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Full Length Research Paper

Levels of nitric oxide in gastric juice of smokers and non-smokers with active peptic ulcer

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Epidemiologic studies have shown that smoking is a major factor in development of malignancies in various human tissues. Smoke from every cigarette contains more than 600 µg nitric oxide radical (NO°) in gas phase. As a result of oxidation of nitrogen in gradients of tobacco and likely atmospheric nitrogen, more than 100 µg NO° is released in cigarette smoke which goes over human palate directly and without any filtering. In this research, we studied levels of nitric oxide in gastric juice of smokers and non-smokers afflicted with active peptic ulcer. Among persons referred to gastroenterology clinic, 43 smoker patients (14 men and 29 women) with average age of 45/30±13/16 who were afflicted with active peptic ulcer were determined as the case group, 43 non-smokers (13 men and 30 women) without peptide ulcer with average age of 42/67±16/04 were determined as the first control group, 43 smokers (16 men and 27 women) without peptic ulcer, with an average age of 44/58±12/07, were determined as the second control group and 43 non-smokers (23 men and 40 women) with peptic ulcer, with an average age of 45/37±13/39, were determined as the third control group. Levels of nitric oxide in gastric juice in the four groups were assessed by means of *Griess* colorimetric method. Compared to control groups 1 and 3, levels of nitric oxide in the case group showed a meaningful increase (in both groups, P<0/0001) while nitric oxide levels in gastric juice of the case group and control group 2 (smokers without active peptic ulcer) did not have any meaningful difference (p=0/656). The results of this study ascertain that damage to the gastric tissue is in direct relationship with toxic elements in cigarette smoke especially NO° radical. It is very likely that peroxynitrite radical ($ONOO^-$), which resulted from rapid reaction between NO° and O_2°, is responsible for these injuries. $ONOO^-$ is a powerful oxidant and nitrating element that can promote reactions of HO°, nitrosoniume (NO_2°) and nitrogen dioxide.

Key words: Active peptic ulcer, cigarette smoking, nitric oxide, nitrosative stress.

INTRODUCTION

Smoking is one of health threatening concerns especially, in progressing countries. Smoking, probably, is due to machine lifestyle and its contrast with traditional lifestyle. According to World Health Organization (WHO), there are 1/15 billion smokers in the world, 80% of which are in progressing countries. Tobacco smoke contains more than 3800 chemicals including toxic chemicals like formaldehyde, acetaldehyde, acrolein (Grafstrom et al., 1986; Dypbukt et al., 1993; Eisenbrand et al., 1995); short lasting radicals and forms of active oxygen which result from oxidation and reduction of cycleofcatechol and hydroquinone; tobacco coordinate carcinogens; carcinogens and tumor causers (International Agency for Research on Cancer, 1986; Yoshi and Ohshima, 1997) like polycyclic aromatic hydrocarbons, aromatic amines (Brunnemann and Hoffmann, 1982) and tobacco

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coordinate nitrosamines. Smoke resulting from every cigarette contains more than 600 µg nitric oxide radical (NO°) in gas phase. Nitric oxide is a radical that binds to Iron and Cupper carrier proteins (Rode, 2000). This radical is produced in various cells including, vascular endothelial cells, neurons, neutrophils and macrophages (Calatayud et al., 2001). NO° concentration in cigarette smoke is in a linear reciprocal correlation with NO° concentrations in every cigarette (Yoshi and Ohshima, 1997; Brunnemann and Hoffmann, 1982). Yet, more than 100 µg of NO° is released in cigarette smoke due to oxidation of nitrogen components of tobacco and presumably oxidation of atmospheric nitrogen and goes over a person's palate without filtering (Yoshi and Ohshima, 1997; Norman et al., 1983). Main radical forms which are conserved in as quinone and hydroquinone (Q/QH_2) in a matrix of tar (Pryor et al., 1983a, b), serve as an active oxidation-reduction system which can reduce molecular oxygen in order to produce superoxide radical (O_2°), and afterward hydrogen peroxide (H_2O_2°) and hydroxyl (HO°) radical will be produced too (Yoshi and Ohshima, 1997).

In recent years, several studies have shown that in aerobic condition, nitric oxide is oxidized instinctively to yield the N_2O_3 which is a powerful nitrozating factor. Nitrozation of secondary amines by N_2O_3 yields N-Nitrozamines which can alkylate nucleic bases and yield mutagens such as O^6-alkyle guanine which causes a guanine base to replace with adenine. There are reports of acute and continual inflammation in animal cases, and also, infection and inflammation in human cases due to increase in N-nitrozamines production *in vivo* (Sawa and Ohshima, 2006). Reaction of NO° with superoxide anion, as a process with limited dispersion potency, yields a very powerful oxidative and nitrating element called peroxinitrite. Peroxynitrite is also produced by reaction between nitroxile anion and O_2 with a controlled progress. Peroxynitrite is strongly active and causes rapid oxidation of sulfidrile and thioether as well as nitration, nitrozilation and hydroxylation of aromatic compounds like tyrosine and tryptophan (Beckman and Koppenol, 1996; Pryor and Squadrito, 1995; Fukuto et al., 2005; Sawa et al., 2000). As mentioned above, while smoking, a large amount of free radicals is released into the human body (Yang et al., 1999; Pryor, 1992; Kodama et al., 1997), and it is estimated that with every cigarette, about 2×10^{14} free radicals are produced including: different types of oxygen radicals, carbon, sulfur, great amounts of nitric oxide and H_2O_2° (Ohshima et al., 2003).

In addition to the harmful effects on various tissues and damage to DNA, these radicals have a coordinated activity along with nicotine and act as an important intermediate for decreasing vascular epithelial cells activity (Nicita-Mauro et al., 2008).

Due to reaction between NO° and various kinds of oxygen radicals, two metabolites, peroxinitrogen ($OONO^-$°) and superoxide (O2 °), are formed which are very toxic and cause severe oxidative pressure in different body tissues (Beckman and Koppenol, 1996; Wink and Mitchell, 1998).

Gastric mucus will also be affected by this oxidative pressure and as a result, peroxidation of lipids, damage to DNA, oxidation of proteins and deactivation of enzymes will occur (Pignatelli et al., 2001). In addition to endoscopic assessment of gastric tissue and lesions resulting from smoking in this tissue, in this study, we also assessed and compared nitric oxide levels in gastric juice of smokers afflicted to dyspepsia with the control groups.

MATERIALS AND METHODS

Patients with symptoms of dyspepsia who conferred to gastroenterology specialist assigned to district of endoscopy of Imam Reza Hospital by indication, were assessed for smoking, and categorized as smoker and non-smoker. With their consent, patients were involved in the study. As the first step, patients were checked for other diseases and conditions such as gastric cancer, use of antioxidant drugs, anti-acid element and drugs such as Bismuth and other elements which can cause false effects. Cases whose history for the mentioned elements was positive were excluded from the study.

In endoscopy, as usual, patients were assessed for existence or absence of active peptic ulcer and then four groups of study were selected. First of all, biopsy samples were taken from antrum and the body of every patient's stomach in fasting condition. These samples were used for rapid (1 h) test of urease. This is a rapid test for distinction of presence or absence of *Helicobacter pylori*. Results of the test are recorded in tables by order of their group. Next, these samples were passed to the Department of Pathology for confirmation of presence or absence of *H. pylori* (as a frame, when both urease and pathologic assessment are negative for a patient, the patient will be considered as negative for *H. pylori* and when one of these tests is positive, he/she will be considered as positive). In order to assess nitric oxide levels, samples of gastric juice were taken from patients of all study groups and transferred to -70°C. In the present study, we perused smokers who had peptic ulcer as group-1 (case group), non-smokers with peptic ulcer as group-2 (control), smokers without active peptic ulcer as group-3 (control) and non-smokers without active peptic ulcer as group-4 (control).

To make the study results extendable to all smokers, we matched the case group with the other three control groups by age, gender, history of disease, income, etc. Nitric oxide levels in gastric juice were measured by Griess colorimetric method.

Statistical analysis of results of the aforementioned measurements was done by SPSS version 23 and the use of one-way variance analysis (one-way ANOVA) and multiple compassions (Tukey HSD).

RESULTS

Out of the 172 persons that were involved in this study, 50% (86 persons) were smokers and 50% (86 persons)

Table 1. Information of comparison mean of age in one, two and three control groups with cases group.

Groups \ Age	n	Mean ± SD (Years)	f	p-value	$CI_{95\%}$
Control group 1	43	42.67 ± 16.04	0.360	0.812	From -10.32 up to 5.06
Case group	43	45.30 ± 13.60			
Control group 2	43	44.58 ± 12.07	0.360	0.995	From -8.41 up to 6.97
Case group	43	45.30 ± 13.16			
Control group 3	43	45.37 ± 13.39	0.360	1.00	From -7.62 up to 7.76
Case group	43	45.30 ± 13.16			

Table 2. Information about comparison of nitric oxide levels in gastric juice of members falling in Control groups 1, 2 and 3 with those who fall in Case group.

Groups	N	Nitric Oxide (Mean ± SD) (µM/L)	F	p-value
Control group 1	43	4.21±1.13	39.30	0.0001
Case group	43	7/90±2/12		
Control group 2	43	7.45±1.54	39.30	0.656
Case group	43	7.90±2.12		
Control group 3	43	5.37±2.26	39.30	0.0001
Case group	43	7.90±2.12		

were non-smokers. 50% of the 86 smokers had active peptic ulcer (case group) and 50% did not have (control group-1). Similar to that of the smokers, 50% of the 86 non-smokers had active peptic ulcer (control group-2) and 50% did not have active peptic ulcer (control group-3). Case group consisted of 29 men (67/5%) and 14 women (32/5%); Control group-1 consisted of 30 men (69/8%) and 13 women (30/2%); Control group-2 consisted of 27 men (62/8%) and 16 women (37/2%) and Control group-3 consisted of 23 men (53/5%) and 20 women (46/5%). There was no significant statistical difference in average age between control groups 1, 2, 3 and case group. In other words, equality gate for age of case group and the three control groups was well calculated Table 1.

According to Table 2 and Figure 1, where nitric oxide levels in gastric juice of smokers with active peptic ulcer (case group) had no significant difference with control group-2 (smokers without active peptic ulcer), nitric oxide levels in gastric juice of smokers with active peptic ulcer (case group) were of significant difference with control groups 1 and 2 (control group 1 = smokers without active peptic ulcer, control group 3 = non-smokers with active peptic ulcer) and they showed a significant increase. Scilicet, nitric oxide levels in gastric juice of case group compared to control group-2 showed a fiddling increase. Results are shown as mean ± standard deviation (in the whole groups except the control group-2: P- value< 0/0001).

DISCUSSION

Strong epidemiological correlations between smoking and increasing rate of different types of cancer along with different experimental studies have shown that carcinogenic nitroze-amines, polycyclic aromatic hydrocarbons, aromatic amines and other toxic compounds existing in smoke Tar cause carcinogenic effects on exposed cells (International Agency for Research on Cancer, 1986; Parkin et al., 1994). Increasing of damage to peptic tissue and DNA is in a direct relationship with toxic components especially NO° in smoke and tar. Muller et al. (1997) demonstrated the formation of peroxinitrite (a known factor damaging DNA) in smoke of cigarette. Matsukura et al. (1991) published an article on direct toxic and mutagenic effects of dense cigarette smoke. Prevost and Shuker (1996) described an ethylating factor which has direct effect on cells and their DNA in tobacco smoke. Considering this factor, we can explain high levels of 3-ethyl-adenin excreted in smokers' urine. Hence, the results of the present study regarding increase in NO° levels in gastric juice of smokers compared to non-smokers are in accordance with the results of the aforementioned studies. Our results suggest that increased level of nitric oxide is one of the effective factors which increase damage to DNA of

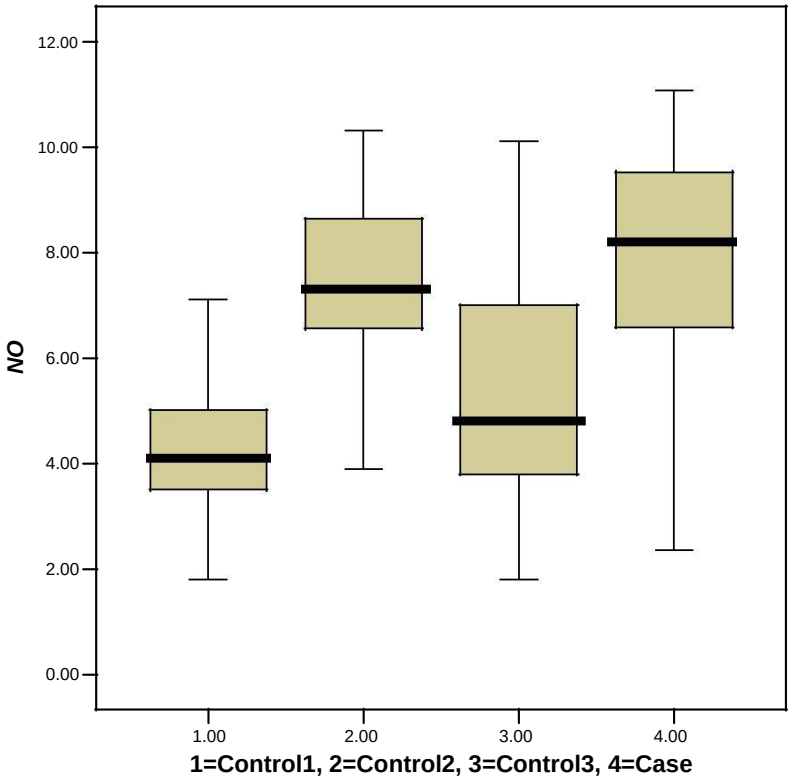

Figure 1. Schematic diagram of comparison between levels of nitric oxide in gastric juice of patients in the four groups of the study.

peptic tissue and subsequently enhance the risk of neoplasia in this tissue in members belonging to the case group.

Yoshi and Ohshima (1997) showed that in the presence of a mixture which releases nitric oxide and concentrate of smokes tar, breakage of plasmid DNA is triggered, but these factors singly, can just cause seldom damage to plasmid DNA. Hence, there may be a new oxidant among metabolites of reaction between NO° and smokes Tar which can be the effective element behind this vast damage in different tissues. It is likely that peroxinitrite radical (ONOO-) which resulted from rapid reaction between NO° and O_2° is the effective factor behind this damage. Peroxide radical (O_2°) can result from instinctive reaction of aromatic poly hydroxides like catechol and 1-4-hydroquinone which are both present in high concentrations in smokes tar (Yoshi and Ohshima, 1997). Peroxinitrite is a strong oxidant and nitrating factor which can initiate the reactions of HO°, Nitrozonum (NO_2°) and nitrogen dioxide (NO_2). It is confirmed that peroxinitrite can cause the breakage of plasmid DNA *in vitro*. These radicals cannot be limited by anti-oxidants such as D-mannitol and Dimethyl sulfoxide (*DMSO*) (Salgo et al., 1995) so they cause more severe oxidative damages to DNA. On the other hand, according to the hypothesis of Pryor (1992), like peroxinitrite, cigarette smoke can also deactivate α -1-protease inhibitor and cause over-activation of the protein digestion process. As a result, destruction of connective tissue in lower respiratory system increases (Pryor, 1992; Evans and Pryor, 1992). Such a destruction of connective tissue has been seen in relation to emphysema in smokers (Evans and Pryor, 1994).

Conclusion

Results of this study show that smoking is associated with considerable increase in nitric oxide radical amounts in gastric juice and that it increases the presence of nitrogen species releaser compounds in gastric tissue. As a result, the existing compounds in cigarette smoke and tar will cause the initiation of DNA-damage in cells and this will subsequently increase the risk of malignancies in these tissues through oxidation-reduction cycle.

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