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Evaluation of manganese superoxide dismutase and thioredoxin2 levels in asbestos-induced pleural mesothelioma

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Abstract

Asbestos is a mineral known as human carcinogenic material. Exposure to asbestos both in an occupational and environmental way causes asbestosis and mesothelioma. ROS contributes to the development of pulmonary-toxicity induced asbestos.We aimed to determine the levels of important mitochondrial substances such as manganese superoxide dismutase (MnSOD) and Thioredoxin (Trx2) in asbestosis and mesothelioma patients. The study was performed with the patients admitted to outpatient clinics of Chest Diseases and Thoracic Surgery, at Medicine Faculty, Bozok University. Group 1 (healthy control group, n=27): Consisting of healthy individuals (54.18±9.89 years old), Group 2 (patients group, n=34): Evaluation of clinical, pathological and radiological analysis, patients who defined as mesothelioma and/or pleural plaques and asbestosis (60.24±15.24 years old). Patients, who were not biopsied or not available for biopsy due to comorbid diseases, were not included in the study.Biochemical analysis was done in Selcuk University Medicine Faculty Research Laboratories.Serum Trx2 and MnSOD levels were determined by the Elisa method. The results of Trx2 were calculated as pg/µg protein. MnSOD samples were determined as ng/µg protein. MnSOD and Trx2 levels in the patients' group were statistically lower than the levels of the healthy group (p=0.000, p=0.048), respectively.Trx2 levels were 1.74±0.33 pg/µg protein in a healthy group whereas were 0.89±0.10 pg/µg protein in asbestosis and mesothelioma group. Serum MnSOD levels were 1.38±0.24 and 0.29±0.11 ng/µg protein in healthy and patients' groups, respectively.These significant changes in malignant mesothelioma patients reflect the impairment of the oxidant-antioxidant balance system. The study presents basic findings for the clinical meaning of mentioned biochemical parameters in mesothelioma patients.

Keywords: Asbestosis, mesothelioma, mitochondrial oxidative, manganese superoxide dismutase, thioredoxin2**.**

Introduction

Asbestos is a well-known human carcinogenic mineral [1]. It is a fibrous silicate and used for optimal material for various construction and covering purposes. The workers are continuously exposed to asbestos who are working to produce these materials. Therefore, asbestosis includes occupational disease classification [1,2].

Turkey Mesothelioma Working Group indicates that all forms of asbestos are serious due to the cause of mortality and morbidity in exposure population and asbestos causes various health problems in Anatolia.

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Exposure is arising from the use of white soil especially in the rural areas. So that, asbestosis is thought not only an occupational disease also is an environmental disease which is thought to occur as exposure to the presence of asbestos-mixed ground plastered and/or roofed. Turkey Mesothelioma Working Group has shown the most intensive region in Turkey which leads to exposure of asbestos as an environmental way; Eskisehir-Mihaliccik, Konya-Eregli, Cankiri-Ilgaz, Yozgat-Sorgun, Sivas. According to 30 provinces in Turkey, the number of mesothelioma cases was determined as 7.787 people for 5 years. In that findings 64.7% male and 58.5% female died [2].

Exposure to asbestos can cause pulmonary fibrosis (asbestosis), pleural abnormalities (effusion and plaques), and malignancies (bronchogenic carcinoma and mesothelioma). Asbestos causes oxidative stress. There are at least three sources of Reactive oxygen

species (ROS) production, including; fiber surface reactivity, release from alveolar macrophage cells, and mitochondria-derived ROS released from inflammatory, lung epithelial cells and mesothelial cells [3]. It has been determined that asbestosis increases the form of nitric oxide species that probably leads to DNA damage and subsequently stimulatesthe development of pulmonary toxicity [4-9]. Superoxide dismutase (SOD), Glutathione peroxidase (GSH-Px), and catalase (CAT) enzyme systems decrease ROS levels,known as complex defense mechanisms against oxidative damage. SOD is a metalloenzyme, an antioxidant that dismute superoxide radicals to hydrogen peroxide (H_2O_2) which is further detoxified by GSH-Px and CATwith the cyclic oxidation and reduction reactions [4,6-9]. The SOD family has 3 forms: Cu-Zn type is the extracellular SOD_3, and the cytoplasmic form is SOD_1, and the mitochondrial Mn (manganese) type SOD_2. Mn-SOD_2 is a homotetramer with each subunit containing an active site surrounding Mn ion. SOD_2 may suppress cell division and cancer growth in tumor cells [8]. Tumor cells have a deficient system for tolerating H_2O2 production from dismutation whereas healthy cells can tolerant [9]. MnSOD has one of the fastest and most efficient reaction rates of all enzymes, with a kcat of 40,000 s^{-1} and a kcat/KM close to 109 $M^{-1}s^{-1}$ [10]. Therefore, The MnSOD form is the most important and valuable enzyme analyzed in the evaluation of mitochondrial oxidative.

As it is known the thioredoxin system consists of Thioredoxin reductase (TrxR) and its main substrate, thioredoxin (Trx/TRX/Tx) [11,12]. The activity of the TrxR enzyme is regulated by NADPH which is produced by the enzyme glucose-6-phosphate dehydrogenase (G6PD) [13].

The Trx / TrxR system reduces free radicals and acts in oxidized ascorbate recycle. TrxR catalysis the enzymatic metabolic process of electrons to oxidized thioredoxin in the presence of NADPH, therefore, thioredoxins are redox-active proteins involved in the oxidation/ reduction reactions of cells [13-18]. Mammalian cells have two distinct forms:Trx1 found in cytoplasm and nuclei. Trx2(mtTrx) is a mitochondrial form. In Trx2 deficient cells, cytochrome C releases from mitochondria into the cytoplasm and activates caspases. It suggests that Trx2 may be involved in the regulation of the mitochondria-mediated apoptosis signal pathway [14].

Trx is an antioxidant protein expressed in most of the tissue [19] and is multifunctional proteins. Expressions of Trx and TrxR were observed in various cell types of the mammalian, including skin keratinocytes, placental cells, liver cells, secretor cells, and leukocytes. A physiological stimulus such as UV light, H_2O_2 and mitogens, viral infection, X-ray irradiation, and chemical carcinogens can stimulate Trx and TrxR expression [14, 20].

Today, mesothelioma is aggressive cancer that is resistant to oncological treatment [1,3,21-23]. So that, exposure to asbestos in an environmental way is a common problem worldwide, including Turkey. Number of the malignant mesothelioma patients continues with increasing acceleration. Butthere are still limited studies investigating basic molecular mechanisms such as redox balance, oxidant-antioxidant mechanism on asbestosis, and mesothelioma. Especially, the mitochondrial form of Trx2 did not analyze in previous studies. Seen from this aspect, we aimed to analyze MnSOD and Trx2 concentrations to figure out the mitochondrial process in asbestosis and mesothelioma patients.

Materials and Methods

Patients

The volunteers who were admitted to Bozok University, Faculty of Medicine, Thoracic Surgery Department, and Chest Disease Department have been included in the study. All the volunteers signed the "Informed Patient Consent Form" as written informed consent conformably to the ethical standards and the Declaration of Helsinki Principles.This study was conducted with the permission of Bozok University, Faculty of Medicine Clinical Research Ethics Committee with the decision number of 15/05 (decision date:21.08.2014). Asbestos exposure begins at birth in rural areas as an environmental way and continues all the life in the Yozgat region of Turkey. Thus, patients included in this study that was from the Yozgat region and live there during the study. The volunteers for both groups who had a chronic illness, the active infection was not included in the study.

Healthy individuals (14 females, 13 male) without any chronic disease and regular use of drugs were designed as Group 1 (n=27, 54.18±9.89 years old).

Patients individuals (17 females, 17 males) after evaluated by clinical, pathological, and radiological analysis who were diagnosed as mesothelioma and/or pleural plaques, and asbestosis and followed up in Bozok University were designed as Group 2 (n=34, 60.24± 15.24 years old). Due to the presence of radiological pleural findings in all of these patients, malign mesothelioma was suspected. So that patient who underwent biopsy were taken into the study. Therefore, we included the patients in group 2 who were diagnosed as a result of the pleural biopsy. Patients,who were not biopsied or not available for biopsy due to comorbid diseases, were not included in the study.

Blood Samples

For the biochemical analysis, no extra blood samples were taken from the patients. The serum samples were used which is taken for their routine analysis. The serum samples were taken at the Bozok University Department of Biochemistry Laboratory. Venous blood samples were collected into Vacutainer-venous blood collection tubes (BD Diagnostic, Preanalytical Systems, USA) and centrifuged at 2000 g for 20 min.After centrifugation, the serum was collected into Eppendorf tubes.

Biochemical analysis

The transfer of the samples was provided with ice batteries placed in the blood tube carrying bag in about 16 hours (cold chain transport) to the Selcuk University, Faculty of Medicine Research Laboratories to perform the biochemical analysis.Then serum samples were kept at -80^{0}C until analyzing.Serum Trx2 levels were determined byElisa commercial test kit with a quantitative sandwich enzyme immunoassay technique (MyBiosource Inc, cat no: MBS919412, San Diego, USA).Intra-assay Precision is CV%<8%. Inter-assay Precision is CV%<10%. The detection range of the test is 31.25-2000 pg/mL. The results of Trx2 were calculated as pg/µg protein. Protein analyzes were carried out with a colorimetric commercial test kit (Abcam, cat. no: ab102536) byReader BMG LABTECH (GERMANY) at 562nm wavelength.

It was calculated as µg/mL. MnSODanalysis was done by Elisa commercial test kit (Abcam, cat no: ab119694). The preliminary preparations of the samples were prepared according to the procedure. Measurements were made at 450 nm for 1-minute intervals for 15 minutes. MnSOD samples were determined as ng/µg protein. The detection range of the test was 4-500 ng/mL. All analysis was performed with Elisa Reader BMG LABTECH (Germany) and Elisa Washer as Rayto Microplate washer (RT-2600, China).

Statistical Analysis

Statistical analyses were carried out with the SPSS program (version 22.0). The results were described as mean±SE. Independent-T test was used to compare differences between two independent groups when normally distributed. Mann-Whitney U test was used when the groups were not normally distributed. Outcomes were statistically evaluated at 0.05 significant level (95% confidence level).

Results

In the present study the mean age of the individuals in group 1 was 54.18±9.89 years old and in group 2 was 60.24±15.24 years old. There were no significant differences between the groups (p>0.05). Gender distributions were equal in both groups. The MnSOD median values of the patient's group were 0.14 ng/µg protein, whereas the healthy group has 0.89 ng/µg protein. The minimum levels of patients and the healthy groups were 0.02 and 0.06 ng/µg protein whereas the maximum levels of patients and the healthy group were 4.15 and 4.40 ng/µg protein, respectively (Table 1).

As shown in Figure 1, MnSOD concentrations (mean±SE) were higher in the healthy group (1.38±0.24 ng/µg protein) than mesothelioma group (0.29±0.11 ng/µg protein) (p=0.000).

In Table, The Trx2 median values of control and patients groups were 1.35 and 0.74 pg/µg protein, respectively. The minimum values of the control group were 0.18 whereas maximum levels 7.76pg/µg protein. We determined in patients group minimum level as 0.06 and maximum level as 2.16 pg/µg protein.

Trx2 concentrations were lower in patients (0.89±0.10 pg/µg protein) than the healthy group (1.74±0.33 pg/µg protein) and the differences between the groups were statistically important (p=0.048). (Figure 1.)

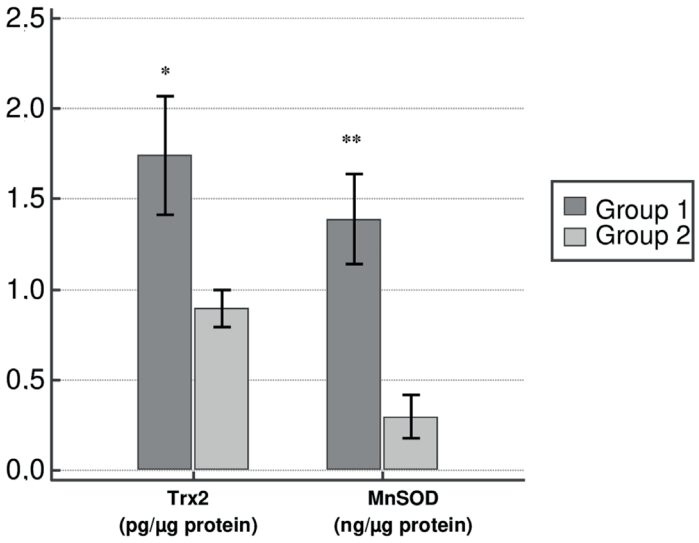

Figure 1. Concentrations of serumMnSOD and Trx2 (Mean±SE)
*Significant differences versus group 1(healthy group) at p=0.048 ≤ α=0.05
**Significant differences versus group 1 (healthy group) at p=0.000 ≤ α=0.05

Discussion

In the present study, to determine the levels of MnSOD and Trx2 in asbestosis and mesothelioma patients was aimed. Followingthis purpose, we observed that two important mitochondrial biochemical parameters as MnSOD and Trx2 have been altered. We think that mitochondrial enzymes may guide future studies and contribute to understanding the basic mechanisms of disease.

It is known that ROS are widely analyzed to explain the oxidative damage in these diseases. Oxidative stress generated by asbestos fibers causes both alterations in antioxidant enzymes and DNA damage in all the relevant target cells (e.g., lung epithelium, and mesothelial cells) in vitro and, in vivo [3].

A significant change in antioxidant enzymes' activitieswas observed during the process of carcinogenesis.Although studies on this subject continuing to investigatebased on biological science in recent years, there is no common conclusion that whether there is the expression of these enzymes decreases or increases. Studies show different and contrast findings, and there are still question marks in this regard.

It is well known that 90% of cellular ROS generation locates in the mitochondrial matrix, MnSOD resides [9] and Mn porphyrins

Table 1. Age, gender distribution and values of MnSOD and Trx2 in Group 1 and Group 2

	Group 1	Group 2	P value	
Age (years old)	54.18±9.89	60.24± 15.24	p>0.05	
Gender distribution	14 female, 13 male	17 female, 17 male	p>0.05	
	MnSOD (ng/µg protein)		**Trx2 (pg/µg protein)**	
	Group 1(n=27)	**Group 2(n=34)**	**Group 1 (n=27)**	**Group 2(n=34)**
Minimum-Maximum	0.06-4.40	0.02-4.15	0.18-7.76	0.06-2.16
Median	0.89	0.14	1.35	0.74
Mean±SE	1.38±0.24	0.29±0.11**	1.74±0.33	0.89±0.10*

*Significant differences versus group 1(healthy group) at p=0.048 ≤ α=0.05
**Significant differences versus group 1 (healthy group) at p=0.000 ≤ α=0.05

are attractive because they lack antigenicity, are extremely stable, andscavenge other ROS such as peroxynitrite[24].

Important research was performed by Zalewska-Ziob et al [4]. They indicated that lung tumor cells had always low MnSOD activity, while had usually low Cu/ZnSOD activity, and almost always low CAT activity compared with the normal tissues [4]. Tumor cells have a deficient system for tolerating H_2O_2 production from dismutation whereas healthy cells can tolerant [9]. However, Hillegasset al. [23] postulated that early increases in the expression of proteinsand the activity of MnSOD occur in human mesothelial cells after exposure to malignant mesothelial-inducing fibers. Thus, increases in H_2O_2 steady-state levels can subsequently lead to increases in cell proliferation, invasion, migration, metastasis, and resistance to apoptosis. They suggested that MnSOD may be a potential biomarker of early response to minerals capable of malignant mesothelioma [23]. Hasagawa et al [25] studied extensive research with different mesothelioma cell lines. It is found highly expressed Mn-SOD compared with MeT-5A cells, and very high expression of the enzyme with a robust activity was observed in the two mesothelioma cells (NCI-H226, NCI-H2452) containing a large amount of Mn [25]. They suggested that expressions of MnSOD altered and showed differencesin each cell line. Furthermore, Kahloset al. [26] found high MnSOD is characteristic of human malignant mesothelioma, and MnSOD immunochemistry can aid the differential diagnosis of mesothelioma [26]. Regarding our result, MnSOD concentrations in the patients' group were approximately 4.75-fold lower than healthy subjects. So that our findings are similar to Zalewsko-Ziobet al. [4]'s findings. The other researchers [23, 25, 26] especially studied different methods and biological samples that is why our findings contrast with them. Based on the findings of us and the researchers mentioned above, we can argue as the following: In early response, MnSOD activities' are increasing [23,25] according to evidence but, in progressive respond, MnSOD doesn't active much moreand inhibition of MnSOD is important due to being a major antioxidant enzyme in mitochondria and this inhibition can result in an increased leak of ROS from respiratory chain. Therefore, an oxidant-antioxidant balance degenerates in mesothelioma and asbestosis patients, caused by asbestos.

Another biochemical parameter analyzed in our study was Thioredoxin2 (Trx2). As it is known, Trx independently inhibits apoptosis, stimulates cell proliferation and angiogenesis, and increases transcription factor activity [11,13,27-29].

Thioredoxins play a role in all of these stages and are considered as an important factor for DNA damage caused by oxidative stress. The selenol group of TrxR can act as a primary sensor for mutagenic H_2O_2 and initiates a signal flow that leads to the transcription of genes encoding antioxidative proteins [30]. Plasma levels of Trx are an indicative inflammatory response against oxidative stress [14]. Asbestos fibers remain in the body lead to changes in the biomarkers of immune function [31,32]. Also,Trx promotes the uptake of cysteine into cells and upregulates the intracellular of GSH. Red blood cells, leucocytes, and platelets contain Trx [14]. The oxidized form of Trx leads to an increase in intracellular oxidized substances and ROS levels, thereby induces apoptosis [33]. So, Trx plays different roles within the cells in the cytoplasm and mitochondrial system [13]. Nowadays, most studies have analyzed Trx1 expression or activity of TRXR.We could not come upon any studies which are directly studied on Trx2 levels on mesothelioma patients. Thompson et al. [34] showed that exposure to crocidolite asbestos leads to irreversible oxidation of Trx1 and depletes reduced Trx1 levels in LP9/hTERT cells.In our study, we found levels of Trx2 as 0.89±0.10 pg/µg protein in patients' group and 1.95 fold times higher levels as 1.74±0.33pg/µg protein in a healthy group. Accordingly, we concluded that the oxidant-antioxidant balance system is impaired in mesothelioma. These data showmultifactor that regulates the antioxidant system in asbestosis and mesothelioma decreases the antioxidant potential of the Trx system. These results may be occurringvia inhibition of TrxR2. H_2O_2 increases through metabolism in mesothelioma and asbestosis.Regarding this knowledge and researches, much more analysis of enzymes' which are in mitochondria in both molecular and biochemical aspects are needed to study for explaining the process in mesothelioma and asbestosis.We suggest that focusing on thioredoxin interacting proteins, enzymes in the Trx system, and its relation between inflammation pathways will be useful for future studies.

Conclusion

MnSOD and Trx2 levels were lower in mesothelioma and asbestosis patients than healthy individuals.These significant changes in malignant mesothelioma patients reflect the impairment of the oxidant-antioxidant balance system.The present study will be a basic aspect ofthe clinical importance of the mentioned biochemical analysis. Although these enzymes appear to be important in these patients, more comprehensive studies are needed to focus on biologic pathways in the mitochondria of these patients.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
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Ethical approval
This study was conducted with permission of Bozok University, Faculty of Medicine Clinical Research Ethics Committee with the decision number of 15/05.

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