OXIDATIVE STRESS AND DNA DAMAGE OF WELDERS

M A Moli*1, S B Dabi1, M Hasan1 and M M H Khan2

1Department of Pharmaceuticals and Industrial Biotechnology, Sylhet agricultural university, Sylhet-3100, Bangladesh
2Department of Biochemistry and Chemistry, Sylhet agricultural university, Sylhet-3100, Bangladesh
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Abstract

Welding consisted of a hazardous process involving high temperatures and generating toxic fumes in the process. Therefore, welders were exposed to many occupational hazards. In the study, oxidative stress and DNA damage of 35 welders, in parallel with 25 control subjects working in Dhaka city was investigated. Oxidative stress level was measured by using five biochemical parameters such as thiobarbituric acid reactive substances (TBARS), phospholipid hydroperoxide (PLHP), total antioxidant status (TAS), superoxide dismutase (SOD) activity and vitamin C level in serum as well as DNA damage by alkaline comet assay. The welders were divided into two group; group I (≤10 years) and group II (>10 years) on the basis of the duration of welding (years). Serum TBARS level (8.0 ± 0.4 vs. 4.0 ± 0.2 nmol MDA equivalent ml⁻¹) and PLHP level (9.0 ± 0.9 vs. 5.0 ± 0.2 nmol ml⁻¹) significantly (p<0.001) increased in welders compared to control subjects. Levels of TAS (0.3 ± 0.02 vs. 0.7 ± 0.03 mmol l⁻¹), SOD activity (74 ± 2.0 vs. 94 ± 0.6 inhibition rate %) and vitamin C (0.4 ± 0.04 vs. 0.8 ± 0.05 mg dl⁻¹) significantly (p<0.001) decreased in welders compared to control subjects. Elevated levels of TBARS, PLHP with decreased levels of TAS, SOD activity and vitamin C indicated that the production of reactive oxygen species (ROS) was higher in welders than that of control subjects. These results suggested that the exposure of welder to welding generated oxidative stress. TBARS level of welders of Group II was significantly higher (p<0.01) than that of welders of Group I (8.0 ± 0.3 vs. 10 ± 0.8 nmol MDA equivalent ml⁻¹). The mean TBARS, mean PLHP, mean SOD Activity, mean vitamin C values significantly differs among smoker control group (SC), non-smoker control group (NSC), smoker welder group (SW) and non-smoker welder group (NSW). This results also supported by the result of comet assay, which indicated that higher DNA damage occurs in welders than that of control subjects. It was found that 15.6% and 6.5% cell’s DNA were moderately and severely damaged in control subjects, whereas in the welders cell’s DNA were 28.2% moderately and 21.0% severely damaged, respectively. When duration of welding (years) was considered, a modest increased in oxidative stress and DNA damage was associated with the higher exposure to welding than that of lower exposure. The evaluated effect of smoking on oxidative stress and DNA damage among all subjects showed that smokers had more DNA damage than non-smokers. In this study correlation was found between oxidative stress and DNA damage of the welders with the duration of exposure of welding fumes and smoking habits of welders.

Keywords: Welders, oxidative stress, DNA damage, comet assay.

Introduction

Welding is one of the key components of numerous manufacturing industries, which pose potential physical and chemical health hazards. Welding fumes consist of a wide range of complex metal oxide particles which can be deposited in all regions of the respiratory tract. According to OSHA (1995), the welding fume may contain manganese (Mn), beryllium (Be), cadmium (Cd), chromium (Cr), vanadium (V), antimony (Sb), zinc (Zn), nickel (Ni), molybdenum (Mo), mercury (Hg), lead (Pb), iron (Fe) and cobalt (Co). Welding fume pulmonary effects have been associated with bronchitis, metal fume fever, pneumoconiosis, airway irritation, fibrosis, cancer and functional changes in the lung (Leonard et al., 2010; Ding et al., 2011; Jeong et al., 2006), manganese may elevate the risk for
neurological diseases (Flynn et al., 2010; Levi et al., 2000). Metal workers were found to have a significantly increased risk of death from lung cancer (Gallagher et al., 1983). Chronic exposure to welding light may cause ocular disorders (Davies et al., 2007). The oxidative status of cells is influenced by welding fumes and oxidative stress index may be useful in predicting disease outcomes (Du et al., 2010).

Metals induce the production of reactive oxygen species (ROS) (e.g., hydroxyl peroxide and superoxide radicals. Oxidative stress is an imbalance between the production ROS and the biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can produce peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Oxidative stress can also cause disruptions in normal mechanisms of cellular signaling (Anderson et al., 2004; Teunissen et al., 2002). The brain is vulnerable to oxidative damage (Halliwell et al., 1985; Cohen et al., 1988). The role of oxidative stress in the generation of several neuro-degenerative diseases is also studied (Halliwell et al., 1992; Gibson et al., 2000). Cadmium can also cause DNA single-strand breaks and cellular DNA damage (Park et al., 2011; Yang et al., 2003). Chronic toxicity from persistent exposure to toxic metals leads to their accumulation in living system (Kim et al., 2010; Clarkson et al., 2006). Cobalt and nickel can cause cytotoxic effect in vitro models (Peters et al., 2007). Although oxidation reactions are crucial for life, they can also be damaging; plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. The objectives of the study were to determine the effects of oxidative stress and DNA damage of welders working in Dhaka city.

Materials and Methods

Study Subjects

The study was conducted with two categories of subjects; Male welding workers (n=35) which directly exposed to the welding for at least 1 year was selected as sample and male adults never exposed to welding (n=25) was selected as control. This study was conducted at different areas in Dhaka city. They were healthy at the time of blood collection, without having any diseases.

Questionnaire

All study subjects completed a structured questionnaire covering information on age, blood pressure, occupational and smoking history and other exposure histories.

Collection of Blood

About 6-7 mL of peripheral blood was collected from each individual with the help of nurse. About 2 mL blood of each sample was transferred into vacuum tube containing anticoagulant. Rest of blood was transferred into a glass test tube. Blood samples were kept in an ice chamber following collection and during transportation.

Separation of Serum

The test tube containing blood was allowed to stand at room temperature for approximately 2 h. The tube was then centrifuged for 15 min at 3,000 rpm and serum was collected. Appropriate aliquots of serum were stored at -20°C until further use.

Biochemical Analysis

Thiobarbituric Acid Reactive Substances (TBARS) value was determined according to the method of Yagi (1998). Phospholipid Hydroperoxide (PLHP) value was determined by colorimetric method based on the oxidation of ferrous to ferric ion in the presence of xylenol orange. Total Antioxidant Status (TAS) was determined according to Miller et al. (1993) using Randox kit (UK). The Serum Superoxide Dismutase (SOD) Activity was determined by SOD Assay Kit. Serum vitamin C was measured by di-nitro phenyl hydrazine method with modification according to Lowry et al. (1945).

Comet Assay Analysis

Single cell gel electrophoresis technique known as “comet assay” was performed for the quantification of the levels of DNA damage in individual cells according to the method of Singh et al. (1988). The slides were examined under
conventional light microscope [(Motic-BA200) (400× magnification)] equipped with CCD camera (Nikon Cool Pix 99F). For comparing the extent of DNA damage in different samples, comet images were analyzed using a software named Computer Assay Software Project (CAPS, version 1.2.2) developed by Konca et al. (2003).

Statistical Analysis of Data

Results were expressed as mean ± SEM (Standard Error of Mean). Data analyses were carried out using the statistical procedure GRAPHPAD PRISM 5 (USA). The statistical method used was student’s t-test (two tailed). The values of p< 0.05 were considered as statistically significant.

Results and Discussion

In Bangladesh, there is very little information available on the effect of environmental exposures of welding to the health of welders. Therefore, the study was undertaken to evaluate oxidative stress and DNA damage of welders working in Dhaka city. To determine the effect of welding on oxidative stress status, the serum level of TBARS, PLHP, TAS, SOD activity and vitamin C were measured. Along with that comet assay to evaluate the DNA damage in lymphocytes was also performed. The effects of smoking and duration of exposure to welding were also determined.

Educational Status of Study Subject

Thirty three welders were taken as study subjects, within this 9 subjects (26%) were illiterate, 21 subjects (60%) and 5 subjects (14%) had completed primary and secondary level respectively. No one in this group had completed higher than secondary educational level. In compare to welders, 7 control subjects (28%) had completed secondary level, 6 subjects (24%) and 12 subjects (48%) had completed graduate and masters level, respectively. Welders were not well educated. So, they didn’t know about health effects of their occupation and that’s why they were not using any protective devices while they were working.

Demographic Characteristic of the Study Subjects

Table 1 shows the characteristic of study subjects. Distribution of study subjects were presented according to age, smoking status and duration of welding. Duration of welding (years) was divided into two groups: equal or less than 10 years (Group I) and more than 10 years (Group II). The number of welders between 15-30 years of age was higher than that of >30-45 years of age. On the basis of smoking status, the number of smoker was higher than non-smoker. Based on duration of welding (years), the number of Group I (≤10 years) welder was higher than that of Group II (>10 years).

Table 1. Study Subjects Characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>15-30</td>
<td>24 (68.6)</td>
</tr>
<tr>
<td>&gt;30-45</td>
<td>11 (31.4)</td>
</tr>
<tr>
<td>Smoking Status</td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>22 (62.9)</td>
</tr>
<tr>
<td>Non-smoker</td>
<td>13 (37.1)</td>
</tr>
<tr>
<td>Duration of Welding (years)</td>
<td></td>
</tr>
<tr>
<td>≤10</td>
<td>22 (62.9)</td>
</tr>
<tr>
<td>&gt;10</td>
<td>13 (37.1)</td>
</tr>
</tbody>
</table>
Status of Oxidative Stress

Mean serum TBARS levels were 4.0 ± 0.2 and 8.0 ± 0.4 (nmol MDA equivalent ml⁻¹) in control subjects and welders respectively. Serum TBARS level in the welders was significantly (p<0.001) higher compared to control subjects. Mean serum PLHP levels were 5.0 ± 0.2 and 9.0 ± 0.9 (nmol ml⁻¹) in control subjects and welders respectively. Serum PLHP level in the welders was significantly (p<0.001) higher compared to control subjects. As TBARS is one of the major products of lipid peroxidation, it indicated high rate of lipid peroxidation in the welders. Another major product of lipid peroxidation, PLHP was also significantly (p<0.001) higher in welders compared to control subjects. These results were consistent with previous findings in human and animal experimental studies showing a significant increase in lipid peroxidation caused by welding fume exposure (Li et al., 2004; Taylor et al., 2003).

Mean serum TAS levels were 0.7 ± 0.03 and 0.3 ± 0.02 (nmol l⁻¹) in control subjects and welders respectively. Serum TAS level in the welders was significantly (p<0.001) lower compared to control subjects. Mean serum SOD activity (inhibition rate %) levels were 94 ± 0.6 and 74 ± 2.0 % in control subjects and welders respectively. Serum SOD activity (inhibition rate %) levels in the welders was significantly (p<0.001) lower compared to control subjects. Mean serum vitamin C levels were 0.8 ± 0.05 and 0.4 ± 0.04 (mg dl⁻¹) in control subjects and welders respectively. Serum Vitamin C levels in the welders was significantly (p<0.001) lower compared to control subjects. Serum TAS level, SOD activity level, vitamin C level were significantly (p<0.05) lower compared to control subjects. So, it can be predicted from results that oxidative stress level was increased in welders compared to that of control subjects. Sung et al. (2005) reported that the total antioxidant status (TAS) were significantly decreased in the welders. This suggests that the increased oxidative stress by welding fumes triggers the up regulation of defenses to protect cells. Contrary to these findings, Li et al. (2004) reported in a study of 37 automobile welders, 24% decline in erythrocyte SOD activity. It indicates that selective biochemical responses may have resulted to cope with the extent and severity of oxidant burden. The results presented in this study provided support for the use of ROS-mediated changes in antioxidant status, free-radical generation potential, and resulting lipid peroxidation.

Table 2 shows results of the status of oxidative stress based on the duration of welding (years). Duration of welding (years) was divided into two groups: equal or less than 10 years (Group I) and more than 10 years (Group II). The mean TBARS value of group 1 and group 2 were 8.0 ± 0.3 and 10 ± 0.8 (nmol MDA equivalent ml⁻¹) respectively. TBARS level of welders working for more than 10 years (Group II) was significantly higher (p<0.01) than that of welders working for less or equal to 10 years (Group I). On the other hand, no significant difference was found on PLHP, TAS, SOD activity and vitamin C levels between Group I and Group II.

Table 2. Status of Oxidative Stress on the basis of the duration of welding (years)

<table>
<thead>
<tr>
<th>Oxidative Stress Parameter</th>
<th>Duration of Welding (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤10</td>
</tr>
<tr>
<td>TBARS (nmol MDA equivalent ml⁻¹)</td>
<td>8.0 ± 0.3</td>
</tr>
<tr>
<td>PLHP (nmol ml⁻¹)</td>
<td>8.0 ± 0.9</td>
</tr>
<tr>
<td>TAS (nmol l⁻¹)</td>
<td>0.3 ± 0.02</td>
</tr>
<tr>
<td>SOD activity (inhibition rate %)</td>
<td>74 ± 3.0</td>
</tr>
<tr>
<td>Vitamin C (mg dl⁻¹)</td>
<td>0.4 ± 0.05</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Student’s t-test was performed to analyze data. Statistically significant at *p<0.01.

TBARS: Thiobarbituric acid reactive substances; PLHP: Phospholipid hydroperoxide; TAS: Total antioxidant status and SOD: Superoxide dismutase.

The mean TBARS values were 5.0 ± 0.1, 3.0 ± 0.2, 9.0 ± 0.5 and 8.0 ± 0.6 (nmol MDA equivalent ml⁻¹) in smoker control group (SC), non-smoker control group (NSC), smoker welder group (SW) and non-smoker welder group (NSW), respectively (Table 3). As shown in Table 3, the TBARS level of SC is significantly higher (p<0.001) compared to than that of NSC. On the other hand, there was no significant different between SW and NSW. TBARS level of SW was significantly higher (p<0.001) compared to than that of SC and also significantly higher (p<0.001)
compared to than that of NSC. TBARS level of NSW was highly significant like SW compared to than that of SC and NSC, respectively.

Table 3. TBARS level on the basis of smoking status

<table>
<thead>
<tr>
<th>Study Subjects</th>
<th>Smoking Status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Smoker</td>
</tr>
<tr>
<td>Welder</td>
<td>9.0 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>5.0 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Student’s t-test was performed to analyze data. Different letter in the superscript means they are statistically significant at p< 0.05.

The mean PLHP values were 6.0 ± 0.1, 5.0 ± 0.2, 9.0 ± 1.0 and 8.0 ± 1.0 (nmol ml<sup>-1</sup>) in smoker control group (SC), non-smoker control group (NSC), smoker welder group (SW) and non-smoker welder group (NSW), respectively (Table 4). PLHP level of SC was significantly higher (p<0.001) compared to than that of NSC. But PLHP level of SW was not significant compared to than that of NSW. PLHP level of SW was not significant compared to than that of SC but significantly higher (p<0.01) compared to than that of NSC. PLHP level of NSW was significantly higher (p<0.05) compared to than that of NSC, but not significant compared to than that of SC.

Table 4. PLHP level on the basis of smoking status

<table>
<thead>
<tr>
<th>Study Subjects</th>
<th>Smoking Status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Smoker</td>
</tr>
<tr>
<td>Welder</td>
<td>9.0 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>6.0 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Student’s t-test was performed to analyze data. Different letter in the superscript means they are statistically significant at p< 0.05.

The mean TAS values were 0.5 ± 0.03, 0.7 ± 0.02, 0.3 ± 0.02 and 0.3 ± 0.03 (mmol l<sup>-1</sup>) in smoker control group (SC), non-smoker control group (NSC), smoker welder group (SW) and non-smoker welder group (NSW), respectively (Table 5). TAS level of SC was significantly higher (p<0.001) compared to than that of NSC. But TAS level of SW was not significant compared to than that of NSW. TAS level of SW was significantly higher (p<0.001) compared to than that of SC and also significantly higher (p<0.001) compared to than that of NSC. TAS level of NSW was highly significant like SW compared to than that of SC (p<0.001) and NSC (p<0.001), respectively.

Table 5. TAS level on the basis of smoking status

<table>
<thead>
<tr>
<th>Study Subjects</th>
<th>Smoking Status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Smoker</td>
</tr>
<tr>
<td>Welder</td>
<td>0.3 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>0.5 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Student’s t-test was performed to analyze data. Different letter in the superscript means they are statistically significant at p< 0.05.

The mean SOD Activity values were 91 ± 0.6, 96 ± 0.4, 72 ± 3.0 and 75 ± 3.0 (%) in smoker control group (SC), non-smoker control group (NSC), smoker welder group (SW) and non-smoker welder group (NSW), respectively (Table 6). SOD activity level of SC was significantly higher (p<0.001) compared to than that of NSC. But SOD
activity level of SW was not significant compared to than that of NSW. SOD activity level of SW was significantly higher ($p<0.01$) compared to than that of SC and also significantly higher ($p<0.001$) compared to than that of NSC. SOD activity level of NSW was highly significant like SW compared to than that of SC ($p<0.001$) and NSC ($p<0.001$), respectively.

**Table 6. SOD activity (inhibition rate %) level on the basis of smoking status**

<table>
<thead>
<tr>
<th>Study Subjects</th>
<th>Smoking Status</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Smoker</td>
<td>Non-smoker</td>
<td></td>
</tr>
<tr>
<td>Welder</td>
<td>72 ± 3.0$^a$</td>
<td>75 ± 3.0$^a$</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>91 ± 0.6$^b$</td>
<td>96 ± 0.4$^c$</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Student’s t-test was performed to analyze data. Different letter in the superscript means they are statistically significant at $p<0.05$.

The mean vitamin C values were 0.7 ± 0.1, 0.9 ± 0.05, 0.4 ± 0.04 and 0.4 ± 0.07 (mg dl$^{-1}$) in smoker control group (SC), non-smoker control group (NSC), smoker welder group (SW) and non-smoker welder group (NSW), respectively (Table 7). Vitamin C level of SC was not significant compared to than that of NSC. Also vitamin C level of SW was not significant compared to than that of NSW. Vitamin C level of SW was significantly lower ($p<0.01$) compared to than that of SC and also significantly higher ($p<0.001$) compared to than that of NSC. Vitamin C level of NSW was significantly lower ($p<0.001$) compared to than that of NSC, but not significant compared to than that of SC.

**Table 7. Vitamin C level on the basis of smoking status**

<table>
<thead>
<tr>
<th>Study Subjects</th>
<th>Smoking Status</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Smoker</td>
<td>Non-smoker</td>
<td></td>
</tr>
<tr>
<td>Welder</td>
<td>0.4 ± 0.04$^a$</td>
<td>0.4 ± 0.07$^{a,b}$</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.7 ± 0.1$^{b,c}$</td>
<td>0.9 ± 0.05$^c$</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Student’s t-test was performed to analyze data. Different letter in the superscript means they are statistically significant at $p<0.05$.

From all results based on smoking status, it can be concluded that smoking increase oxidative stress level. Oxidative stress levels were much higher in smoker group compared to the non-smoker group. In conclusion, this study demonstrated that exposure to welding fumes enhanced the generation of ROS and induced oxidative stress, causing some oxidant damage in target cells and resulting in changes in certain well characterized and exploratory markers of oxidative stress.

**Evaluation of DNA Damage by Comet Assay**

The alkaline comet assay or single cell gel electrophoresis was performed to assess the DNA damage of the lymphocytes cells of Welders and control subjects.

**Oxidative Stress Induced DNA Damage**

Fig. 1 is the representative picture of comet assay for the lymphocytes of Welders and control subjects. As evident from the figure, most of the nuclear DNA of control cells was perfectly round and almost all DNA was concentration inside the head region of comet (%) indicating there was no DNA damage (Fig. 1 A). On the other hand, the nuclear DNA of the Welders were severely fragmented i.e., DNA moved in the tail region during electrophoresis (Fig. 1C). Some lymphocytes that were moderately damaged are common in both groups (Fig. 1 B).
Fig. 1. Representative comet images of control and welders lymphocytes. Fig. 1(A) represent the comet image of control subject; Fig. 1 (B) and 1(C) moderately and severely damage lymphocytes of welders, respectively.

From the observation of slide under microscope it was found that 15.6% and 6.5% cell’s DNA were moderately and severely damaged in control subjects, whereas in the welders cell’s DNA were 28.2% moderately and 21.0% severely damaged, respectively. The increased DNA damage in the lymphocytes of the welders indicated that exposure to working on welding induced DNA damage.

Quantitative Analysis of Comet

The comet image of both welders and control subject’s lymphocytes were analyzed by using CASP computer software in order to reveal the DNA damage. The DNA damage was presented as the percent of DNA fragmentation (percent of DNA in the comet tail and comet head); the higher the percentage of DNA fragmentation, the more severe is the damage. Similarly, the longer the comet tails, the higher the DNA fragment, the more severe is the damage. The percentage of DNA damage was also presented based on duration of welding and smoking status.

DNA Percentage in Comet Head and tail

Number of severely damaged cells (present of <10% DNA in head region) of welders were 25 ± 2.0, whereas in control subjects it was 9.0 ± 2.0. Some cells of both welders and control group showed moderate damage. On the other hand, number of less damaged cells (present of >60% DNA in head region) of welders were 55 ± 3.0 and control subjects had 76 ± 6.0 cells. The number of severely damaged cells had significantly \( p<0.001 \) increased in welders as compared to control subjects. The number of less damaged cells had significantly \( p<0.001 \) decreased in welders as compared to control subjects. Number of severely damaged cells (present of >60% DNA in tail region) of welders were 17 ± 3.0 and have higher DNA percentage (>60) in their tail region in welders in compare to control subjects (10 ± 2.0). Some cells of welders and control subjects showed moderate damage. On the other hand, number of less damaged cells (present of <10% DNA in tail region) of welders were 61 ± 5.0 and control subjects had 73 ± 6.0 cells. The number of severely damaged cells had significantly \( p<0.001 \) increased in welders as compared to control subjects. The number of less damaged cells had significantly \( p<0.001 \) decreased in welders as compared to control subjects.

Number of severely damaged cells (present of <10% DNA in head region) of group 1 (≤10 years) were 25 ± 2.0 and had lower DNA percentage (<10) in their head region in Group I (≤10 years) in compared to Group II (>10 years).
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(33 ± 3.0). Some cells of Group I (≤10 years) and Group II (>10 years) showed moderate damage. On the other hand, number of less damaged cells (present of >60% DNA in head region) of Group I (≤10 years) were 58 ± 5.0 and Group II (>10 years) have 51 ± 4.0 cells. The number of severely damaged cells had significantly (p<0.001) increased in Group II (>10 years) as compared to Group I (≤10 years). The number of less damaged cells had significantly (p<0.001) increased in Group I (≤10 years) as compared to Group II (>10 years).

Number of severely damaged cells (present of >60% DNA in tail region) of Group I (≤10 years) were 15 ± 2.0 and had lower DNA percentage (>60) in their tail region in Group I (≤10 years) in compared to Group II (>10 years) (22 ± 3.0). Some cells of Group I (≤10 years) and Group II (>10 years) showed moderate damage. On the other hand, number of less damaged cells (present of <10% DNA in tail region) of Group I (≤10 years) are 65 ± 6.0 and Group II (>10 years) had 55 ± 5.0 cells. The number of severely damaged cells had significantly (p<0.001) increased in Group II (>10 years) as compared to Group I (≤10 years). The number of less damaged cells had significantly (p<0.001) increased in Group I (≤10 years) as compared to Group II (>10 years).

Table 8 shows the effects of smoking on head DNA (%) measurement in study subjects. Number of severely damaged cells (present of <10% DNA in head region) of smoker control group (SC) were highly significant (p<0.01) compared to that of non-smoker control group (NSC) and also highly significant (p<0.001) compared to that of smoker welder group (SW). Number of severely damaged cells (present of <10% DNA in head region) of smoker welder group (SW) were highly significant (p<0.001) compared to that of non-smoker welder group (NSW). Number of severely damaged cells (present of <10% DNA in head region) of NSW were highly significant (p<0.001) compared to that of NSC. So, the numbers of severely damaged cells were increased much higher in SW compared to other group and numbers of severely damaged cells were decreased in NSC compared to other group.

Number of less damaged cells (present of >60% DNA in head region) of SC were highly significant (p<0.001) compared to that of non-smoker control group (NSC) and also highly significant (p<0.001) compared to that of smoker welder group (SW).

Number of less damaged cells (present of >60% DNA in head region) of SW were highly significant (p<0.01) compared to that of non-smoker welder group (NSW). Number of less damaged cells (present of >60% DNA in head region) of NSW were highly significant (p<0.001) compared to that of NSC. So, the numbers of less damaged cells were decreased in SW compared to other group and numbers of less damaged cells were increased much higher in NSC compared to other group.

Table 8. Head DNA (%) measurement on the basis of smoking status

<table>
<thead>
<tr>
<th>Head DNA (%)</th>
<th>SC</th>
<th>NSC</th>
<th>SW</th>
<th>NSW</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10</td>
<td>14 ± 0.6</td>
<td>9.0 ± 0.7<strong>b</strong>*</td>
<td>30 ± 0.4<strong>b</strong>*</td>
<td>21 ± 0.6</td>
</tr>
<tr>
<td>10-60</td>
<td>21 ± 0.2</td>
<td>11 ± 0.1**<strong>b</strong>*</td>
<td>16 ± 0.6**b*</td>
<td>19 ± 0.3</td>
</tr>
<tr>
<td>&gt;60</td>
<td>65 ± 0.3</td>
<td>80 ± 0.1**<strong>b</strong>*</td>
<td>54 ± 0.2****b**</td>
<td>60 ± 0.1</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Letters in the superscript, a = vs smoker control group (SC) and b = vs non-smoker welder group (NSW). Student’s t-test was performed to analyze data, they are statistically significant at *p <0.05, **p <0.01, ***p<0.001. SC (smoker control group), NSC (non-smoker control group), SW (smoker welder group), NSW (non-smoker welder group).

Table 9. shows the effects of smoking on tail DNA (%) measurement in study subjects. Number of severely damaged cells (present of >60% DNA in tail region) of smoker control group (SC) were highly significant (p<0.01) compared to that of non-smoker control group (NSC) and were not significant compared to that of smoker welder group (SW). Number of severely damaged cells (present of >60% DNA in tail region) of smoker welder group (SW) were not significant compared to that of non-smoker welder group (NSW). Number of severely damaged cells (present of >60% DNA in tail region) of NSW were not significant compared to that of NSC. So, the numbers of severely damaged cells were increased in SW compared to other group and numbers of severely damaged cells were decreased in NSC compared to other group.
Number of less damaged cells (present of <10% DNA in tail region) of SC were highly significant ($p<0.001$) compared to that of non-smoker control group (NSC) and also highly significant ($p<0.001$) compared to that of smoker welder group (SW).

Number of less damaged cells (present of <10% DNA in tail region) of SW were highly significant ($p<0.001$) compared to that of non-smoker welder group (NSW). Number of less damaged cells (present of <10% DNA in tail region) of NSW were highly significant ($p<0.001$) compared to that of NSC. So, the numbers of less damaged cells were decreased in SW compared to other group and numbers of less damaged cells were increased much higher in NSC compared to other group.

Table 9. Tail DNA (%) measurement on the basis of smoking status

<table>
<thead>
<tr>
<th>Tail DNA (%)</th>
<th>SC</th>
<th>NSC</th>
<th>SW</th>
<th>NSW</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10</td>
<td>68 ± 0.6</td>
<td>77 ± 0.7</td>
<td>57 ± 0.4</td>
<td>67 ± 0.6</td>
</tr>
<tr>
<td>10-60</td>
<td>19 ± 0.2</td>
<td>14 ± 0.1</td>
<td>29 ± 0.6</td>
<td>22 ± 0.3</td>
</tr>
<tr>
<td>&gt;60</td>
<td>13 ± 0.3</td>
<td>9.0 ± 0.1</td>
<td>14 ± 0.2</td>
<td>11 ± 0.1</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Letters in the superscript, a = vs smoker control group (SC) and b = vs non-smoker control group (NSC). Student’s t-test was performed to analyze data, they are statistically significant at *$p<0.05$, **$p<0.01$, ***$p<0.001$. SC (smoker control group), NSC (non-smoker control group), SW (smoker welder group), NSW (non-smoker welder group).

Welders were investigated for genotoxic effects in the current study using the comet assay. The comet assay is increasingly being used to monitor genotoxic effects in occupationally exposed humans (Kassie et al., 2000). We observed that there was considerable loss in the integrity of DNA in comet head region due to single strand breaks, as evident from Fig 1.

All the results indicated that higher DNA damage occurs in welders than that of control subjects. Smokers showed more DNA damage than non-smokers (both for welders and control subjects). In the present study, a significant increase in DNA damage was observed in welders when compared with controls by the comet assay. These results indicated that the level of exposure in the workplace is sufficiently high and also highlights the sensitivity of the assay used. Studies of welders utilizing the comet assay were lacking. However, elevated levels of DNA protein cross-links were observed in a study of welders (Costa et al., 1993). A statistically significant enhancement in the frequency of protein crosslinking and DNA strand breaks was noticed in the blood lymphocytes of welders (Popp et al., 1991). Similarly, welders showed a significantly higher level of DNA single-strand breaks with the alkaline filter elution method (Werfel et al., 1998). The results were in line with these studies.

Smoking did not have a significant effect on DNA damage in the present study. The lack of an influence of smoking on the comet assay results of this study is supported by a report on lead-exposed workers in which smoking did not significantly affect the comet assay values (Fracasso et al., 2002).

The present results indicated a significant increase in mean DNA damage in the welders with increasing duration of work in the polluted environment. In a study of lead exposed workers, DNA damage increased significantly with increase in years of exposure (Danadevi et al., 2003). A significant effect of age on DNA damage was not observed in welders. Many studies have shown that the development of lung cancer in chromate workers usually starts at a relatively old age, but one study reported a patient who developed cancer at 37 years of age and who had 15 years of occupational exposure to chromate. Thus the period of exposure to the carcinogen was the factor that should be considered, rather than the age of the worker (Ewis et al., 2001).

On the basis of all results, it appeared that TBARS level and PLHP level in serum were much higher in welders than that of control subjects. Whereas, the TAS, SOD activity and vitamin C level in serum were much lower in welders than that of control subjects. Both the findings suggested that the oxidative stress increased in welders exposed to welding works. On the basis of smoking status, all the smoking subjects (both welders and control) had higher oxidative stress status and higher DNA damage rate than that of non-smokers. Along with that higher DNA damage occurs in welders exposed to welding for higher duration of time. The disruption of homeostasis induced by oxidative stress may promote the development of a disease state with continued occupational exposure to welding fumes. These results document that exposure to welding can cause changes in serum biomarkers of oxidative stress...
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that may be valuable in clinical monitoring of disease development and in assessing whether further reduction of worker exposures is needed.

References

OSHA, Welding fumes (Total Particulate) [Web page on the Internet] Chemical sampling Information. 1995.


