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Full Length Research Paper

Evaluation of oxidative damage to peptic tissue DNA and gastric juice levels of nitric oxide and oxidative stress in smokers and non-smokers with signs of dyspepsia

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Smoking elevates risk of peptic ulcer and gastrointestinal cancer in human being. In this study, the severity of the damage was assessed by detection of nitric oxide levels, oxidative stress of gastric juice in smokers and non-smokers. 43 smoker patients with active peptic ulcer as case group and 43 non-smokers without peptic ulcer, 43 smokers without peptic ulcer and 43 non-smokers with active peptic ulcer as control groups were selected for this study. The levels of nitric oxide in gastric juice and the rate of DNA damage, those of total antioxidant capacity and the activities of superoxide dismutase and glutathione peroxidase in gastric mucosa were determined using standard methods. The rate of DNA damage in case group was significantly higher than those of controls groups. Comparing with two control groups, increase in nitric oxide levels of case group was noticed. Comparing with control group, significant elevation in the mean activities of superoxide dismutase and glutathione peroxidase was observed in the case group. Total antioxidant capacities of control groups were higher than that of case group. Results of the study shows that damage rate of DNA have a direct correlation with the presence of toxic agents in cigarette smoke and tar especially NO^{o}. Increase in activity of antioxidant enzymes, superoxide dismutase and glutathione peroxidase, and decrease in total antioxidant capacity in gastric juice; confirm the presence of oxidative stress in smokers' gastric juice.

Key words: Cigarette smoking, DNA damage, oxidative and nitrosative stress, nitric oxide, dyspepsia.

INTRODUCTION

Epidemiologic studies have revealed that smoking is an important factor in forming malignancy in different tissues of the body (Levitz et al., 2004; Parkin et al., 1994). Although the exact mechanisms about the relationship between smoking and cancer has not been proved yet, it is obvious that smoke of tobacco has more than 3800 types of toxic, carcinogenic materials (Levitz et al., 2004; Yoshi and Ohshima, 1997) including aromatic polycyclic hydrocarbons, aromatic amines (Brunnemann and Hoffmann, 1982), tobacco nitrose amines (Norman et al., 1983) and among toxic complexes formaldehyde, acetaldehyde, acroleine (Grafstrom et al., 1986; Dypbukt et al., 1993; Eisenbrand et al., 1995), short-life radicals and reactive oxygen species which are produced by oxidation and hydrogenation of catechole and hydroquinone (Yang et al., 1999; Pryor, 1992; Kodama et al., 1997), are from this category. In a study on rat population (Lindell et al., 1997) it was concluded that nicotine accumulation in gastric juice stimulates central nervous system and oxidative stress in the gastrointestinal system of these animals and it was reported that the dual reaction between stress and nicotine worsens peptic ulcer (Lindell et al.,

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1997). Gastric mucosa prostaglandin, nitric oxide and other adhesive gastric mucosal complexes take part in protecting stomach from damages, nevertheless, it has been reported that neutrophils go through the gastric septum due to the stress caused by nicotine in the stomach. This accelerates the injury to the stomach (Nishida et al., 1997). The fresh smoke produced by each cigarette has more than 600 micrograms of nitric oxide (NO^o) radical in its gas phase. NO^o concentration in smoke has a linear relationship with nitrate level in a cigarette (Yoshi and Ohshima, 1997; Brunnemann and Hoffmann, 1982).

Nonetheless, more than 100 micrograms of NO^o as a result of nitrogenic compartments in tobacco or probably atmospheric nitrogen oxidation, enters body without filtering (Yoshi and Ohshima, 1997; Norman et al., 1983). On the other hand, it has been reported that cigarette tar has high values of reactive oxygen species (Pryor, 1992; Kodama et al., 1997), peroxynitrite (Pryor, 1992; Muller et al., 1997) and ethylating agents (Prevost and Shuker, 1996; Kopplin et al., 1995) that have a direct destroying effect on DNA (Yoshi and Ohshima, 1997). Main radical forms, quinone (Q) and hydroquinone (QH2) molecules held in a tar matrix (Pryor et al., 1983, Pryor et al 1983) are an active oxidation-reduction system which can reduce oxygen to radical superoxide (O_2^o) which is the precursor of hydrogen peroxide ($H_2O_2^o$) and hydroxyl (HO^o) (Yoshi and Ohshima, 1997). Recently it has been proved that gas phase of cigarette smoke is the main agent in damaging strain injury and basal change in DNA (forming xanthine and hypoxanthine) in epithelium of human respiratory system (Spencer et al., 1995) which shows that active forms of nitrogen can have inducing effects in injury to DNA. Nevertheless, there is not any study preformed on the damages due to cigarette smoke to DNA using synchronous gas phase and tar concentrations of tobacco. Reaction between NO^o and superoxide anion which is a limit-distribution reaction creates a very powerful oxidizing and nitrating factor called peroxynitrite. This product can also be created by a reaction between nitroxile anion and molecular oxygen with an almost controlled progression. Peroxynitrite is a very active agent and leads to very quick oxidation of sulfidril, tio-eter and also nitration, nitrosilation and hydroxylation of aromatic complexes like tyrosine and triptophan (Beckman and Koppenol, 1996; Pryor and Squadrito, 1995; Fukuto et al., 2005; Sawa et al., 2000).

Smoking produces many free radicals in the body estimating 2×10^{14} per cigarette which includes: Different oxygen, carbon and sulfur radicals, nitric oxide and hydrogen peroxide (Ohshima et al., 2003). These radicals with the nicotine act as a united mediator to decrease vessels epithelial activity in addition to their harmful affects on several tissues and damage to DNA (Nicita-Mauro et al., 2008).

We still do not have any absolute and definite index for oxidative stress but there are many indices which can partly indicate this condition (Herbert, 1999). Recently, some studies have shown that measuring superoxide dismutase activity of mucus shows oxidative damage for a large part (Noguchi et al., 2002).

On the other hand in mucosal injuries, rise in free radicals is stimulated in antrum and corpus caused by simple voiding of glutathione and α-tocopherol; this condition leads to extension of lesions in antrum and corpus (Jung et al., 2001; Yoshidawa et al., 1997). There is another antioxidant enzyme in clearance of these free radicals called glutathione peroxidase. This enzyme is categorized in selenoproteins group and has an important role in defending mechanisms against oxidative lesions in mammals, birds and fishes (Yoshidawa et al., 1997).

Therefore, because tobacco smoke contains high concentration of gas phase NO^o and a Q/QH_2 system which produces radical superoxide in liquid tar, our objective was to identify the synchronous effects of smoke and tar of cigarette on the induction of DNA damaging. We evaluated the level of peptic juice nitric oxide and oxidative stress of gastric tissue in smokers and non-smokers as well as its relationship with damage rate to DNA in these tissues.

MATERIALS AND METHODS

This is a cross-sectional case-control study which its objects were smokers' patient with dyspepsia. Patients who visited by the specialist were referred to endoscopy ward of Emam Reza educational health center based upon their indication and then divided into two smoker and non-smoker groups. Patients were involved in this study by a prior consent and evaluation surrounding other diseases like peptic cancer, antioxidant, bismuth and antiacid drugs use and other cases which might influence our study by making false positive results, so that these patients were excluded from the study. Then 43 smoker patients with active peptic ulcer (14 men and 29 women) with the average age of45.30±13.6 as case group and 43 non- smokers without peptic ulcer (13 men and 30 women) with average age of 42.67±16.04 as control group 1 and 43 smokers without peptic ulcer (16 men and 27 women) with average age of 44.58±12.57 as control group 2 and 43 non-smokers with active peptic ulcer (20 men and 27 women) with average age of 45.37±13.39 as control group 3.

As a rule, selected patients were evaluated in case they had active peptic ulcer by endoscopy. Gastric juice was taken from each individual in fasting condition and then 2 other biopsy samples from antrum and corpus were obtained. One of these samples was used in quick urease test (1 h) which is a quick test to detect the presence of *Helicobacter pylori*. Then this sample was sent to pathology ward to confirm presence of *H. pylori* and malignancy (mainly in cases who both urease test and pathologic evaluation were negative, the result would be negative but in case one of these tests was positive, the result would be positive). Second sample was kept in freezer in -70°C to study damage rate to DNA, and gastric juice sample was used to assess NO^o, SOD (superoxide dismutase), GPX (glutathione peroxidase) and TAC (total antioxidant capacity). Damage to gastric tissue DNA was measured by DNA damage kit (Kimiya biomedical, Cat. No. DN-004) measuring with CECIL 3000 SCANING SPECTROPHOTOMETR [measured the apurinic/apyrimidinic (AP) site] in all groups. The levels of NO° in gastric juice were measured colorimetrically by Griess method (Dolatkhah et al., 2011; Ansari et

Table 1. Data related to comparison between control groups 1 to 3 with case group.

Age group	n	Mean±SD (Years)	f	p-Value*
Control-1	43	42.67±16.04	0.360	0.812
Case	43	45.30±13.16		
Control-2	43	44.58±12.07	0.360	0.995
Case	43	45.30±13.16		
Control-3	43	45.37±13.39	0.360	1.00
Case	43	45.30±13.16		

*- The P<0.05 was meaningful.

al., 2006; Green et al., 1982) with BDSL Immunoskan MS ELISA Reader. The activities of SOD, GPX and total gastric juice antioxidant capacity were determined in Hitachi 911 Auto Analyzer using Randox kits (Dolatkhah et al., 2011; Brown et al., 1992; Gotz et al., 1996). The levels of protein in the samples were measured colorimetrically by Lowry method (Dolatkhah et al., 2011; Ansari et al., 2006; Lowry et al., 1951). The results of the enzyme analysis were reported as IU/mg protein.

To analyze considered parameters, we determined presumed factors' averages in each group separately and then compared these values by one-way variance ANOVA using SPSS version 12. The P<0.05 was meaningful in this study.

RESULTS

One hundred and seventy two individuals were used for the study. 50% of them were smokers and the rest were non-smokers. Each group had been divided into 2. 50% of smokers had active peptic ulcer (case group) and the rest did not (control group 1). The 50% of 86 non-smokers had active peptic ulcer (control group 2) and 50% did not (control group 3). No meaningful differences were noticed between the mean age of control groups 1 to 3, and case group (Table 1).

To analyze considered age, averages in each group were determined separately and then compared by one-way variance ANOVA using SPSS version 12. According to Figure 1, damage rate to stomach tissue DNA in comparison with control groups had a meaningful difference in case group or smokers with active peptic ulcer. According to Figure 2, it is obvious that peptic juice nitric oxide in case group has a meaningful difference compared to control groups 1 and 3, but these values did not have meaningful differences between case group and control group 2.

In other words, peptic ulcer nitric oxide in case group has a little rise compared to control group 2. In smokers with active peptic ulcer, superoxide dismutase activity and glutathione peroxidase levels were significantly high in case group rather than control groups 1, 2 and 3 (Figures 3 and 4).

According to Figure 5, also in smokers with active peptic ulcer, a total antioxidant capacity level was significantly low in case group rather than control groups 1 to 3.

DISCUSSION AND CONCLUSION

There is a strong epidemiologic relationship between smoking and many cancers. It has been shown that carcinogenic nitrose amines, aromatic polycyclic hydrocarbons and other toxic complexes present in smoke and tar of cigarette can cause carcinogenic effects on target cells (Kocyigit et al., 2011; Parkin et al., 1994). In the present study, damage to peptic tissue DNA due to smoke and tar of cigarette appeared on apurinic/ apyrimidinic (AP) site. As it is indicated in results, AP site had a meaningful increase in case group rather than control groups 1 to 3 (in all individuals p<0.0001). This increase in damage rate to DNA has a direct relationship with the presence of toxic complexes in cigarette specially NO°. Muler et al. (1997) showed formation of peroxynitrite in cigarette smoke (which is a known agent in damage to DNA). Matsukura et al. (1991) has reported a direct relationship between toxic and mutagen effects of smoking and several diseases. Our findings based upon increase in peptic juice NO° in smokers rather than non-smokers were consistent with the aforementioned studies and therefore, one of the causes of increase in damage rate to peptic tissue DNA and risk of malignancies in case group would be rise in nitric oxide radicals.

On the other hand, in a study conducted by Yang et al. (1999), the presence of free short-life radicals and complexes releasing reactive oxygen species were admitted. Because active oxygen mediators coming from smoke are also one of the factors that cause DNA damage in different cells, our findings in this study is consistent with the process that radicals produced by tobacco smoke initiate damage to DNA using oxidation-reduction cycle, therefore this finding supports Tsutsui et al. (1997) study considering mutations and other genetic injuries caused by catecholamine and 1-4 hydroquinone in hamster cells (Tsutsui et al., 1997).

In another study by Yoshi and Ohshima,, it has been shown that DNA strain rupture in plasmid is stimulated in presence of a complex releasing NO° and tar concentrate, but these factors cause little injuries when they are acting alone. Therefore a new oxidant produced by reaction between NO° and tar can be responsible for this vast lesions in various tissues. It is highly probable that radical peroxynitrite which is a production of the quick reaction between NO° and O_2°, is responsible for this injury. Radical peroxide (O_2°) can be produced by spontaneous oxidation of hydroxy aromatics like catechole and 1-4hydrocoinone that both are present in high concentration in tar of cigarette (Yoshi and Ohshima, 1997). Peroxynitrite is a very powerful oxidant and nitrating agent that is able to initiate reactions associated with (HO°), nitrososnium (NO_2°) and nitrogen dioxide

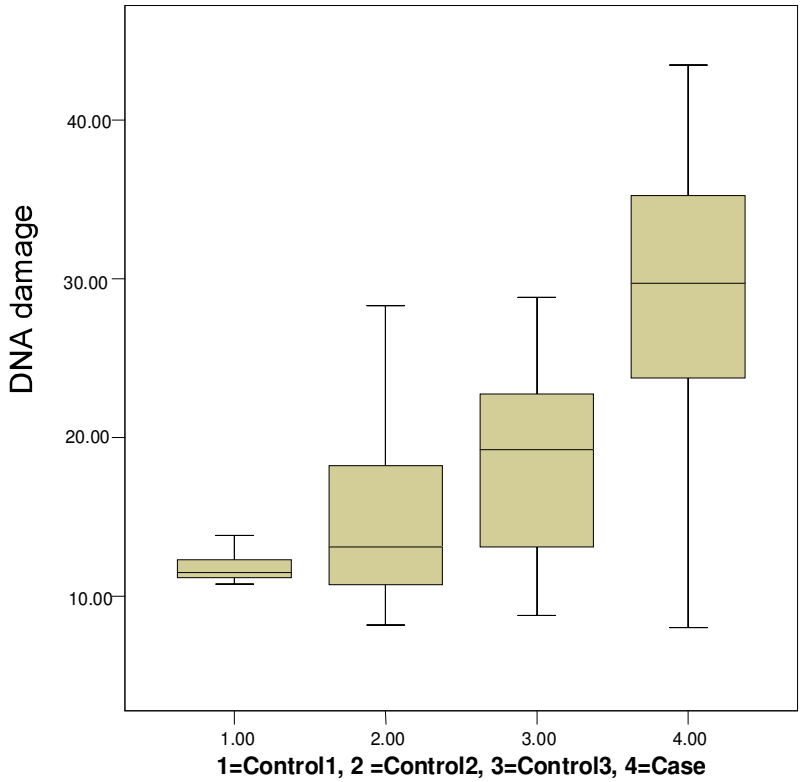

Figure 1. Graph related to mean damage rates to stomach tissue DNA in 4 studied groups.

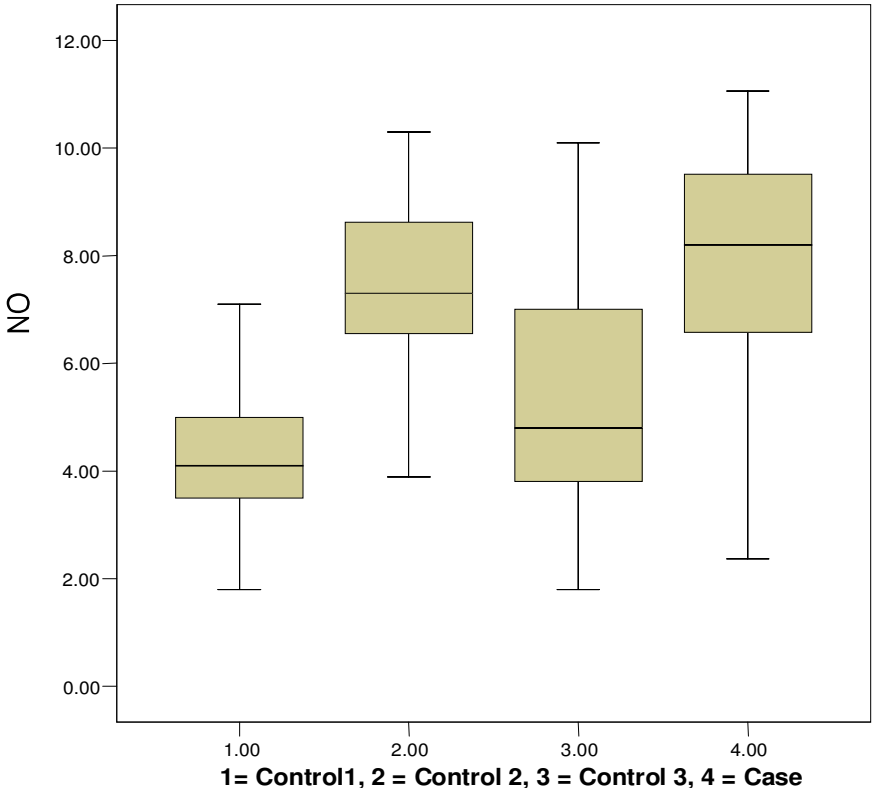

Figure 2. Graph related to mean peptic juice nitric oxide among the studied groups.

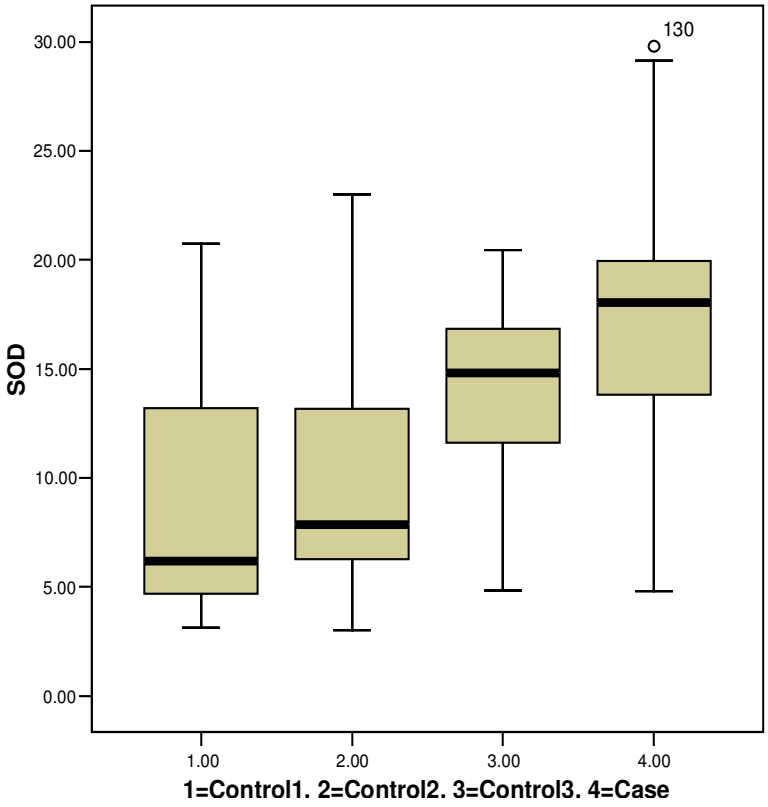

Figure 3. Graph related to mean peptic juice superoxide dismutase activity and glutathione peroxidase in 4 studied groups.

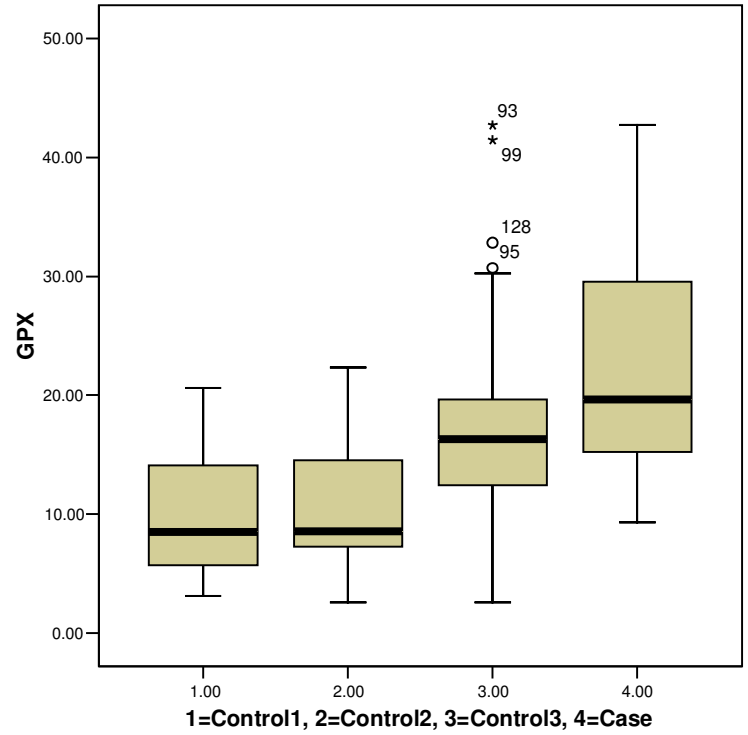

Figure 4. Graph related to comparison between mean peptic juice glutathione peroxidase specific activities among 4 studied groups.

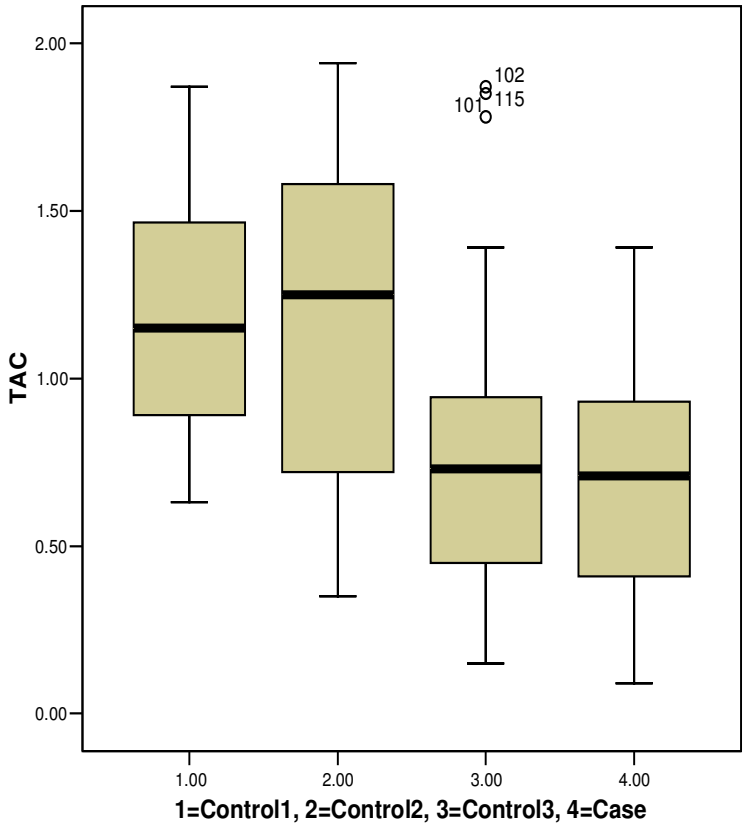

Figure 5. Graph related to comparison between mean peptic juices total antioxidant capacities among 4 studied groups.

(NO_2) radicals. It had been proved that peroxynitrite is able to induce rupture in plasmid DNA strains in *in-vitro* condition. But the radical is not inhibited by antioxidants such as D-manitol and dimethyl sulfoxide (Salgo et al., 1995), injuries caused by this radical would be worsened. On the other hand, based upon Pryor et al. 1992, theory, cigarette smoke like peroxynitrite can block α-1-protease inhibitor and lead to over activation of protein lyses and therefore destruction of connective tissue in lower respiratory system (Pryor, 1992; Evans and Pryor, 1992). This destruction is associated with emphysema in smokers (Evans and Pryor, 1994). Exact mechanisms of free radicals production fallowing smoking is not understood completely and as it was mentioned, there are some theories surrounding this process. Anyway, it is obvious that smoking will give rise to the increase in some free radicals in different body tissues of a smoker that leads to severe oxidative pressure on tissues; specific activity of two antioxidant enzymes had a significant increase in smokers with peptic ulcer compared to control groups.

It seems that reactive oxygen and nitrogen species like peroxide and peroxynitrite radicals and new unknown complexes which are produced in the reaction between tar and nitric oxide have an important role in damage to DNA and related diseases like gastric cancer.

It can be concluded that smoking, first by increasing peptic juice nitric oxide and secondly by increasing presence of free radicals and complexes releasing reactive oxygen and nitrogen species in gastric tissue which are produced and mediated by complexes present in smoke and tar of cigarette, can initiate damage to cells' DNA and consequent rise in risk of malignancies in tissues.

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