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Evaluation of Serum Catalase Activity and Malondialdehyde Level as Stress Oxidative Biomarkers among Iranian Welders

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Abstract

Background: Oxidant/antioxidant imbalance results in oxidative stress which plays a major role in many diseases. Inhalation of metal-enriched fumes and exposure to electromagnetic fields of welding device could induce oxidative stress in welders. Therefore, it is necessary to determine the level of oxidative stress among welders. Our study is aimed at estimating serum catalase and Malondialdehyde (MDA) levels in Iranian welders. **Materials and Methods:** Serum catalase activity and MDA levels were measured in 30 Iranian welders and 30 healthy non-welder subjects via catalase and MDA kit (Abcam). **Results:** The catalase activity (mU/L) and MDA levels (nmol/mL) were found to be 7.19±2.30 and 0.97±0.55, respectively in welder subjects, 10.73±1.08, and 0.58±0.38 in the control subjects. Catalase activity among welders was significantly lower than the control subjects (P<0.0001). In addition, plasma MDA level was significantly higher in welders, compared to the control subjects (P=0.0028). There was no significant difference between welder subgroups when the catalase activity and plasma MDA levels were compared. **Conclusion:** This study indicates that oxidant/antioxidant balances alter in Iranian welders. In other words, these results imply that the threshold limit of the resistance of the welder body against oxidative stress and damage has decreased.**[GMJ. 2015;4(3):62-66]**

Keywords: Catalase; Oxidative Stress; Reactive Oxygen Species; Malondialdehyde

Introduction

The influence of low frequency electromagnetic fields on human health is a very significant scientific research topic. Individuals exposed to such fields for prolonged times are prone to affliction with different types of cancer, depression and miscarriage [1, 2]. Some studies have shown the incidence of brain cancer and leukemia in people working under electrical fields [3, 4]. Welders are a group that are exposed to electric and magnetic fields during manual metal arc and flux core arc welding [5]. High electrical currents of 50-60 Hz were employed to induce a plasma arc [2] (usually making some sparks) and electromagnetic fields of welding device are formed near connecting cables, arcs and sparks. Welding operators are in close contact with these fields and directly handle the cables

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and clamp grasps [2]. A survey on magnetic field measurements showed approximately several hundred micro tesla of field intensity in 10 cm area around these welders [2]. A study showed that low frequency electromagnetic fields exposure induces oxidative stress in cells [6]. On the other hand, fusion of metals by high temperature of an electrical arc resulted in the formation of metal-enriched fumes. Welding fumes include complex mixture of gases and small particulates of metal oxides [7, 8]. Inhalation of these particles can also cause a variety of adverse respiratory diseases, inflammation and oxidative stress [7, 8]. Studies have reported that transition of metal particles induce production and release of reactive oxygen species (ROS) [7, 8]. These reactive compounds can reduce antioxidants and cause cellular damage, lung injury and inflammation [9]. Free radical-mediated oxidative damage and oxidative stress are produced under an imbalanced condition between the productions and detoxification of ROS. These conditions cause body's inability to quickly remove ROS and repair the resulting damages [10] and the pathogenesis of neurodegenerative disease such as amyotrophic lateral sclerosis, Alzheimer's and Parkinson's diseases [11,12]. This oxidant/antioxidant imbalance also plays a key role in aging.

There has been a great deal of attention to the development and validation of biomarkers including superoxide dismutase, catalase, malondialdehyde (MDA) and glutathione peroxidase. Changes in these biomarkers in body fluids can be a sign of specific kinds of disease, pathology or trauma [12]. The analysis and measurement of these biomarkers may provide a simple and quick way for prognosis and diagnosis of damage and disease in human [13]. In this study, we aim to investigate the oxidant/antioxidant balance markers in Iranian welders. For this reason, we determined serum MDA level as an index of lipid peroxidation and catalase activity in welders.

Materials and Methods

Subjects

The catalase activity and MDA levels of serum were determined in 60 subjects divided into two groups as follows: the healthy, non-welder subjects (30 males) as the control group and the healthy welder subjects (30 males) with an average age of 27 and seven years of welding experience at Aboughaddareh Industrial Complex-Shiraz-Iran. Catalase and MDA assay kits were purchased from Abcam (USA).

Determination of Plasma Catalase Activity

Catalase was colorimetrically determined using a catalase assay kit containing a stopping solution based on Jeong method [14]. The stop solution caused the complete stop of catalase activity. Catalase content of the samples reacted firstly with hydrogen peroxide to produce water and oxygen and the unconverted hydrogen peroxide reacted with OxiRed™ probe solution to produce a product which was measured at 570 nm. 12 µL 1 mM fresh hydrogen peroxide solution was added to 40 µL serum samples or high control serum samples (HC sample); stopping solution was firstly added to HC serum samples and then the catalase activity was assayed. All samples were incubated at 25°C for 30 min and then 10 µL stop solution was added into sample vials. HC and standard samples already contained the stop solution. OxiRed™ probe solution was added to the samples and incubated at 25°C for 10 min. Then, the absorbance at 570 nm was measured. A standard curve was prepared by adding 10 µL stop solution to 0, 2, 4, 6, 8 and 10 µL 1 mM hydrogen peroxide solution.

Determination of Serum MDA Level

The MDA levels, as an index of lipid peroxidation, were determined using lipid peroxidation (MDA) assay kit, according to An *et al* [15]. The MDA in these samples reacted with thiobarbituric acid (TBA) to generate MDA-TBA adducts at 95°C for 60 min. The MDA-TBA adduct was easily quantified colorimetrically at 532 nm. For this purpose, to 10 µL serum sample, 500 µL 42 mM H_2SO_4 solution was added and 125 µL of the kit's phosphotungstic acid. After incubation at room temperature for 5 min, samples were centrifuged for 3 min at 13000 g. The pellet was collected and re-suspended in 98 µL deionized water + 2 µL of the kit's butylated hydroxytoluene (BHT) on ice. Then, sample volumes were adjusted to

200 µL with deionized water. Then, 600 µL of TBA solution was added to the samples. It was incubated at 95°C for 60 min. and cooled to room temperature in an ice bath for 10 min; then we measured the absorbance of a sample at 532 nm. The concentration was calculated using the standard curve. Standard curve for MDA was prepared by adding TBA to different concentrations of MDA solution (0, 0.4, 0.8, 1.2, 1.6 and 2.0 nmol MDA).

Statistical Analysis

Catalase enzyme activity and MDA levels were reported as mean ± standard deviation (SD). In order to compare two groups of welder and non-welder, independent sample T-test was employed [GraphPad software]. A P-value less than 0.05 was considered significant. The correlation among samples in one group was determined using one sample t-test.

Results

The values of the catalase and MDA levels of serum samples [for the welders and control groups] are reported in Table 1. A decrease in the catalase activity was observed in the experimental group compared to the control group. This decrement was 3.54, which was statistically significant ($P<0.05$) in comparison with the control group. An increase in plasma MDA levels, in comparison with the control group, was observed in the welder group. This increment in MDA serum levels for the welder group was 0.38 which was statistically significant ($P<0.05$) in comparison with the control group. Moreover, there was no significant difference between welder subgroups when the catalase activities and plasma MDA levels were compared.

Table1. Levels of serum MDA and catalase in welder and control groups

Group	MDA (nmol/mL)*	Catalase (mU/L)†
Control group	0.58±0.38	10.73±1.08
Welder group	0.97±0.55	7.19±2.30

*Values significantly different from control (two-tailed P value equals 0.0028).

†Values extremely significantly different from control (two-tailed P value is less than 0.0001).

Discussion

There are a few valid methods to estimate the true level of oxidative stress, including the assessment of the activity of antioxidant enzymes such as catalase and the level of end products formed as a result of lipid peroxidation reactions such as MDA [16, 17]. Catalase, which is found in all cells, contains protoporphirin and degrades hydrogen peroxide to inhibit oxidative damage. Hydrogen peroxide is produced as a result of consumption of glutathione in cells [17, 18]. Production of superoxide radical, hydrogen peroxide and hydroxyl radical induces oxidative stress in human body. ROS reacts with macromolecules, leading to oxidative damage and lipid peroxidation of membranes and production of MDA [19]. Therefore, MDA has been well known as a stress oxidative biomarker in both in-vitro and in-vivo evaluations.

The impact of electromagnetic field exposure on human health depends on different variables such as duration of exposure and field frequencies. Welders are exposed to an extremely low frequency electromagnetic field of about 50 Hz during operation [2]. Low-frequency electromagnetic fields and gases of welding fumes cause oxidative stress in welders [6, 9].

Our results showed that the level of serum MDA was remarkably increased in welders' blood serums ($P=0.0028$) and the serum catalase activity was significantly lower in welders, compared to the control subjects ($P=0.0001$). A similar simultaneous decrease in the plasma catalase activity and increase in the MDA plasma levels were observed under antibiotic administration [20], exposure to extremely low frequency electromagnetic fields [21] and in cataract disease [22]. Increase of MDA plasma results in increased peroxidation of unsaturated fatty acids in the membrane.

It should be pointed out that the physiological level of ROS is useful for the body, but its high concentrations are cytotoxic [23]. Various conditions such as radiation, electromagnetic fields, some drugs, hypoxia, inflam-

matory conditions, infection and carcinogenic processes induce formation of high ROS levels. It was shown that high ROS level has a role in the development of some disease states [24]. Excessive production of ROS and an imbalance in antioxidant enzyme activity resulted in oxidative stress conditions [6]. ROS can peroxide membrane lipid and produce MDA as a by-product. Higher MDA levels are due to the increase in free radicals and an evidence of oxidative stress conditions [21,25]. Body defends against oxidative stress via antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase. Decrement in serum catalase activity reveals decreased protection capacity of the body against hydrogen peroxides and the increase of ROS. Catalase scavenged excess free radicals via enzymatic and chemical mechanisms resulting in decrement in the severity of lipid peroxidation. This behavior leads to depletion of catalase. Therefore, catalase activity loss indicates the conditions of long-term oxidative stress, increased susceptibility to oxidative stress or instability in enzyme structure [9]. In these conditions, individuals may be susceptible to development of a variety of late-onset disorders such as type 2 diabetes [26].

Actually, the case of oxidative impulse due to increase in MDA levels and decrease in catalase activity compared to the control group (in spite of insignificance of these differences) implies free radical formation at levels that are tolerated by the body.

Conclusion

In conclusion, this study shows that oxidant/antioxidant balance changes in welders and they might be prone to oxidative stress, with increment in MDA serum levels and decrement in antioxidant enzyme activity. This study supports the hypothesis that welding could cause oxidative disorders such as cataract, diabetes, Alzeimer and so on.

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Conflict of Interest

Authors declared no conflicts of interest.

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