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Evaluation of Oxidative Stress Status in Workers with Toluene Exposure

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are well-known group of chemical carcinogens and can cause environmental pollution. Toluene is a polycyclic aromatic hydrocarbon which is found in plastics, paints and solvents like benzene. In this study, we evaluated the association between toluene exposure and TAS (Total Antioxidant Status), TOS (Total Oxidant Status), OSI (Oxidative Stress Index) measurements in workers with toluene exposure.

The workers with toluene exposure were admitted to Ankara Occupational Diseases Hospital from 46.6% painting (n = 14), 33.3% automotive and repair (n = 10) and 20% furnishing (n = 6) sectors. Oxidative stress status was evaluated using TAS, TOS, OSI markers in 30 toluene exposed workers and 30 control subjects. OSI was calculated by the TOS/TAS formula.

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TOS levels and OSI value in toluene exposed group were significantly higher (P = 0.017, P = 0.013; respectively) and TAS levels were significantly lower (P = 0.040) than the control subjects.

Our study showed that toluene exposure cause increased oxidative stress. Taking measures to reduce exposure to toluene may reduce incidence and severity of oxidative stress based diseases like cancer, inflamatuar disease, infection and aging.

Keywords: Toluene exposure, Total Antioxidant Status, Total Oxidant Status, Oxidative Stress

Conflict of interest: Authors do not have any conflict of interest

1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs), a group of toxic chemicals, are common pollutants in the environment, due to widespread use of petroleum, plastic, tobacco, coal, paint, varnish and metal [1, 2). Toluene is an aromatic hydrocarbon especially found in the adhesive and paint and it passes systemic circulation with its rapid absorbtion from the lungs [3-5].

Toluene has local and systemic adverse effects [5]. Brain, heart, liver, kidney, lung and bone marrow are affected organs. Following absorption, toluene is accumulated in oil-rich and well vascularized tissues in the body [6, 7]. Due to their lipophilic properties, toluene affects the myelin sheath and the lipid structure of the cell wall [8]. The main effects of toluene appears on lipid-rich nervous system [8, 9]. Toluene has been shown to cause chronic encephalopathy and seizures. Various studies demonstrated ventricular arrhythmias, myocardial infarction, sudden death, sinus bradycardia and dilated cardiomyopathy in rats and patients with toluene exposure [10, 11]. The increase in endogenous catecholamines and catecholamine sensitivity in heart, induced coronary vasospasm by causing an increase in lipid peroxidation and consequently it causes ischemic events. Toxic effects of toluene on the kidney causes electrolyte disturbances, and it contributes to the development of serious arrhythmias. It has irritative effects on the respiratory tract, eyes and skin [5]. Most of the toluene (about 80%) is metabolized by the liver cytochrome P-450 oxidases enzyme system and main metabolites hippuric acid and are excreted in the urine [8, 12].

Inside these metabolites, hippuric acid is a conventional biomarker in the monitorization of occupational exposure to toluene [12, 13] . Toluene can induce toxicity after it is metabolically activated to form reactive

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oxygen species. These reactive oxygen species including superoxide,hydrogen peroxide(H_20_2),hydroxyl (OH^-)can make covalent bonds with DNA or participate in redox cycling, and can reveal oxidative damage to DNA, proteins, carbonhydrates, lipoproteins, lipids, aminoacids and also connective tissue macromolecules [1, 7, 14]. Generally, oxidative stress is indicated as a degenerated balance between free radical production and antioxidant capacity resulting in excess oxidative products. In recent studies, plasma malondialdehyde (MDA), Glutathione S-transferase (GST), and urinary 8-hydroxydeoxyguanosine (8-OhdG), superoxide dismutase (SOD), catalase (CAT), total antioxidant substances (TAS) were measured as biomarkers of oxidative stress [3, 7, 15, 16]. TAS, TOS and OSI are the parameters that evalaute oxidative status in organism and give information about the overall status instead of reflecting the specific enzymes or metabolites.The aim of the study was to examine TAS, TOS and OSI levels to evaluate oxidative stress in patients with toluene exposure.

2. MATERIALS AND METHODS

In this cross-sectional study, blood samples were collected from workers in paint- factories in Turkey, during August - November 2014. This research was approved by the Ethics Committee of the Ankara Numune Education and Research Hospital.

2.1. Study population and sample collection

The study population consisted of an exposed group and a control group. The exposed group was formed from 30 workers. The workers with toluene exposure were admitted to Ankara Occupational Diseases Hospital from 46.6% painting (n = 14), 33.3% automotive and repair (n = 10) and 20% furnishing (n = 6) sectors. In study, inclusion criteria for the exposed group were: paint workers who had occupational contact with the aerosol spray paints during work for at least 1 year. Toluene exposure group were 14-57 years of age (median, 46.0) and all workers are male. The unexposed group (30 persons) was selected from the general population that did not have occupational contact paints during work. Control group included male subjects with 18-69 years of age (median, 38.0)

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2.2. Sample collection

Urine samples of 60 participants (30 exposed; 30 nonexposed) were collected in the dry tubes and were centrifuged at 1300 g for 5 minutes, supernatant was frozen at - 80 C^0 until analysis. Blood fasting specimens were collected in serum separator tube (BD Vacutainer®) and centrifuged at 1300 g for 10 minutes after comletion of clotting and stored at - 80 C^0 until analysis.

2.3. Laboratory analysis

Toluene exposure levels in paint workers were determinated via urine hippuric acid levels. Hippuric acid in urine was determined with Agilent 1200 series HPLC with a commercial kit (Eureka, Italy) in Ankara Occupational Diseases Hospital toxicology laboratory.

TAS method shows the antioxidative effect of the sample against the potent free radical reactions, which is initiated by the produced hydroxyl radical [17]. TOS can be measured spectrophotometrically and colour intensity is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide (H_2O_2) [18]. OSIcan be described as powerful parameter reflecting both oxidative and antioxidant status [19]. Serum TAS (Total Antioxidant Status) levels were determined by using a colorimetric method. Analysis was carried out by using Rel Assay Diagnostics TAS (Total Antioxidant Status) kit (catalog no: RL0017) in Beckman Coulter AU680 autoanalyzer (Measuring range: 1.50 to 1.20 mmol / L).Serum TOS (Total Oxidant Status) levels were determinedby using spectrophotometric method. Analysis was carried out by using Rel Assay Diagnostics TOS (Total Oxidant Status) kit (catalog no: RL0024) in the Beckman Coulter AU680 autoanalyzer (measuring range: 4-6 micro mol / L).OSI(Oxidative Stress Index) was calculated by the TOS / TAS formula.

2.4. Statistical analysis

The findings of the study were analyzed with "The Statistical Package for Social Sciences for Windows ver.18" (SPSS Inc, Chicago, USA, 2009) software. The conformity of continuous variables to normal distribution was

tested with Kolmogorov-Smirnov test. The descriptive statistics of continuous variables were expressed as mean ± SD or median (min-max). The presence of a statistically significant difference between the groups in terms of continuous variables was examined with the Student t test for parametric and Mann-Whitney U test for nonparametric variables. P value of < 0.05 was considered statistically significant for all tests.

3. RESULTS

The workers were exposed to toluene in an average time of 3-4 years. All participants were male and toluene exposure group were 14-57 years of age (median, 46.0) and control group were 18-69 years of age (median, 38.0).

Table-1 shows the median values of serum TAS, TOS, OSİ as oxidative stress markers in exposed and unexposed groups. TOS levels and OSI value of toluene exposed group were significantly higher (respectively P = 0.017, P = 0.013) and TAS levels were significantly lower (P = 0.040) in comparison with control subjects.

4. DISCUSSION

In our study, we showed that OS (Oxidative Stress) parameters of exposed group were significantly different from the controls to indicate the presence of an increased oxidative stress.

Volatile organic compounds (VOCs) are toxic pollutants that are present in chemicals such as paints, thinners, adhesives, aerosol sprays, inks, fuels and gasoline [2, 14, 20]. Toluene exposure can cause many diseases related with immune, respiratuary, reproductive, neurologic, and cardiologic systems by forming ROS [2, 7, 14, 21].

Mechanism of these diseases is not clear, but it is believed that oxidative stress and DNA damage have a basic role [7, 8, 15, 16].

The possible damage mechanism of these compounds are related to their lipophilic structure, they pass easily from cellular membranes and accumulate in fatty-rich tissue [6, 7, 20]. Previous studies showed that toluene accumulating in cell causes induced ROS production and increased ROS is related with DNA damage [2, 6, 7, 14, 22]. The oxidative metabolism of toluene produces cytosolic NADH. NADH is oxidized by mitochondria

electron transport chain and this product causes the formation of soperoxide radical in the electron transport chain. Also, toluene exposure induced quinones are produced by cytochrome P-450. These quinones accumulate cytotoxic ROS via futile redox cycle [8, 23].

Organic solvents are metabolised by cytochrome P-450 2E1 systems [24]. These enzymes use molecular oxygen, it generates reactive oxygen species (ROS), which are very reactive hydroxyl radicals [23, 25]. Hydroxyl radicals can react with proteins, lipids, nucleic acids [8, 12].

Studies showed that elevated oxidative stress and DNA damage was present in patients with low levels of toluene exposure [7, 22]. The reasons for increase in oxidative stress are changes in lipid peroxidation and endogenous antioxidants [6, 26, 27].Similarly, our study showed the elevation of oxidative stress markers in toluene exposed workers.

Researchers evaluated oxidative stress status and genotoxicity in paint workers with lead and toluene exposure during short time and this damage was analyzed using thiobarbituric acid reactive substances (TBARS) and carbonylated protein (CP) assays, superoxide dismutase (SOD) and catalase (CAT).In the study, researchers observed decreased SOD activity. It is known that SOD is an antioxidant system enzyme and SOD rises in increased oxidative stress status but this study found low SOD levels related that superoxide radical in paint lead to enzyme depletion. Also they showed increased DNA damage in exposed group [15].

One of the study assessed malondialdehyde (MDA) concentration for lipid peroxidation and protein carbonyl (PCO) assay for protein oxidation. Ischemia-modified albumin (IMA) and albumin (ALB) levels were evaluated to determine the effect of reactive oxygen species in the induction of genotoxicity.Study showed significant increase DNA damage index (DI), MDA, PCO levels, IMA concentrations in painters when compared to control subjects [22] . Another one indicated that exposure to toluene, benzene and styrene can induce endogenous ROS production in cells [3]. Similarly, present study demonstrated that TOS levels and OSI values in toluene exposed group were significantly higher and TAS levels were significantly lower than the control subjects.

Our study confirmed that toluene exposure causes increased oxidative stress. One strength of the study is the methods used in this study provides information about the overall oxidative status, and these methods can be used to determine and follow up of the oxidative stress in the workers with automatic biochemistry analyzers.

Taking measures to reduce exposure to toluene may reduce incidence and severity of oxidative stress based diseases like cancer, inflamatuar disease, infection and aging.

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Table-1: Median values of serum TAS, TOS, OSİ in toluene exposed and control group.

	Patient group (n=30)	**Control group (n=30)**	**P**
Age (years)	46 (14-57)*	38 (18-69)*	
TOS levels (μmol H2O2 equivalent/L)	3.91 (1.19-24.59)*	2.85 (1.17-6.87)*	0.040*
TAS levels (mmol Trolox equivalent/L)	1.42 (1.20-1.91)*	1.55 (1.35-2.12)*	0.017*
OSI (arbitrary unit)	244.34 (86.23-1517.90)*	179.33 (81.0-431.57)*	0.013*

*Statistically significant (P<0.05)