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. 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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oxidative stress but there are many indices which can
Mauro et al., 2008).

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
with an almost controlled progression. Peroxynitrite is a
concentrations of tobacco. Reaction between NO
reduce oxygen to radical superoxide (O
are an active oxidation-reduction system which can
reduce oxygen to radical superoxide (O
and hydroxyl (HO
which is the
precursor of hydrogen peroxide (H
2
) and hydroxyl
(\text{HO}^2\text{\textsuperscript{\scriptstyle 2}})\) (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
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
triphtohan (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 2x10^{10}\text{s} 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\text{\textsuperscript{\scriptstyle 2}} and a Q/\text{OH}_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 Eman 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\text{\textsuperscript{\scriptstyle 2}} and a Q/\text{OH}_2 system
correlation with damage to DNA. These samples were measured
spectrophotometrically by Griess method (Dolatkhah et al., 2011; Ansari et

SPECTROPHOTOMETR [measured the apurinic/apyrimidinic (AP)
...
Table 1. Data related to comparison between control groups 1 to 3 with case group.

<table>
<thead>
<tr>
<th>Age group</th>
<th>n</th>
<th>Means±SD (Years)</th>
<th>f</th>
<th>p-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-1</td>
<td>43</td>
<td>42.67±16.04</td>
<td>0.360</td>
<td>0.812</td>
</tr>
<tr>
<td>Case</td>
<td>43</td>
<td>45.30±13.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control-2</td>
<td>43</td>
<td>44.58±12.07</td>
<td>0.360</td>
<td>0.995</td>
</tr>
<tr>
<td>Case</td>
<td>43</td>
<td>45.30±13.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control-3</td>
<td>43</td>
<td>45.37±13.39</td>
<td>0.360</td>
<td>1.00</td>
</tr>
<tr>
<td>Case</td>
<td>43</td>
<td>45.30±13.16</td>
<td></td>
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</tr>
</tbody>
</table>

* The P<0.05 was meaningful.

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 O₂°, is responsible for this injury. Radical peroxide (O₂°) can be produced by spontaneous oxidation of hydroxy aromatics like catechole and 1-4 hydroquinone 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°), nitrososnum (NO₂°) 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.
(NO₂) radicals. It had been proved that peroxynitrite is able to induce rupture in plasmid DNA strains in \textit{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 lyzes 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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\textbf{REFERENCES}


