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Evaluation of paraoxonase-1 enzyme activity and oxidative stress relations in malignant mesothelioma cases

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Abstract:

BACKGROUND: Malignant pleural mesothelioma (MPM) is the most common cancer in the pleura and highly aggressive with a very poor prognosis. Asbestos, known as a carcinogenic mineral with fiber structures, is the main cause of MPM formation. Exposure to asbestos causes an increase in reactive oxygen species, deficiency of antioxidant enzyme levels, and DNA damage. As a result of asbestos pathogenesis, all of these changes cause pulmonary fibrosis, pleural diseases, and malignancies. The endogenous antioxidant paraoxonase-1 (PON-1) is a calcium-dependent esterase involved in the hydrolysis of lipid peroxides, and PON-1 has been shown to have protective properties in oxidative stress and inflammatory diseases in various studies.

OBJECTIVE: The study aimed to examine the relationship of MPM with PON-1 enzyme activity and oxidative status using total oxidant status (TOS) and total antioxidant status (TAS).

MATERIALS AND METHODS: The study population was formed of 33 retrospectively examined mesothelioma patients as MPM group and 33 age- and sex-matched healthy individuals as controls. PON-1 activity was measured spectrophotometrically by enzyme-linked immunosorbent assay method. Total antioxidant and oxidant status was determined using Rel Assay Diagnostics kit. Oxidative stress index (OSI) was estimated as the ratio of the TOS to the TAS levels.

RESULTS: In the present study, PON-1, TOS, TAS, and OSI levels were adjusted by comorbidity and smoking. The results indicated that TOS and OSI of MPM patients increased compared to healthy controls ($P < 0.001$ for both). The results also demonstrated the decrease of PON-1 activity and TAS in MPM cases ($P < 0.001$, for both).

CONCLUSION: These results suggested that oxidative stress occurring as a result of inhalation of asbestos fibers may reduce the level of PON-1.

Keywords:

Malignant pleural mesothelioma, oxidative stress index, paraoxonase-1, total antioxidant status, total oxidant status

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Introduction

Malignant pleural mesothelioma (MPM) is primary malignant tumors with poor prognosis and originates from the mesothelium lining of the pleura. It has been reported that approximately 80% of patients diagnosed with MPM have been exposed to asbestos in environmental and/or occupational aspects.[1-3] Asbestos is a group of naturally occurring fibrous silicate mineral fibers characterized with a ≥3:1 length to diameter ratio.[4-6] It is known as a carcinogenic mineral, and its fibers induce the generation of reactive oxygen species (ROS) and reactive nitric species that are included in the pathogenesis of asbestos-related diseases.[7] Especially ROS production is known as a second messenger of asbestos toxicity and results in pulmonary fibrosis, pleural diseases, and malignancies.[8] Exposure to asbestos allows the production of ROS in different ways. ROS can be produced as a result of the interaction of the asbestos fibers with the surface or can be released as a result of the stimulation of the inflammatory cells, as well as mitochondrial-derived ROS release in other affected target cells such as mesothelial cells. The elevation of oxidative stress is occurred by the increased ROS production and reversal/inadequacy of the antioxidant enzymes. The increase in oxidative stress causes p53 activation and DNA damage.[5] Moreover, several studies have shown that ROS triggers apoptosis, inflammation, and cell damage.[6,8] Recent studies indicate that increased production of ROS caused by asbestos plays an important role in MPM pathogenesis. Especially asbestos stimulates breakage of DNA strand through iron-catalyzed free radicals. Several events that progress the mutations in cells are also indirectly affected by ROS production of asbestos fibers.[1,8,9]

Several studies have shown that the failure of antioxidants and/or related systems, resulting in oxidative stress is associated with cancer development.[10,11] The endogenous antioxidant paraoxonase-1 (PON-1) is a calcium-dependent esterase with lipophilic antioxidant property and widely distributed among tissues such as the liver, kidney, intestine, and serum.[12,13] PON-1 binds to high-density lipoprotein (HDL) and hydrolyzes toxic metabolites of insecticides, nerve agents, and aromatic esters such as lactones.[14] It protects low-density lipoprotein molecules from oxidation through hydrolysis. PON-1 also considerably hydrolyze hydrogen peroxide and lipid-soluble radicals from lipid peroxidation due to structural similarities to lactones.[14-17] Due to its ability to hydrolyze oxidized metabolites, PON-1 has been shown to have protective properties in oxidative stress and inflammatory diseases in various studies. Although many clinical and animal studies demonstrated that PON-1 has a role for protection against oxidative stress,[10] there are relatively limited studies on PON-1 activity in cancer. In particular, associated with mesothelioma, PON-1 serum activity has not been reported elsewhere.

Accordingly, the objectives of the present study were to determine the relationship of MPM with PON-1 enzyme activity and oxidative status using total antioxidant status (TAS) and total oxidant status (TOS).

Materials and Methods

Subjects and study design

Adhering to the objective, the study population was formed of retrospectively examined 33 healthy individuals who did not smoke and did not have any chronic disease such as diabetes, cardiovascular disease, or any cancer type (16 males and 17 females) as a control and 33 mesothelioma patients with asbestos exposure, consisting of 16 males and 17 females who admitted to and definitively diagnosed by histopathological as MPM in Eskişehir Osmangazi University, Faculty of Medicine Lung and Pleural Cancer Research and Application Center as MPM group. None of the participants received any treatments before the blood collection. The study was approved by the Ethics Committee of the Medical Faculty in Eskisehir Osmangazi University, Turkey, and written informed consent was obtained from each participant before the study according to the Helsinki Declaration (ethics committee approval, January 11, 2016; 80558721/01).

Sample collection

Peripheral blood samples were collected by needle venipuncture in serum tubes and allowed to clot for 30 min. After centrifugation at 3000 rpm for 10 min, serum was separated and stored at −80°C until measurement.

Measurement of paraoxonase-1 activity, total antioxidant status, and total oxidant status

PON-1 activity was measured by commercially available enzyme-linked immunosorbent assay kit with antihuman PON-1 monospecific antibodies spectrophotometrically at a wavelength of 450 nm (USCN Life Sciences Inc., Wuhan, China). The activity of PON-1 in the samples was then determined by comparing the optical density (OD) of the samples to the standard curve.

TAS and TOS were measured using the Rel Assay Diagnostics Kit (Rel Assay®, Diagnostics kits, Mega Tıp, Gaziantep, Turkey) spectrophotometrically at 520 nm as described previously.[18,19]

The total oxidant assay kit principle is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidant species in acidic medium and the

measurement of the ferric ion by xylenol orange. Briefly, oxidants in the sample oxidize the ferrous ion–o-dianisidine complex to ferric ion. Glycerol molecule in the reaction medium is used for the prolongation of the oxidation reaction. The ferric ion forms a colored complex with xylenol orange in an acidic medium. Spectrophotometrically, the evaluation of the color intensity is related to the total amount of oxidant molecules in the sample. The assay is calibrated with hydrogen peroxide, and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2Equiv./L).[19]

The principle of the total antioxidant assay kit is based on the decolorization of dark blue-green-colored 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid [ABTS]) cationic radical to a colorless reduced form of ABTS by antioxidant molecules in the sample. The decolorization rate is proportional to the amount of the antioxidant molecule in the sample. The assay is calibrated with a stable antioxidant standard solution, which is conventionally named as Trolox equivalent, a Vitamin E analog. The data were expressed as μmol Trolox equivalents/L.[18,19]

Oxidative stress index calculation

Oxidative stress index (OSI) is defined as the ratio of the TOS to the TAS levels. OSI value was calculated according to the following formula: OSI (Arbitrary Unit) = TOS/TAS.[20]

Statistical analysis

The Kolmogorov–Smirnov test was used to verify the normality of the distribution of continuous variables. Normally distributed continuous variables were compared using the Student's *t*-test, and Mann–Whitney U-test was used for the nonnormally distributed continuous variables in independent samples. All results are expressed as mean ± standard deviation. IBM SPSS Statistics 21 software (Statistical Package for the Social Sciences; SPSS Inc., Chicago, IL, USA) was used for the statistical analyses, and $P < 0.05$ was considered statistically significant.

Results

The statistical study made regarding the age and gender of the individuals forming groups showed no significant differences between the groups in terms of age and gender ($P = 0.97$, $P = 1$, respectively). The mean age of MPM group is 63.51 ± 11.25 years and the mean age of the control group is 63.45 ± 5.81. Table 1 demonstrates the characteristics of the MPM and healthy groups. While none of the healthy individuals participated in the study smoked, 19.7% of the individuals in the MPM group smoked ($P < 0.001$). In addition, none of the healthy individuals included in the study had a different secondary disease, whereas 51.5% of the patients in the MPM group had comorbid diseases that could affect oxidative stress such as hypertension, diabetes, atherosclerosis, and psoriasis ($P < 0.001$). Table 2 demonstrates the PON-1, TAS, TOS, and OSI levels in the MPM and control groups that were adjusted by comorbidity and smoking. As shown in Table 2, the MPM group exhibited higher TOS and OSI levels compared to control group ($P < 0.001$, both). The results of the study also demonstrated that PON-1 activity and TAS decreased in MPM cases ($P < 0.001$, both).

Table 1: Characteristics of the malignant pleural mesothelioma and healthy groups

	Healthy (%)	MPM (%)	*P*
Age	63.45±5.81	63.51±11.25	0.97*
Sex			
Male	16 (48.48)	16 (48.48)	1.00**
Female	17 (51.52)	17 (51.52)	
Smoking status			
Yes	0 (0)	13 (19.7)	<0.001**
No	33 (100)	20 (80.3)	
Comorbidity			
Yes	0 (0)	17 (51.5)	<0.001**
No	33 (100)	16 (48.5)	

*Student's *t*-test, **Pearson χ^2. Categorical variables are expressed as *n* (%), numerical variables are expressed as mean±SD, SD: Standard deviation, MPM: Malignant pleural mesothelioma

Discussion

During the process between asbestos exposure and tumor development, mesothelial cells are widely sensitive to various processes (toxicity, pathogenetic alteration, and oncogenic transformations) that contribute to cancer formation.[1,21] Recent studies are reported that MPM pathogenesis is closely associated with oxidative stress. There are a lot of articles about oxidative stress caused by free radicals which play critical roles in the development of asbestos-induced mesothelioma. The mutagenic effects of free radicals produced on the surface of asbestos fibers in the pleura, oxygen radicals released from damaged cells as a result of lipid peroxidation, and oxidative DNA damage caused by direct physical effects of asbestos fibers increase the risk of mesothelioma formation. In addition, asbestos fibers-surface interaction and/or stimulation of the inflammatory cells leads to excessive ROS production.[5,17,22,23] Superoxide anion, hydrogen peroxide, and hydroxyl radical are the most important reactive metabolites contributing the asbestos-related lung diseases.[6,24]

Oxidative stress occurs in cells through antioxidant-oxidant imbalance.[21] Previous studies on cell culture and animals have shown that asbestos exposure reduces antioxidant enzymes while increasing oxidant markers.[8,9,25,26] In the

Table 2: The paraoxonase-1, total oxidant status, total antioxidant status, and oxidative stress index levels in the malignant pleural mesothelioma and healthy groups

	PON-1 (μg/ml)	TOS (μmol H_2O_2 equivalents/L)	TAS (μmol Trolox equivalents/L)	OSI
Healthy	160.27±0.41	6.29±0.04	0.53±0.004	12.48±0.11
MPM	130.66±4.47	135.44±4.86	0.39±0.07	504.34±15.31
P	<0.001*	<0.001**	0.001**	<0.001**

*Student's *t*-test; **Mann–Whitney U-test. PON-1, TOS, TAS, and OSI levels were adjusted by comorbidity and smoking. PON-1: Paraoxonase-1, TOS: Total oxidant status, TAS: Total antioxidant status, OSI: Oxidative stress index, MPM: Malignant pleural mesothelioma

present study, compared to healthy individuals, MPM group has increased TOS and decreased TAS values which are consistent with the previous studies. These results indicate that as a result of exposure to asbestos, the increase in oxygen radicals and inadequacy of the antioxidants for scavenging the ROS generate oxidative stress. This is also supported by the assessment of the OSI value that demonstrated the higher OSI of MPM group compared to controls.

The evaluation of PON-1 enzyme activity and detection of the polymorphisms in the genes coding PON-1 have become popular research subjects for oxidative stress-related diseases.[10] PON-1 polymorphisms that participated in enzyme production have a role to the increased risk of cancer depending on pollutants and other environmental chemicals.[27] Selek *et al*. demonstrated that serum HDL and PON1 activities were decreased, whereas oxidative stress was increased in pulmonary tuberculosis patients.[28] Elkiran *et al*. reported that lung cancer patients have lower serum PON-1 activity than healthy individuals.[15] The result of the current study was also indicated that PON-1 enzyme activity was decreased in MPM patients as in lung cancer patients. These results suggested that oxidative stress occurring as a result of inhalation of asbestos fibers may reduce the level of PON-1, which could be related to increased lipid peroxidation and oxidative stress.

Conclusion

To our knowledge, this is the first study showing oxidative stress and PON-1 enzyme association in MPM. The decreased level of the PON-1 having a protective effect against the oxidative stress and elevated level of oxidative stress indicated the possible relationship between MPM and PON-1 in our study. PON-1 protects against oxidative stress and inflammatory diseases because of the ability to hydrolyze oxidized metabolites.

Further researches, including larger cohorts of patients and multicentered studies, are needed to explore the possible mechanisms that can be specified as an alternative in the treatment of the disease and to identify the molecules involved in oxidative stress mechanisms associated with MPM.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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