Estimation of Serum Malondialdehyde and Uric acid levels in Smokers and non-Smokers

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Received in : 26 September 2012 , Accepted in : 4 December 2013

Abstract

Malondialdehyde (MDA) is one of many low molecular weight end-products of lipid peroxidation; it is as an index of lipid peroxidation. Uric acid is one of the endogenous oxidant-antioxidant paradoxes.

The aim of this study is to evaluate the levels of serum MDA and uric acid in smokers and non smokers.

This study was carried out from January to July 2012 on (30) smokers and (30) non smokers. Serum MDA level was measured spectrophotometrically using thiobarbituric acid method, whereas serum uric acid was measured using enzymatic colorimetric method.

The results of the study revealed a significant increase ($P<0.001$) in uric acid value in smokers subjects compared to non smokers (6.54 ± 0.37 Vs 4.68 ± 0.22) respectively, while no significant difference ($P>0.05$) In MDA value was observed in smokers and non smokers subjects (2.227 ± 0.394 Vs 1.912 ± 0.32) respectively.

Key words: Smoking, MDA, Uric acid.

Introduction

Tobacco smoking has been claimed to cause a wide variety of health problems such as atherosclerosis, vascular diseases, including coronary heart disease and stroke, mutagenesis of exposed cells, and cancer in the upper respiratory system as well as lungs. One of the putative mechanisms of the hazardous effect of tobacco smoking is oxidative stress, which is caused by the numerous reactive chemicals both in tar and gas phases [1-5].

Cigarette smoke is a complex mixture of chemicals containing more than 4000 different constituents. Some of the compounds identified include pyridine alkaloids such as nicotine, ammonia, acrolein, phenols, acetaldehyde, N-nitrosamine, polycyclic aromatic hydrocarbons, combustion gases such as carbon monoxide, hydrogen cyanide and trace elements [6].

As a consequence of these chemicals, cellular macromolecules such as, lipids, proteins, carbohydrates, and DNA are oxidized and degraded [4]. When oxidizing compounds attack lipids, peroxidation of lipids initiates by abstraction of a proton from fatty acid side-chains and this process results in several degradation products: small molecule alkanes, alkenes and aldehydes[1]. MDA is an aldehydic product of this process and its determination via the thiobarbituric acid assay is commonly used as a test for evaluating oxidative stress in the body [2].

Uric acid is a final enzymatic product of degradation of purine nucleosides in humans [7]. In other mammals, the last enzymatic product of purine degradation chain is allantoin, which is excreted in urine; as a consequence, humans have to cope with the higher levels of uric acid in the blood (200—400 µM / L) and are prone to hyperuricaemia and gout [8].

The high concentration of urate was proposed to be one of the major antioxidant of the plasma and protects cells from oxidative damage, thereby contributing to an increase in life span of our species and decreasing the risk for cancer [9].

The initial experiments by Ames *et al* [9], showed that when urate at physiological concentration (300µM / L) was exposed to singlet oxygen, it's rapidly degraded (55% in 5 minutes), and the end product of this reaction was allontoin. The same authors reported that the chemistry of urate oxidation by radicals is much more complicated and can form free radicals in a variety of radical-forming systems. But Sautin *et al* [23] reported that uric acid may work as oxidant-antioxidant paradox. Thus, uric acid can become a pro-oxidant and pro-inflammatory factor by forming radicals in reactions with other oxidants [10].

The aim of this study is to demonstrate the possible effects of smoking on serum MDA and uric acid concentrations.

Materials and Methods

1. Subjects:

This study was proceeded over a period of six months, from January to July 2012, in collaboration between the Dept. of Clinical Analysis/ College of Pharmacy and Dept. of Medical Biochemistry/ College of Medicine/ Hawler Medical University on (60) apparently healthy smoker and non smoker volunteers, which were divided into two groups. Informed - consent was obtained from all participants and they were interviewed to obtain their smoking histories, including the number of cigarettes smoked / day and the number of years of smoking. Inclusion criteria were as follows: > 18 years of age, smoking more than 5 cigarette/ day (smokers only). Exclusion criteria of chronic diseases, regular use of medicine, use of vitamins or other dietary supplements within the last 2 years, and smoking (non smokers only) this study was approved by ethical committee/ Medical College/ Hawler Medical University .

Details concerning number, age, number of cigarettes smoked/ day and duration of smoking are illustrated in Table (1).

2. Samples:

Five ml of venous blood samples were drawn in the morning from each individual, using disposable syringes. The samples were transfer into glass tubes, quitting for 30 minutes for clotting, and centrifuged for 15 minutes at 3000 round per minute. The separated serum was used for measurement of MDA and uric acid.

3. Methods

A. Evaluation of serum MDA :

The evaluation of serum MDA was based on the reaction with thiobarbituric acid, forming red, fluorescent MDA-TBA2 which absorbed at wavelength 532 nm [11-12].

B. Evaluation of serum uric acid:

Uricase acts on uric acid to produce allantoin, carbon dioxide and hydrogen peroxide. Hydrogen peroxide in the presence of peroxidase reacts with a chromogen (amino-antipyrine and dichloro-hydroxybenzene sulphonate) to yield quinoneimine, a red colored complex, which absorbed at 520 nm (490-530) which is proportional to the amount of uric acid in the specimen [13].

4. Statistical analysis:

The statistical analysis of the results [mean, standard deviation (S.D), and standard error of mean (S.E.M)], were calculated using the SPSS system version 13.0 for windows. The different variables were compared to each other. The unpaired student test (t test) was used. Only P values < 0.05 were considered as statistically significant [14].

Results

Table (2) provides the mean ± S.E.M. of serum. MDA of both groups. The obtained results reveal that the mean± S.E.M. of serum MDA concentration is 2.227± 0.394 µg/L, and 1.912± 0.32 µg/L in smokers and non-smokers respectively, the statistical analysis indicated that the mean value of serum MDA in smokers is no significant comparing with non-smokers ($P>0.05$) P-value = 0.673.

Table (3), shows the mean ± S.E.M. values of serum uric acid in smokers (6.54 ±0.37 mg/dl) and non-smokers (4.68 ± 0.22 mg/dl). The mean level of serum uric acid in smokers group was significantly higher ($P < 0.001$) than that of non-smokers group.

Discussion

The present study showed that there was a non-significant elevation of serum MDA level in smokers compared to non-smokers.

Cigarette smoking leads to the uptake of many hazardous compounds or their metabolites which may be electrophorus which is able to react with biological macromolecules, or they may causes an oxidative stress by formation of reactive oxygen species (ROS) that are capable of initiating the self-perpetuating chain reactions of lipid peroxidation in the membranes [4] .

There is a study shows that there is an increased level of lipid peroxidation products such as MDA in smokers compared to non-smokers [9].

Lin *et al* [15] demonstrated that smoking of 10 cigarettes daily on an average, for 6-8 years was associated with enhanced lymphocyte DNA strand breaks as well as greater urinary excretion of MDA compared with non-smokers.

The data obtained in this study for serum MDA is disagreement to those obtained by Flemming *et al* [16], Ermis *et al* [17], Klara *et al* [18]. These authors studied the effects of smoking on the serum and milk levels of MDA and found an elevation.

On the other hand Bahri *et al* [19] conduct study showed the influence of smoking on maternal and neonatal serum MDA and found no significant difference between smokers and non-smokers.

The results obtained in this study for serum MDA is also not consistent with that reported by Jain *et al* [5] and Jens *et al* [20], who studied the influence of smoking on the serum level of MDA and found an elevation in smokers compared with non smokers and suggested that is attributed partially to lower intake of fruits and vegetables by smokers which is due to the effect of smoking (via nicotine) on their sense of taste.

Furthermore, other epidemiologic studies showed also that cigarette smokers consume fewer fruits, vegetables and vitamin supplementation than do non-smokers [4].

The mean serum level of uric acid in smokers was significantly higher ($P < 0.001$) than that of non-smokers. This result is similar to results obtained by Boshtam *et al* [21], and Krishnan *et al*[28]. These authors compared the serum levels of C- reactive proteins (CRP) and uric acid in active, passive and non-smokers and reported an elevation of the two variables in smokers, and suggested that this increase is due to the harmful effects of nicotine. Kristine *et al* [22] showed the effect of smoking on serum uric acid and other metabolic markers throughout normal pregnancy and found an increase of these variables in smokers compared to non-smokers and suggested that this increase in the serum level of uric acid may be secondary to increased production through xanthine oxidase pathway. Yuri and Richart [23], Rodrigues *et al* [29], Tamariz *et al* [30], Suzuki *et al* [31], and Soletsky *et al* [32], reported that uric acid, despite a major antioxidant in the human plasma, both correlates and predicts development of obesity, hypertension and cardiovascular disease, conditions associated with oxidative stress in smokers, and they suggested that this paradox (antioxidant primarily in plasma or pro-oxidant primarily within the cell) could be that a rise in serum uric acid represents an attempt protective response by the host or uric acid being activated as a defense mechanism against oxidative stress.

Bagnati et al [24] and Muraoka [25] reported also that uric acid can become a pro-oxidant by forming radicals in reactions with other oxidants, and these radicals seem to target predominantly lipids (LDL-C and membranes) rather than other cellular components. At the same time, they found that the hydrophobic environment created by lipids is unfavorable for the antioxidant effects of uric acid and oxidized lipids can even convert uric acid into an oxidant.

However, in contrast to these findings, some investigators have not found any significant difference in the serum level of uric acid in smokers compared to non-smokers [16], whereas others have found significant lower serum levels of uric acid in smokers and suggested that this is attributed to a reduction of the endogenous production as a result of the chronic exposure to cigarette smoking that is a significant source of oxidative stress [26-27].

Conclusions

This study revealed a significant increase in the serum level of uric acid in smokers compared to non-smokers. However, further investigations involving a large number of participants and analysis of other oxidants and antioxidants are required to confirm the extent of the free radical load generated by smoking.

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Table (1): The number, age, number of cigarettes smoked / day and duration of smoking of the smokers and non smokers subjects.

Groups	No.	Age /years, (Mean ± S.E.M)	No. of cigarette smoked/ day	No. of years of smoking (Mean ± S.E.M.)
Group 1 (Non smokers)	30	35 ± 2.61	0	0
Group 11 (Smokers)	30	39.73 ±2.84	10--- 20	21.23 ±2.00

Table (2): The (mean± S.E.M.) of serum MDA (µg/L) of the smoker and non-smoker groups.

Groups	No.	MDA (Mean± S.E.M.)
Smokers group	30	2.227 ± 0.394
Non smokers group	30	1.912 ± 0.32

Table (3): The (mean± S.E.M.) of serum uric acid (mg/dl) of the smoker and non-smoker groups.

Groups	No.	Uric acid (Mean± S.E.M.)	P value
Smokers group	30	6.54 ± 0.37	< 0.001
Non Smokers group	30	4.68 ± 0.22	

تقدير مستوى المالونداياالديهايد وحامض اليوريك في مصل دم المدخنين وغير المدخنين

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استلم البحث في : 26 أيلول 2012 ، قبل البحث في : 4 كانون الأول 2013

الخلاصة

مالوندايالديهايد هو احد المركبات ذات الوزن الجزيئي الواطيء والناتج النهائي لعملية مافوق الاكسدة للدهون ويعتبر كدليل لاكسدة الدهون أما حامض اليوريك فانه يعمل بشكل متناقض كمؤكسد وكمضاد للأكسدة داخل خلايا الجسم.

الهدف من البحث هو تقدير مالوندايالديهايد وحامض اليوريك في مصل دم المدخنين وغير المدخنين.

تم أجراء البحث بين فترة كانون الثاني الى تموز 2012 على (30) من المدخنين و(30) غير المدخنين. وتم قياس مستوى مالوندايالديهايد في المصل بواسطة جهاز (spectrophotometer) بأستخدام طريقة (thiobarbituric acid) وقياس حامض اليوريك في مصل الدم بأستخدام الطريقة اللونية (colorimetric).

أثبتت نتائج البحث على أن الزيادة في قيمة حامض اليوريك في المدخنين عالية معنوياً مقارنةً بغير المدخنين (6.54 ± 0.37 Vs 4.68 ± 0.22) على التوالي. وأما الزيادة في قيمة مالوندايالديهايد ليس معنوية في المدخنين مقارنةً بغير المدخنين (0.32 ± 1.912 Vs 0.394 ± 2.227) على التوالي.