Changes in the Antioxidant Defense System of the Smokers

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SUMMARY

To determine whether tobacco use is associated with certain alterations involving antioxidant system and some of its related parameters 50 subjects who have been smoking more than a pack of cigarette per day for at least five years and 50 healthy individuals who had never smoke in their life were examined. Serum catalase, erythrocyte superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and glucose 6-phosphate dehydrogenase (G6PD) activities, and malondialdehyde (MDA) concentration in both serum and erythrocyte of all subjects were measured. Periferic blood count, serum uric acid, and albumin levels were also measured. In comparison with the non smokers, catalase and GSH-Px enzyme activities were found significantly lower in the smokers while SOD and G6PD activities were not significantly different. In addition, MDA was found significantly higher in both serum and erythrocytes (p < 0.001) of the smokers compared with nonsmokers. While serum albumin and uric acid levels of smokers were found not significantly lower (p > 0.05), hemoglobin (Hb) and white blood cells (WBC) were found higher (p < 0.01 and p < 0.001, respectively). From these results, it can be suggested that antioxidants can be depleted against fighting with oxidants, or they can be inhibited by certain chemical compounds of cigarette smoke.

Key Words: Catalase, glutathione peroxidase, malondialdehyde, superoxide dismutase, smoking, antioxidant system.

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Cigarette smoke contains oxygen radicals and causes formation of new radicals in the body. The smoke is formed by dispersing of the products as a consequence of melting and distillation in hot medium at gas or droplet state (1). If the smoke is passed through Cambridge glass fiber, 99.9 percent of the particles larger than 0.1 μm remain in the filter. The part which passes through the filter makes gas phase, and the remaining part makes the tar phase (2,3).

Tar and gas phases of cigarette have very different characteristics. While the gas phase has strong oxidants, tar phase has the reduction system. The radicals of gas phase including reactive oxygen species, nitrogen dioxide, epoxide, peroxide, peroxynitrite etc may be both organic and inorganic. In comparison with the stable radicals in cigarette tar, the radicals of gas phase are the radicals with reactive carbon and oxygen centered.

The remaining part after dissociation of water steam and nicotine from the particle phase of cigarette is tar, and this is major cancerogen of the smoke. Nicotine is one of the most important substances being in droplet state of the smoke (1).

Tar contains stable organic radicals such as semiquionon radical generally at very high concentrations (4). The radical species with interesting chemical characteristics have been described as semiquionon at relatively balance with quionon and hydroqionones in the viscous tar. In fact, the chemistry of tar radical is described as a semiquionon (QH) (2,3,5). These radicals form hydrogen peroxide and hydroxyl radical by forward reactions by reducing oxygen to superoxide. Furthermore, tar radicals, them selves, reduce ferric ion to ferrous ion (4).

If oxidant stress exceeds the antioxidant capacity, oxidative damage may occurs. Using some biochemical parameters, it is possible to obtain the information about oxidative damage. Malondialdehyde (MDA), the last product of the lipid peroxidation, is accepted as an indicator of lipid peroxidation. On the other hand, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and Catalase are antioxidant enzymes. SOD protects the cells against harmful effects of superoxide radicals by catalyzing the conversion of superoxide radical to hydrogen peroxide (6). GSH-Px catalyzes reduction of hydrogen peroxide and organic hydroperoxides by glutathione. Enzyme activity depends on NADPH produced in penthose phosphate pathway (PPP). Hydrogen peroxide at low concentrations is cleared firstly by GSH-Px (7,8). Catalase decomposes hydrogen peroxide to oxygen and water (3). Glucose 6-phosphate dehydrogenase (G6PD) is also necessary for formation of NADPH which is required for detoxification of free radicals and peroxides formed within the cell.

Additionally, besides enzymatic antioxidants, some molecules act as nonenzymatic antioxidants, such as albumin, urate and hemoglobin (9-15).

In order to find what changes occur in antioxidants in the body against the oxidative effects of cigarette smoke, some parameters were determined in serum, plasma and whole blood. The level of albumin, urate and MDA and the activity of Catalase were studied in serum whereas the activities of SOD, GSH-Px and G6PD and the level of MDA were determined in erythrocytes. The amount of hemoglobin, WBC and RBC were also studied in whoe blood.

**MATERIALS and METHODS**

This study was performed with a survey of diabetes. First of all, a face to face inquiry was applied on 243 people who were living in a district of Isparta. Some information such as health, life style, income, habits, family history etc of subjects were obtained using this inquiry. Bloods were drawn in the day after the inquiry done day in their working place or in our laboratory. The study group and control group consisted of smokers and nonsmokers, respectively. Each of the groups consisted of 50 men who are 35 years old or more. Individuals included to the control group had never smoked or not had been smoking at least five years. Individuals who belong to the study group had been smoking one packed or more a day at least five years. Persons were matched between the study and the control groups according to their ages, their incomes that were classified as low, medium, and
high, and the state of being married or unmarried. Because of the number of female who was smoker and over 35 years was very few, females were not included to the study. From the bloods, serum glucose, aspartate amino transferase, alanine amino transferase, urea, and creatinine levels were studied. Persons who had abnormal level of at least one of these parameters were excluded to the study. On the other hand, individuals who have any systemic illness or who were taking any medication or antioxidant for prophylaxis were not included to this study even their routine tests were normal.

All samples were taken from the persons in the same day. The samples that belong to the both groups were performed in one series. Collecting the samples and determining the levels of G6PD, albumin, bilirubin, urate, and blood counting were done in the same day. The samples for GSH-Px, Catalase, SOD, and MDA studies were kept in deep freeze (-70°C), and studied in a week.

Sample Preparation
Blood samples were taken into the empty tubes and ones with EDTA, following ten to twelve hours fasting. Blood serum in the empty tubes was separated by centrifuging for 10 minutes at 3000 round/min. From this serum, uric acid, and albumin were measured at the same day. For serum catalase and MDA, it was left in deep freeze.

After blood counting, 3 mL blood from tubes with EDTA were pulled into an injector that has 10 cc volume and contains 2 mL solution from Dextran 70 and then this mixture was shakened. The injector was kept waiting for 60 minutes in vertical position at room temperature. At the end of this time, superior phase containing leukocytes was thrown out. Inferior phase containing erythrocytes was taken into a glass tube. This tube was centrifuged at 2000 round per minute for 10 minutes. Supernatant was excluded. Inferior part was washed three times with cold saline. Thus, erythrocyte packet was obtained. Blood counting was made from the packet with Coulter Max M mark blood counter device. From this packet G6PD was measured daily. The samples for GSH-Px, SOD and MDA measurements were kept in deep freeze.

Assays
The determination of GSH-Px and G6PD were made by using commercial kits (Randox Laboratories, UK) whose principles were based on generally accepted enzymatic methods (7,16). Catalase determination was made by the modification of Goth’s method (17). In this method, calculation was made via this formula. [Catalase activity (kU/L)= (A_{B1} - A_{S})/(A_{B2} - A_{B3}) x 271]. The formula misprinted in the literature was changed as above. The estimation of SOD was studied according to Flohe et al method (18). MDA was studied by using the method of thiobarbituric acid reactivity (19). Uric acid and albumin levels were measured by using Vitrous commercial kit in Vitrous-750 autoanalyzer.

The spectrophotometer Shimadzu UV-1202V (Japan) was used for GSH-Px, catalase, SOD, G6PD, and MDA analysis.

Statistics
Statistics were performed by using Student’s t-test and Pearson correlation test in “SPSS for Windows 6.0” packet program.

RESULTS
The mean ± SD of ages of the study and control groups were 50.06 ± 16.5 years and 52.44 ± 15.9 respectively. There was no difference between ages (p> 0.05). In the study group, mean SD of smoking time was 23.74 ± 16.6 years and the packet a day was 1.12 ± 0.40. The comparison of both groups were given in Table 1.

As seen in Table 1, serum catalase and erythrocyte GSH-Px activities were found significantly lower, but serum and erythrocyte MDA levels were significantly higher in the study group compared to the controls. There was no significant difference in other parameters between both groups.

In Pearson correlation test, a positive correlation was found between G6PD and GSH-Px, and smoking time and G6PD. However, there was a negative correlation between GSH-Px and erythrocyte MDA, G6PD and erythrocyte MDA, and hemoglobin and G6PD.
DISCUSSION

Oxidative injury caused by free radicals may involve in the pathogenesis of some diseases occurred in smokers. The damaging effect of cigarette may develop as a result of either the direct effect of its toxic chemical contents or the release of toxic free radicals from activated inflammatory cells (20). There are several oxidants that cause membrane damage in the content of the cigarette smoke. It has been shown that the cigarette smoke causes lipid peroxidation in plasma and teratogenic alterations in lipoproteins. Cytotoxic activities of free oxygen radicals show themselves by causing peroxidation of the membrane phospholipids which cause alterations in the membrane fluidity, increase in the permeability and loss of membrane integration (21).

We have found that MDA was significantly higher in both serum and erythrocyte in the smokers (p< 0.001). High lipid peroxidation values in both serum and erythrocyte suggest that oxidative injury occurs in smokers.

We have also found that peripheral leukocyte number was high in smokers. In previous studies, it had been found also higher in smokers (22). The cause of this increase may be the increase in catecholamine due to nicotine or inflammation resulted from the smoke irritation in airways.

Although blood and serum MDA have been found to be high in smokers in several studies, some researches observed that there was no difference in lipid peroxidation between smokers and nonsmokers (10,23,24).

Antioxidants have an important role in preventing the free radical injury occurred in smokers. These are intracellular enzymes such as SOD, catalase, and GSH-Px, vitamins such as tocopherol and ascorbate, and trace elements (3,25).

In the study, erythrocyte GSH-Px activity was significantly lower in smokers. However, there was no significance for G6PD activity between the groups although a slight elevation was observed in smokers. Similarly, Peirce et all also found increased G6PD activity in cigarette smoke exposed rats (26). Thus, it can be suggested that G6PD activity increases to reduce increased oxidized glutathione and GSH-Px is depleted while it eliminates increased H2O2 in smokers. Furthermore, a positive correlation was found between GSH-Px and G6PD, supporting these findings.

GSH-Px in erythrocytes is the most effective antioxidants against oxidative stress. Decreasing GSH-Px activity causes to increase in H2O2 and severe cell injury (7). Oxidative stress may cause to increase in GSH-Px activity, which subse-

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<tr>
<th>Table 1. Comparison of study and control groups.</th>
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<tbody>
<tr>
<td><strong>Parameters</strong></td>
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<tr>
<td>GSH-Px (U/gHb)</td>
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<td>Catalase (kU/L)</td>
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<td>SOD (U/gHb)</td>
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<td>G6PD (mU/10⁹ erythrocyte)</td>
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<td>Serum MDA (nmol/mL)</td>
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<td>Erythrocyte MDA (nmol/10¹⁰ erythrocyte)</td>
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<td>Albumin (g/dL)</td>
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* p< 0.01
** p< 0.001
quent oxidizes glutathione. In order to reduce this oxidized glutathione, it is expected that PPP will be activated and G6PD will be increased. Supportedly, a positive correlation was observed between GSH-Px and G6PD. Inspide of this correlation, decreased level of GSH-Px in smokers supports the idea that this enzyme is being depleted.

Negative correlation between erythrocyte MDA and GSH-Px, and erythrocyte MDA and G6PD suggest that G6PD and GSH-Px increase to protect erythrocytes against oxidative injury increased in erythrocytes. Why these protective enzymes have not high levels may be the result of their depletion while they prevent oxidative injury.

A positive correlation was determined between G6PD and smoking time in smokers. We have thought that more smoking time, higher levels of G6PD as the erythrocyte protection.

Different results has been found in the studies concerning SOD. Some researchers suggested that SOD didn’t change but some said it decreased (27,28). In this study, no significant difference was found in erythrocyte SOD activities between groups. Leonard et al couldn’t find any difference in SOD and GSH-Px activities between smokers and nonsmokers like us, and they concluded that free radicals occurred in long-time smokers were being cleared sufficiently (24).

In some studies, it has been observed that smoke did not change Catalase activity. Herein, we found lower serum catalase activity in the smokers compared to the control group. GSH-Px and Catalase are the enzymes which detoxify H$_2$O$_2$. Lower levels of both enzymes may be the result of depletion of enzymes while they are eliminating the excessive H$_2$O$_2$ production of cigarette.

Furthermore, hydroxyl radical can cause enzyme inactivation by breaking the structure of metal-containing enzymes (Catalase, SOD, GSH-Px). This inactivation influences the catalase much more than others. Again SOD and Catalase becomes inactivated by singled oxygen. Inactivation of these enzymes at low oxidant concentrations resembles each others. Although catalase is more reactive, it shows more enzyme inactivation than SOD as a result of protein and radical interaction (29).

Serum hemoglobin level was statistically higher in the smokers. Hemoglobin increase may be due to its protective effect against toxic effect of cigarette or the carbonmonoxide level increased by cigarette. However, negative correlation between Hb and G6PD may indicate that hemoglobin increases for its antioxidant action. Furthermore, it may be thought from this negative correlation that Hb and G6PD act in complementary fashion each other for antioxidant defense. If increase in hemoglobin were due to increasing CO, the number erythrocytes of the smokers would increase, but such an increase could not be observed.

Depending on the findings above, it can be suggested that antioxidant compounds are depleted while they resist to oxidative injury of cigarette smoke or inhibited by some of several chemical compounds of cigarette.

REFERENCES


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