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Comparison of levels of nitric oxide in gastric juice of smoker and non-smoker patients with dyspepsia.

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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^0) in gas phase. As a result of oxidation of nitrogen in gradients of tobacco and likely atmospheric nitrogen, more than 100 µg NO^o 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 referring to gastroenterology clinic, 43 smoker patient(14 men and 29 women)with average age of 45/30±13/16who afflicted with active peptic ulcer were determined as case group, 43 non-smoker(13 men and 30 women) without peptide ulcer with average age of 42/67±16/04 were determined as first control group, 43 smoker(16 men and 27 women)without peptic ulcer, average age of44/58±12/07,were determined as second control group and 43 non-smokers (23 men and 40 women) with peptic ulcer, average age 45/37±13/39, were determined as third control group. Levels of nitric oxide in gastric juice in four groups were assessed by means of Griess colorimetric method. Compared to control groups 1 and 3, levels of nitric oxide in Case group showed a meaningful increase (in both groups P<0/0001)while nitric oxide levels in gastric juice of Case group and Control group 2 (smokers without active peptic ulcer) didn't have any meaningful difference(p=0/656). Results of this study as certain that damage to the gastric tissue is in direct relationship with toxic element's in cigarette smoke specially NO^o radical. It's very likely that Peroxynitrite radical ($ONOO^-$), resulted from rapid reaction between NO^o and O_2^0, be responsible for these injuries. $ONOO^-$ is a powerful oxidant and nitrating element that can promote reactions of HO^0, Nitrosoniume (NO_2^0) and Nitrogen dioxide.

Key Words: Cigarette smoking, nitric oxide, nitrosative stress, active peptic ulcer

INTRODUCTION

Smoking is one of health threating concerns specially, 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(5-7) short lasting radicals and forms of active oxygen which are resulted from oxidation and reduction cycleofcatechol and hydroquinone; tobacco coordinate carcinogens; carcinogens and tumor causers (1,2) like polycyclic aromatic hydrocarbons, aromatic amines(3) and tobacco coordinate nitrosamines. Smoke resulted from every cigarette contains more than 600µg Nitric Oxide radical (NO^o) in gas phase. Nitric oxide is a radical that binds to Iron and Cupper carrier proteins (11). This radical is produced in various cells including, vascular endothelial cells, neurons, neutrophils and macrophages (12). NO^o concentration in cigarette smoke is in a linear reciprocal correlation with NO^o concentrations in every cigarette (2and3). Yet, more than 100 µg of NO^o is released in cigarette smoke due to oxidation of nitrogen components of tobacco and presumably oxidation of atmospheric nitrogen and goes over person palate without filtering (2and4). Main radical forms which are conserved in as quinone and hydroquinone(Q/QH_2) in a matrix of Tar(13,14), serve as an active oxidation-reduction system which can reduce molecular oxygen in order to production of Superoxide radical (O_2^0) and afterward Hydrogen peroxide ($H_2O_2^o$) and Hydroxyl (HO^0) radical will be produced too(2).

In recent years, several studies have shown that in aerobic condition, nitric oxide is oxidized instinctively to yield the N_2O_3 which is powerful nitrozating factor. Nitrozation of secondary amines by N_2O_3 yields N-Nitrozamines which can alkylate nucleic bases and yieldmutagens 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 (15). Reaction of NO^o 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 (16-19). As mentioned above, while smoking, a huge amount of free radicals release in human body(8-10), and it's 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 $H_2O_2^o$(20).
In addition to 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 (21).

As mentioned above, owing to reaction between NO^o and various kinds of oxygen radicals, two metabolite, peroxinitrogen ($OONO^{-o}$) and superoxide ($O2^{-o}$) result which are very toxic and cause severe oxidative pressure in different body tissues (16 , 22).
Gastric mucus will be also affected by this oxidative pressure and as a result, peroxidation of lipids, damage to DNA, oxidation of proteins and deactivation of enzymes will occur (23).

In addition to Endoscopic assessment of gastric tissue and lesions resulted 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 control groups.

MATERIAL AND METHODS

Patients with symptoms of dyspepsia who conferred to gastroenterology specialist assigned to district of endoscopy of Imam Reza Hospital by indication, assessed for smoking, and categorized as smoker and non-smoker. With their consent, patients were involved in 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 mentioned elements, were positive were excluded from 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 Body of every patient's stomach in fasting condition. These samples were used for rapid (1 hour) test of urease. This is a rapid test for distinction of presence or absence of Helicobacter Pylori. Results of the test recorded in tables by order of their group. Next, these samples passed to 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 assessment of nitric oxide levels, samples of gastric juice were taken from patients of all study groups and transferred to-70^{oC}. In present study, we perused smokers who had peptic ulcer as Case group (group-1), 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 making the study results extend able to all smokers, we matched the Case group with other three Controls

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Table: Information about comparison of nitric oxide levels in gastric juice of members falling in Control groups1, 2, 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		

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 aforementioned measurements was done by SPSS version 23 and using one-way variance analysis (one-way ANOVA) and multiple compassions (Tukey HSD).

RESULTS

Sum of 172 person were studied in this study of whom, 50% were smokers and 50% were non- smokers. 50% of 86 smokers had active peptic ulcer (case group) and 50% hadn't (control group-1). As like as smokers, 50% of 86 non-smokers had active peptic ulcer (control group-2) and 50% hadn't active peptic ulcer (control group-3). Case group consisted of 29 men (67/5%) and 14 women (32/5%), Control group-1consisted 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 word, equality gate for age of case group and three control groupswere well done.

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), according to Table.1 and Figure.1, nitric oxide levels in gastric juice of smokers with active peptic ulcer (case group) were of significant difference with two control group 1 and 2 (control group 1= smokers without active peptic ulcer, control group 3= non-smokers with

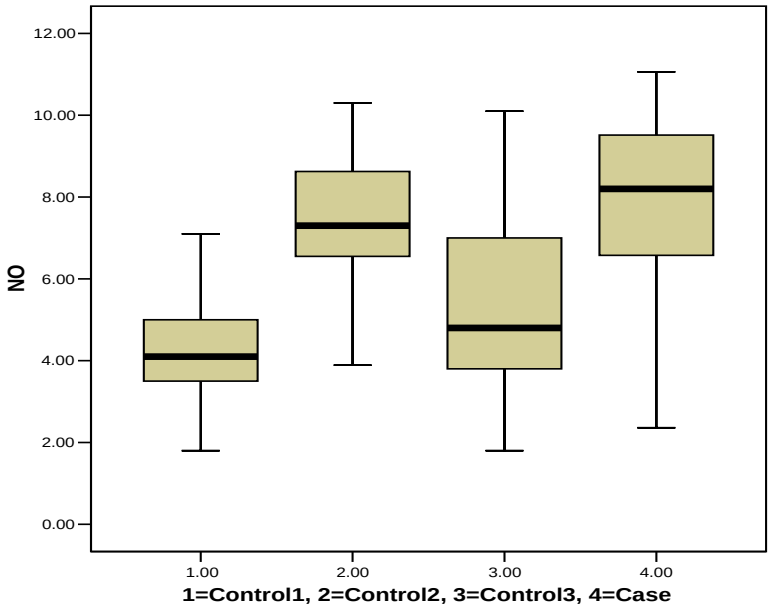

Fig.1: schema of comparison between levels of nitric oxide in gastric juice of patients in four groups of study

active peptic ulcer) and 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 whole groups except 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 Tarcause carcinogenic effects on exposed cells (24, 25). Increasing of damage to peptic tissueand DNA is in a direct relationship with toxic components specially NO^{o} in smoke and Tar. Muler and et al (26) demonstrated the formation of peroxinitrite (a known factor damaging DNA) in smoke of cigarette. Matsucora and et al (27) published an article on direct toxic and mutagenic effects of dense cigarette smoke. Peri vast and Shoker described a ethylating factor which has direct effect on cells and their DNA inTobacco smoke. Considering this factor we can explain high levels of 3-ethyl-adenin excreted in smoker's urine (28). Hence, results of present study regarding increase in NO^{o} levels in gastric juice of smokers compared to non-smokers are in accordance with results of aforementioned studies. Our results suggest that increased level of nitric oxide is one of effective factors which increases damage to DNA of peptic tissue and subsequently enhances the risk of neoplasia in this tissue in member's belonging to case group.

In a study by Youshi and et al (2), they showed that, in 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^{o} and smokes Tar which can be the effective element behind this vast damage in different tissues. It's likely that peroxinitrite radical (ONOO-) resulted from rapid reaction between NO^{o} and O_2^{o} be the effective behind this damage. Peroxide radical ($O2^{o}$) can be resulted from instinctive reaction of aromatic poly hydroxides like catechol and 1-4-hydroquinone which both present in high concentrations in smokes Tar(2).Peroxinitrite is a strong oxidant and nitrating factor which can initiate the reactions of HO^{o}, Nitrozonum(NO_2^{o}) and nitrogen dioxide (NO_2). It is confirmed that peroxinitrite can cause the breakage of plasmid DNA in vitro. These radicals can't be limited by anti-oxidants such as D-mannitol and Dimethyl sulfoxide (DMSO) (29) so, they cause more sever oxidative damages to DNA. In other hand, according to hypothesis of Pryor and his coworkers, like peroxinitrite, cigarette smoke can also deactivate α-1-protease inhibitor and cause over-activation of protein digestion process. As a result, destruction of connective tissue in lower respiratory system increases (30, 31). Such a destruction of connective tissue has been seen in relation to Emphysema in smokers (32).

Considering the results of this study we can conclude that smoking is along with considerable increase in nitric oxide radical levels in gastric juice. This increase is due to compounds exist in smoke and Tar which initiate procedure of DNA damage in different cells and subsequently increase the rate of malignancies in this tissue through oxidation and reduction cycle.

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