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Increased serum malondialdehyde levels among cigarette smokers

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Abstract

Cigarette smoking is recognized as a serious health hazard as each cigarette tears away 7-11 minutes of human life. The present study aimed to investigate the serum MDA levels in non-smoker and smoker subjects and its association with cigarette consumption. A total of 114 subjects, 52 smokers and 62 non-smokers matched with respect to age, lifestyle and socio-economic status were selected for the present study. Serum samples were analyzed for MDA level. Statistical analysis was done using Independent sample t-test, ANOVA with Duncan post hoc test and Pearson correlation coefficient. The serum MDA level was observed to be significantly ($P < 0.001$) elevated in smokers as compared to non-smokers. There was a significant positive relationship between serum MDA level and cigarette consumption among smoker subjects.

Keywords: Lipid peroxidation, Malondialdehyde, Oxidative stress, Smoking.

1. Introduction

Cigarette smoking is a major health issue and causes large number of deaths worldwide. Smoking has been reported to be a risk factor for oral cancer, oesophageal cancer, lung cancer and even liver cirrhosis [1]. Cigarette smoke constitutes 7357 chemical compounds from many different classes such as alkenes, nitrosamines, aromatic and heterocyclic hydrocarbons and amines, etc. in both the gaseous form and condensed particle [2]. Smoking leads to generation of enormous amount of reactive oxygen species such as hydrogen peroxide, hydroxyl ion, superoxides and peroxy radical, which in turn causes oxidative stress. Lipid peroxidation is a naturally occurring process generating malondialdehyde (MDA) in small amounts in the body, mainly initiated by various reactive oxygen species. These reactive oxygen species readily attack the polyunsaturated fatty acids (PUFA) of the plasma membrane which result in alteration in the membrane fluidity, increased permeability and loss of membrane integrity, ultimately reducing the viability of cells [3]. The increased levels of MDA due to lipid peroxidation is known to be crucial step in the pathogenesis of large number of pathological states like lung cancer [4], asthma [5], diabetes mellitus [6], coronary heart disease [7], oral cancer and precancer [8]. Thus, MDA concentration in serum is a reliable biomarker to evaluate lipid peroxidation status. The aim of the present study was to estimate the serum MDA levels in non-smoker and smoker subjects. The effect of cigarette consumption on serum MDA level in smoker subjects was also investigated.

2. Materials and methods

2.1 Subjects

The present study included a total of 114 healthy male subjects, out of them 62 were non-smokers (average age 53.194 ± 1.276 years) and 52 were smokers (average age 52.500 ± 1.250 years). The individuals who smoked were classified into two groups with respect to the number of cigarettes consumed per day as follows:

- 1) Group 1: persons who consumed 1-5 cigarettes per day
- 2) Group 2: persons who consumed >5 cigarettes per day

The healthy subjects having never smoked and not being exposed to passive smoking were selected as control subjects. All the subjects included in the present study were non-alcoholics, who were not suffering from any disease and were not on any medication. The present study was performed at Human Genetics Laboratory, Kurukshetra University, Kurukshetra, Haryana during the period of May 2014-Oct 2014. The epidemiological data regarding the gender, age, smoking habits, drinking habits, dietary habits and other lifestyle parameters of subjects were recorded in the form of questionnaire. A written informed consent was taken from all the subjects prior to sampling. This study was approved by Institutional Ethics Committee, Kurukshetra University, Kurukshetra.

2.2 Sample collection

For each subject, 2ml of blood sample was collected in glass tube with the help of registered medical practitioner. For serum extraction, blood samples were allowed to clot for 1 hour at room temperature and then centrifuged at 3000 rpm for 10 minutes.

2.3 Malondialdehyde (MDA) concentration

For evaluating the level of lipid peroxidation, serum MDA level was estimated according to the standard protocol described by Beuge and Aust [9]. The serum sample (0.1 ml) was mixed thoroughly with 0.1 ml Tris HCl buffer, 0.1 ml FeSO₄ and 0.1 ml Ascorbic acid. After adding 0.6 ml distilled water, the mixture was incubated at 37°C for 15 minutes. Then, 1 ml Trichloroacetic acid (TCA) and 2 ml Thiobarbituric acid (TBA) were added to the reaction mixture. Tubes were plugged with cotton and incubated for 15 minutes in boiling water. Centrifugation was done at 3000 rpm for 10 minutes. After cooling, readings were taken for light pink coloured supernatant at 532 nm using nanophotometer (IMPLEN). The concentration of MDA was calculated by using the extinction coefficient of MDA-TBA complex, 1.56 X 10⁵ nmol L⁻¹ cm⁻¹ and expressed as nmoles/ml of serum. All the reagents were prepared in laboratory. No commercial kit was used for the analysis of samples.

2.4 Statistical analysis

Statistical analysis was performed using statistical software SPSS v16.0 and Microsoft Excel 2007. The independent sample t-test and ANOVA with Duncan post hoc test were used to study the difference between the mean values in different groups. Pearson correlation coefficient was estimated to find out correlation between the parameters.

3. Results

The average age of non-smokers (n=62) was 53.194±1.276 years and that of smokers (n=52) was 52.500±1.250 years, showed no significant difference for age between the two groups. The serum MDA level was observed to be significantly higher (P<0.001) in smokers (0.852±0.062) as compared to non-smokers (0.418±0.043) (Figure 1). Serum MDA level showed a marked difference (P<0.05) between the sub-categories (i.e. Group 1 and Group 2) of smoker subjects. Group 2 (>5 cigarettes/ day) had higher serum MDA levels as compared to Group 1 (1-5 cigarettes/ day) (Table 1).

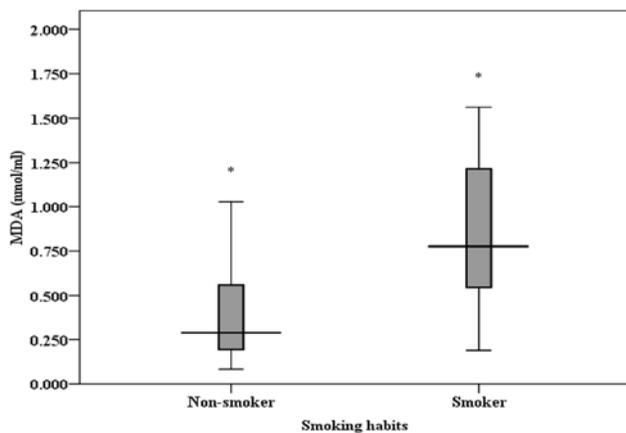


Fig 1: Comparison of serum MDA levels (nmol/ml) in non-smoker and smoker subjects.

*Significant (p<0.001) (Independent sample t-test).

Table 1: Serum MDA levels (nmol/ml) in smoker subjects in relation to number of cigarettes smoked per day.

	Group 1 1-5 cigarettes/ day	Group 2 >5 cigarettes/ day
N	32	20
Average age (years)	52.688±1.934	52.200±1.060
MDA (nmol/ml)	0.729±0.059*	1.049±0.121*

*Significant (p<0.05) (Independent sample t-test).

Figure 2 depicts the comparison of serum MDA levels between groups of non-smoker and smoker subjects on the basis of number of cigarettes smoked per day. It is clear that the serum MDA levels showed a dose dependent relationship with the consumption of cigarettes revealing elevated lipid peroxidation with increased cigarette consumption (P<0.05). In correlation analysis, there was a positive and significant (P<0.001) correlation between serum MDA levels and number of cigarettes consumed among smoker subjects. We have observed that as the number of cigarettes consumed increased, the serum MDA levels also increased (Figure 3).

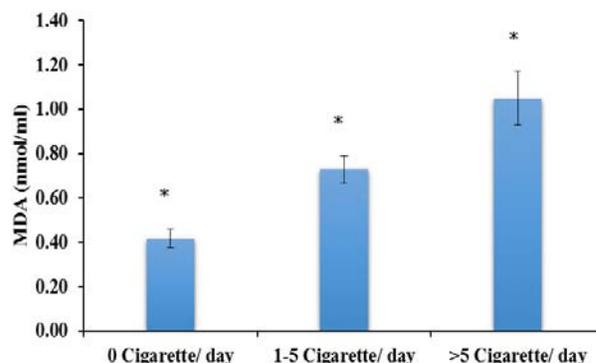


Fig 2: Dose dependent relationship of serum MDA levels (nmol/ml) in non-smoker and smoker subjects.

*Significant (p<0.001) (Independent sample t-test).

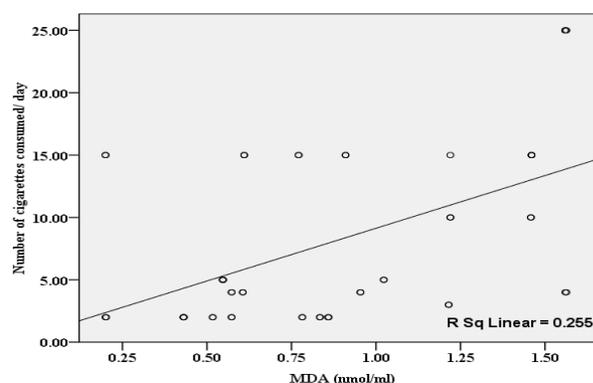


Fig 3: Pearson correlation of serum MDA levels (nmol/ml) with respect to number of cigarettes smoked per day in smoker subjects.

4. Discussion

Smoking may enhance oxidative stress through generation of reactive oxygen species, thereby causing lipid peroxidation. One of the frequently used biomarker of lipid peroxidation is the serum MDA concentration, a byproduct of the lipid peroxidation process. In the present study, the serum MDA level was remarkably higher (P<0.001) in smokers as compared to non-smokers. These results are in accordance with the earlier studies, showing elevated lipid peroxidation

among smoker subjects ^[10-12]. Chole *et al.* ^[8] reported association of lipid peroxidation with the habit of either chewing betel nut or betel leaf or tobacco or smoking in the control subjects. In another study, significantly ($P < 0.001$) elevated MDA levels were reported in smokers than non-smokers in patients with lung cancer ^[4]. In the present study, smokers did not differ from non-smokers in age, so the effect of age on serum MDA levels was omitted. To eliminate the effect of alcohol consumption and gender, the subjects consuming alcohol were excluded and all the subjects included in the current study were males.

In the present study, dose-response relationship of serum MDA level was observed in non-smoker and smoker subjects. Similar to the findings of the present study, Jain *et al.* ^[13] reported two-fold increase in thiobarbituric acid reactive substances level in mild bidi smokers and four-fold increase in heavy bidi smokers as compared to control subjects. In this study, a significant positive correlation was observed between consumption of cigarettes and serum MDA levels. Nielsen *et al.* ^[14] and Solak *et al.* ^[15] also reported that MDA levels in plasma also correlated with the daily exposure to cigarette smoke. While Kurty and Gokpinar ^[16] observed the similar results in saliva of smokers. In contrast to the findings of the present study, Craig *et al.* ^[17] reported lack of correlation between lipid peroxidation and cigarette smoke exposure in healthy subjects.

5. Conclusion

From the results obtained, we conclude that oxidative stress as indicated by serum lipid peroxidation is more intense in smoker subjects as compared to non-smoker subjects. There is a strong association between increased lipid peroxidation and cigarette consumption in smoker subjects. Evaluating the serum MDA levels might serve as a valuable biomarker to identify the high risk population, which may deserve further investigation for early diagnosis and treatment.

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