each antioxidant vitamin), without any relation to age or sex. There was no significant difference between the antioxidant levels of healthy controls of Tuzkoy and Ankara. In conclusion; our findings suggested an increased risk of MPM being associated with low levels of α-tocopherol and ascorbic acid in patients with MPM.

Keywords: Malignant mesothelioma - β-carotene - α-tocopherol - retinol - ascorbic acid - fibrous mineral fibers

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Introduction

Malignant mesotheliomas are highly aggressive neoplasms arising primarily from the surface serosal cells of the pleural, peritoneal, and pericardial cavities. Malignant pleural mesothelioma (MPM) is frequently caused by environmental and occupational exposure to asbestos. Asbestos fibers play an important role in the pathogenesis of many lung diseases including malignant mesothelioma, lung cancer, asbestosis, pleural calcification, benign pleural effusion, diffuse lung fibrosis, benign asbestos pleurisy and pleural plaques (Yazicioglu et al., 1978; Carbone et al., 2002).

Erionite, a natural fibrous zeolite, which can be found in volcanic tuffs, is an environmental contaminant in the Cappadocia region of Central Anatolia (Emri et al., 2002: 2004). In this region, some villages such as Karain, Tuzkoy, and Sarıhıdır, with environmental exposure to erionite are known as 'erionite villages'. Environmental tremolite type asbestos related pleuro-pulmonary diseases also constitute health problems in some part of rural Anatolia due to domestic usage of white soil to make a whitewash or stucco to surface the walls, floors and roofs of houses and also as a substitute for baby powder and gripe water. Kureysler is a village in the western Turkey where tremolite exposure was detected (Coplu et al., 1996). The high potential of erionite to induce MPM has been confirmed by both epidemiological and experimental studies (Artvinli et al., 1979: 1982; Barış et al., 1981: 1987; Suzuki et al., 1982; Poole et al., 1983; Johnson et al., 1984; Wagner et al., 1985). According to epidemiological studies, the incidence of malignant mesothelioma in erionite villages is 800-1000 times higher than the reported general population incidence elsewhere in the world (Mc Donald et al., 1970: 1977).

Since free radicals have been considered a potential pathogenetic mechanism in asbestos-related pulmonary diseases, one possibly important determinant in the development of lung injury caused by fibers is the antioxidant capacity of the cells (Mossman et al., 1983: 1989a: 1989b: 1990). There are limited information about the relationship between serum level of antioxidants and mesothelioma. In this study, we aimed to compare the serum levels of retinol, β-carotene, ascorbic acid and α-tocopherol between patients with MPM and healthy controls.

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Materials and Methods

Patients and Controls

A total of 160 subjects including 42 subjects with biopsy proven MPM and 118 healthy subjects without MPM from Tuzkoy village (n=59) and Kureysler village (n=35), where the exposure risk to the erionite and tremolite fibers respectively is high, and Ankara (n=24), where the exposure risk is very low, were enrolled in the study. Healthy subjects consisted of people who were performed respiratory function test because of preoperative evaluation and who were relatives of patients admitted to Department of Chest Disease. Forty-two patients with MPM were individually matched to control subjects on the basis of age, sex, smoking status and life-style. A comparison was made between the MPM group and three healthy control groups of which two were exposed (Tuzkoy and Kureysler) and one was unexposed to mineral fibers (Ankara). All patients and healthy control groups are ≥20 years old. Lifestyle factors, habitual dietary intake, and physical activity were similar between and within the groups.

Sample Collection and Preservation

From each patient at the time of diagnosis and healthy age-and sex-matched person, 10 ml of blood from antecubital vein were taken. Blood was always collected while fasting in the morning, and centrifuged at 4°C for 10 min at 2,500 rpm to obtain serum. The serum samples were freshly frozen under nitrogen and stored at -70°C until analysis. Samples were not stored longer than six months. The samples were thawed at room temperature only once at the time of assay.

Determination of Vitamin levels

Serum α-tocopherol concentration was measured by using the method described by Desai et al. (1984). In short, the mixture containing 0.5 ml volume of serum, 0.5 ml of ethanol and 0.25 ml of 25% ascorbic acid was preincubated at 70°C for 5 min. Following incubation, 0.3 ml of saturated potassium hydroxide was added and the mixture was further incubated at 70°C for 30 min. Tubes were cooled immediately in an ice-bath. A 4.0 ml of hexane was added into tubes. Duplicate samples were extracted in glass-stoppered centrifuge tubes, followed by vigorous vortex mixing for 1 min and centrifugation at 1500 rpm for 5-10 min. After separation of phases, hexane extracts were used to estimate the concentration of the vitamin level on a spectrophotometer. Extraction and emission wavelengths were 286 and 330 nm, respectively. Alpha-tocopherol concentration was obtained directly from the standard curve. The intra- and inter-assay of coefficient variation for the measurement were 1.9 and 3.1%, respectively. β-carotene and retinol were determined by using the method of Neeld and Pearson (Neeld et al., 1969). Briefly, duplicate serum samples (0.5 ml) were mixed with 1.0 ml of cold ethanol and then extracted with 2.0 ml of hexane, followed by being vortex mixed for 2 min. β-carotene was determined spectrophotometrically at 450 nm (unicam 8,700 series). Retinol was extracted into petroleum ether in the presence of ethanol and was reacted with trifluoracetic acid, which leads to the dehydrated anhydrovitamin, which was then measured spectrophotometrically at 620 nm (unicam 8,700 series). Both β-carotene and retinol concentrations were obtained directly from their standard curves. Intra- and inter-assays of coefficient variation in β-carotene measurements were calculated as 1.6% and 1.7%, and retinol measurement were 2.6 and 3.1 respectively. Serum ascorbic acid was measured spectrophotometrically at 520 nm after its reaction with 2,6 dichlorophenolindophenol (Kalaycı et al., 2000). The intra- and inter-assays variations for the vitamin C assay in this study were 2.1 and 3.2% respectively.

Statistical Analysis

All statistical analysis was conducted using "SPSS 10.0 for Windows" (SPSS Inc., USA) statistical program. Statistical results were given as mean±standart deviation for continuous variables. Probability values $p \leq 0.05$ were considered statistically significant. Multivariate ANOVA and Student Newman-Keuls tests were used for comparison of mesothelioma patients and controls. Correlations between parameters were studied by using Pearson's test. We also performed logistic regression analysis to investigate the relationship between the levels of antioxidants and risk of mesothelioma occurrence.

Results

One hundred sixty subjects were enrolled in the study, 42 (26.3%) were diagnosed with MPM and 118 (73.7%) were healthy subjects from Tuzkoy (n=59), Kureysler (n=35), and Ankara City (n=24). The study population consisted of 82 males (51%) and 78 females (49%). The mean±SD age was 44.8±14 years (range; 20-65 years). The baseline characteristics age, sex, and smoking status were similar to between all groups (Table 1). For all study population, mean age of female was similar to that of male (44.7±10.1 and 44.9±10.8, respectively; p=0.884). None of the subjects used antioxidant supplementation. The measured serum antioxidant levels are shown in the Table 2 and Figure 1A-1D. There was no correlation between the serum antioxidant levels and age (p= 0.976 for

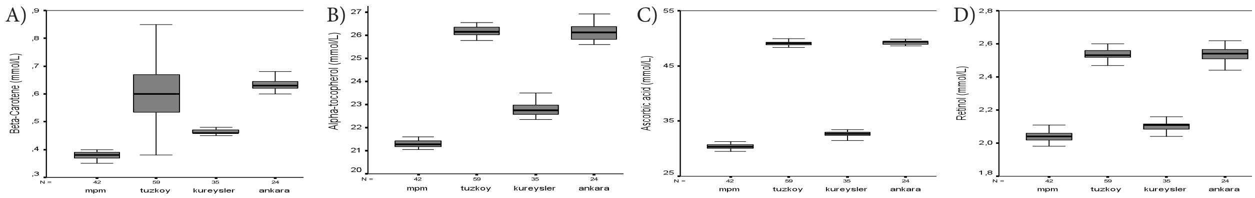

Figure 1. A) Serum β-Carotene Levels between the Groups. B) Serum α-Tocopherol Levels between the Groups. C) Serum Ascorbic Acid Levels between the Groups. D) Serum Retinol Levels between the Groups.

Table 1. Baseline Characteristics of the Study Population

	MPM	Tuzkoy	Kureysler	Ankara	p
Female/ Male (%)	48/52	51/49	46/50	50/50	0.966
Age (mean±sd)	44.7±11.0	45.0±9.6	44.8±9.9	44.6±11.6	0.998
Smoking (n / %)	27/64.3	41/69.5	22/63.0	16/66.7	0.224

Table 2. β-Carotene, α-Tocopherol, Ascorbic Acid and Retinol in Serum by Groups (mean±sd)

Group	n (%)	β-Carotene (μmol/L)	α-Tocopherol (μmol/L)	Ascorbic acid (μmol/L)	Retinol (μmol/L)
Tuzkoy Control	59 (37)	0.62±0.01	26.2±0.2	49.1±0.4	2.53±0.03
Kureysler Control	35 (22)	0.46±0.01	22.8±0.3	32.7±0.6	2.09±0.04
Ankara Control	24 (15)	0.63±0.02	26.2±0.4	49.1±0.7	2.54±0.05
MPM Patients	42 (26)	0.38±0.01	21.3±0.2	30.4±0.5	2.04±0.03

Table 3. Serum Antioxidant Levels According to City and Sex

	Female		Male		p‡
	Mean±sd	p†	Mean±sd	p†	
β-carotene (μmol/L)					
MPM	0.38±0.01	<0.001	0.38±0.01	<0.001	0.444
Tuzkoy	0.62±0.10		0.60±0.09		
Kureysler	0.46±0.01		0.46±0.01		
Ankara	0.63±0.02		0.63±0.02		
α-tocopherol (μmol/L)					
MPM	21.40±0.19	<0.001	21.27±0.16	<0.001	0.737
Tuzkoy	26.14±0.22		26.21±0.21		
Kureysler	22.83±0.30		22.83±0.36		
Ankara	26.03±0.34		26.23±0.40		
Ascorbic acid (μmol/L)					
MPM	30.34±0.40	<0.001	30.43±0.45	<0.001	0.618
Tuzkoy	49.03±0.47		49.07±0.34		
Kureysler	32.82±0.36		32.54±0.43		
Ankara	49.28±0.62		49.14±0.52		
Retinol (μmol/L)					
MPM	2.03±0.03	<0.001	2.04±0.030	<0.001	0.594
Tuzkoy	2.54±0.03		2.53±0.030		
Kureysler	2.11±0.03		2.09±0.040		
Ankara	2.53±0.05		2.55±0.060		

†Within female and male, ‡Between female and male

β-carotene, p=0.862 for ascorbic acid, p=0.773 for retinol, and p=0.770 for α-tocopherol). There was no association between the serum antioxidant levels and sex (Table 3).

The baseline levels of β-carotene, ascorbic acid, retinol, and α-tocopherol were lower in MPM patients than control groups (MPM vs control groups combined, p<0.0001 for each antioxidant vitamins). The lowest serum levels of antioxidants were in MPM group and the highest level was in Ankara control group, but there was no significant difference between antioxidant levels of Tuzkoy and Ankara groups.

For β-carotene, the lowest serum level was measured in MPM group (0.38±0.01). Serum levels of β-carotene were similar in Tuzkoy and Ankara control groups (0.62±0.01 vs 0.63±0.02, p>0.05), but it was different from MPM and Kureysler groups. It was 0.46±0.01 in Kureysler group. In MPM group, serum level of ascorbic acid was significantly lower than the other groups. Serum levels of ascorbic acid in Tuzkoy and Ankara groups were similar (49.1±0.04 vs 49.1±0.07), but it was lower in the other groups. In Ankara and Tuzkoy groups, serum levels of α-tocopherol were significantly higher compared to those of MPM and Kureysler groups. The lowest level of α-tocopherol was in the cancer group (21.3±0.02) and the highest level was in the Tuzkoy group. Serum levels of α-tocopherol were not different between Ankara and Tuzkoy groups (26.2±0.04 vs 26.2±0.02). Similarly, the lowest level of serum retinol was in MPM group (20.4±0.03) and it was different than that the other groups. The observed difference in these serum vitamins levels between controls and the patients with MPM was not due to age and sex.

Table 4. CLogistic Regression Model

Variable	B	S.E.	Wald	df	p
Variables in the Equation					
Ascorbic	0.2975	0.0996	89,329	1	0.0028
Alpha-Tocopherol	0.5443	0.2049	70,589	1	0.0079
Constant	-215,399	53,246	163,651	1	0.0001
Variable	**Exp(B)**	**Lower**	**Upper**		
95% CI for Exp(B)					
Ascorbic acid	13,465	11,079	16,367		
Alpha-Tocopherol	17,235	11,535	25,752		
Variable	**Score**			**df**	**p**
Variables not in the Equation					
Retinol	0.8124			1	0.3674
β-Carotene	0.5863			1	0.4439

The results of the last step of the logistic regression analysis using the forward stepwise method are given in Table 4. From this table, it is observed that the levels of α-tocopherol and ascorbic acid are statistically significant factors on mesothelioma, whereas the levels of retinol and β-carotene have no important affect on mesothelioma. By the logistic regression analysis, we see that a patient, who has low level of ascorbic acid or α-tocopherol, has respectively 1.3465 and 1.7235 times higher risk for mesothelioma than the one who has normal levels. Note that the accuracy of the logistic regression model is 88.24%. This model can be written as **mesothelioma = -21,5399 + 0,2975 ascorbic + 0,5443 tocopherol + e**

where e is the error term of the model. Using this equation, when mesothelioma* >0.5 is obtained for a patient, this patient can be diagnosed as mesothelioma in 88.24% accuracy. **mesothelioma*=1/[1+exp(-mesothelioma)]**

Discussion

Exposure to fibrous mineral fibers associates with an increased risk of malignant mesothelioma, lung cancer, and cancer of other organs. All types of asbestos fiber can cause mesothelioma, but erionite (fibrous zeolite) is the most potent carcinogenic fiber so far (Emri et al., 2002: 2004). Tuzkoy in the Cappadocia region of Central Anatolia is, however, highly rich in erionite. The estimated incidence of MPM was 996 per 100,000 inhabitants in the population exposed to erionite in erionite villages in Cappadocian region (Baris et al., 1987; Albelda et al., 1998). In a recent study the authors was shown that the median survival was 17.0 months for localized disease, 18.4 months for regional disease, and 17.2 months for distant metastasis (Milano et al., 2010).

Several plausible explanations have been proposed as to how asbestos fibers cause malignant change in mesothelium cells (Robinson et al., 2005). These include pleural irritation, interference with mitosis, persistent kinase-mediated signaling and generation of reactive oxygen and nitrogen species (Heintz et al., 2010). Individual variability in the antioxidant and/or detoxifying mechanisms probably has an important role in the development of asbestos-related lung disease. Currently, the data regarding the relationship between antioxidant vitamins such as β-carotene, α-tocopherol, retinol, and ascorbic acid and mesothelioma is limited. The aim of this study was, therefore, to investigate the possible changes in antioxidant vitamin levels and their relationship to the oxidative stress produced by mesothelioma. For this purpose, we measured the serum levels of anti-oxidant vitamins, β-carotene, α-tocopherol, retinol, and ascorbic acid in the patients with mesothelioma and the healthy subjects.

In the last few years, the relationship between the antioxidants and malignancies such as lung cancer was clinically studied. Antioxidants were used as prophylaxis in patients with high risk of cancer. The CARET study (The Carotene and Retinol Efficacy Trial) was a large two-arm study that was designed to compare the effects of a combination of β-carotene and retinol with those of placebo on lung cancer incidence in subjects who were at high risk for the development of lung cancer (Omenn et al., 1996). The study was stopped in January 1996 because participitants who were in active intervention group were found increased lung cancer, death, and cardiovascular disease frequency compared with placebo group. The results of the study showed that there was an inverse relationship between cigarette smokers and β-carotene levels. The use of β-carotene was not shown to prevent lung cancer. A pilot study of 1,029 high-risk persons demonstrated the safety and tolerability of β-carotene 50 mg/day, retinol 25,000 IU/day and the combination of the two (Goodman et al., 1993). The ATBC trial was designed on the effect of α-tocopherol and β-carotene on the incidence of lung cancer. No effect of α-tocopherol on lung cancer incidence was observed but β-carotene arm was associated with the increased lung cancer (Heiononen et al., 1994). However, some study showed that there was no significant difference in cancer incidence with β-carotene supplementation (Lee et al., 1999; Cook et al., 2000). A recent meta-analysis of randomized controlled trials showed that antioxidant supplements had no effects on either primary or secondary prevention of cancer (Myung et al., 2010).

There are no enough studies about to use of antioxidants to prevent mesothelioma in people with known asbestos exposure. In Australian study where 1,024 asbestos workers were enrolled in, the subjects were randomized to either β-carotene or retinol groups (De Klerk et al., 1998). After the follow-up period, no difference in lung cancer incidence was found, but the authors found that daily retinol was associated with lower rate of mesothelioma in crocidolite-exposed individuals, compared with daily β-carotene, which seemed to have no significant effect. In another study, the relationships between plasma concentrations of retinol, β-carotene and vitamin E and the incidence of mesothelioma and lung cancer were examined (Alfonso et al., 2006). The results of study did not show any significant relationship between the plasma levels of vitamin E and β-carotene with either mesothelioma or lung cancer. But, low plasma levels of retinol associated with very modest increased risk of developing mesothelioma and lung cancer. However, a recent experimental study revealed that dietary supplementation with the antioxidant vitamins A and E, and selenium did not alter the rate of mesothelioma development and survival in transgenic mouse model (Robinson et al., 2012).

In this study, we compared to the serum vitamin levels in patients with MPM and healthy control groups from two different regions with high risk of mesothelioma. The serum levels of all antioxidants were significantly lower in mesothelioma patients than those in the control groups. The antioxidant levels in Tuzkoy and Ankara control groups were similar. Logistic regression analysis indicated that low levels of α-tocopherol and ascorbic acid in patients living in this area leads to high risk of mesothelioma than the others. But, those of lower levels of retinol and β-carotene have no such higher risk of mesothelioma. Interestingly, the serum levels of all antioxidants were lower in Kureysler group than the other control groups, but higher than MPM group. We asked habitual dietary of the people in this region and learned that total consumption of dietary vegetables and fruits was lower in Kureysler village than Ankara and Tuzkoy region. The lower serum levels of antioxidants in Kureysler group may be due to less frequent consumption of vegetables and fruits, which is rich from antioxidants. While smoking does not increase the risk of mesothelioma, smoking may modify the association between serum antioxidant levels of risk of MPM. According to a recent survey, the proportion of smokers who live in urban areas is higher than the smokers in rural part of Turkey. There is no regional difference for the male smokers (48%). However, the proportion of female smokers is lower in rural areas (7%) compared to urban areas (19%). In our study population, smoking rates were similar with the findings according to the results of Global Adult Tobacco Survey conducted in November 2008 (http://www.cdc.gov/tobacco/global/gats/countries/eur/fact_sheets/turkey/2009/pdfs/turkey_2009.pdf).

Vitamin C, also known as ascorbate, has been showed strongly cytotoxic to different mesothelioma cell lines (Martinotti et al., 2011; Ranzoto et al., 2011), and in vitro drug cytotoxicity tests revealed synergistic effects of ascorbate/gemcitabine combinations.

This is a limitation of our study along with other potential confounders such as stage of the mesothelioma for cases and documentation of histological subgroups and nutritional status of the cases such as body mass index, and malnutrition parameters.

In conclusion, in summary, our findings suggested that levels of α-tocopherol and ascorbic acid were lower in patients with MPM than the others. The number of enrolled patients to the study was limited. The results should be supported by other studies with high number of patients.

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