

Effect of Waterpipe (hookah) Smoking on Lipid Profile, Lipid Peroxidation, and Homocysteine Levels

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ABSTRACT

BACKGROUND & OBJECTIVE: Tobacco smoke promotes atherosclerosis which is the major cause of death due to cardiovascular diseases, which include coronary artery disease, cerebrovascular disease, and peripheral arterial disease. Waterpipe smoking is one of the classical forms of tobacco smoking. The objective of our study was to examine the effect of waterpipe (hookah) smoking on serum lipid profile, homocysteine, and oxidant status in male waterpipe smokers.

METHODOLOGY: The cross-sectional comparative study was performed at the Post Graduate Medical Institute at the Department of Physiology, Lahore. All 60 volunteers (30 waterpipe smokers and 30 non-smokers) participated included in the study using convenient sampling. Total cholesterol, high-density lipoprotein, low-density lipoprotein, triglyceride, homocysteine, and malondialdehyde levels were measured by the ELISA method in serum samples. Results were analyzed using SPSS. Mann-Whitney U-test (non-parametric test) was applied to compare differences between the two groups.

RESULTS: Serum cholesterol ($p \leq 0.001$), low-density lipoprotein ($p \leq 0.001$), triglyceride ($p = 0.001$), Serum Malondialdehyde ($p = 0.001$), and homocysteine ($p \leq 0.001$) levels were higher, and the difference was statistically significant while high-density lipoprotein ($p = 0.003$) was significantly lower in waterpipe smokers than non-smokers.

CONCLUSION: This study concludes that serum cholesterol, triglyceride, LDL, homocysteine, and MDA levels increase and HDL decrease in male waterpipe smokers.

KEYWORDS: Waterpipe smoking, Cholesterol, Homocysteine, Malondialdehyde.

INTRODUCTION

Tobacco use is a widespread global issue. Apart from generating social and economic burdens, it is responsible for a major health problem. It is the reason behind 8 million deaths every year due to co-morbidities associated with tobacco smoking [1]. Cardiovascular events caused by active tobacco smoking include acute myocardial infarction, certain microvascular complications, and stroke, which may lead to sudden death [2]. Waterpipe smoking is a common practice in Pakistan, India, Bangladesh, and some regions of China.

Waterpipe is known as “shisha” in countries like Saudi Arabia and Egypt, “narghile” in Jordan and Syria, and “hookah” in Africa, Pakistan, and India [3]. In this study, “waterpipe” refers to classical hookah used in rural areas of Punjab, Pakistan.

Tobacco smoking may promote atherosclerosis and acute cardiovascular events. Free radicals in tobacco smoke cause oxidative stress in endothelial cells. Cellular components such as proteins, lipids, and DNA react with free radicals [4]. Lipid peroxides formed because of this peroxidation are very unstable and degrade easily. One of these degradation products is malondialdehyde (MDA) which can be used to estimate the extent of oxidative stress [5].

Nicotine in tobacco smoke increases the sympathetic nervous activity and catecholamines release, resulting in lipolysis and increasing hepatic re-esterification of free fatty acids [6]. In turn, this contributes to increased levels of hepatic low-density lipoprotein (LDL) and triglycerides. Lipid abnormalities in hookah smokers seem important to get attention because they are considered a risk for cardiovascular disease.

How to cite this: Latif S, Saeed M. Effect of Waterpipe (hookah) Smoking on Lipid Profile, Lipid Peroxidation, and Homocysteine Levels. *Journal of University Medical & Dental College*. 2023;14(2):596-600.



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In tobacco smokers' homocysteine undergo oxidation and causes the formation of free radicals, which make them more prone to arterial endothelial damage leading to vascular inflammation, atherogenesis, and ischemic injury [7].

There are very few previous studies available locally addressing these parameters in classical hookah smokers. Most of the available studies address other forms of tobacco use, like cigarette smoking and smokeless tobacco use. This study aims to investigate the effects of traditional hookah smoking on lipid profile, MDA, and homocysteine in the male adult Pakistani population.

METHODOLOGY

Our cross-sectional comparative study was accomplished in the Physiology wing of Post Graduate Medical Institute, Lahore, for 2 years. The protocol of our study was accepted by the Ethical Review Committee of the institute (research no. 00-16-S-2016). We included sixty healthy males in the age range of 30-50 years in our study using convenient sampling. The sample size was calculated using serum MDA level through the following formula [8].

$$n = \frac{\left(z_{1-\frac{\alpha}{2}} + z_{1-\beta}\right)^2 (\sigma_1^2 + \sigma_2^2)}{(\mu_1 - \mu_2)^2}$$

Participants were recruited from rural areas near Shahkot, Faisalabad. They were approached at social gatherings where they used to smoke hookah together. All participants gave informed written consent. Complete family and medical history were taken and recorded in a questionnaire proforma. Those having a history of drug intake, cigarette smoking, diabetes mellitus, and cardiovascular diseases were excluded from the study. They were divided into two study groups: non-smokers (group I) and waterpipe smokers (group II).

Water pipe smokers were those using a classical form of hookah at least one time per day for the last five years. A single episode of about one hour involves almost 200 puffs and inhalation of approximately 90,000 ml of smoke [9]. They were compared with group I, containing age-matched healthy non-smoker males.

Serum cholesterol, triglyceride, LDL, HDL, Hcy, and MDA levels were measured by the enzyme-linked immunosorbent assay (ELISA) method. Serum triglyceride tests were performed using triglyceride M.R. kit ref. 1155005, linear chemicals Barcelona, Spain. Serum cholesterol tests were performed using CHEMELEX, S.A. Cholesterol CHOD-POD. Liquid kit, ref.30183, Barcelona, Spain. Serum HDL test were performed using HDL cholesterol liquicolor kit, Ref#:10084 by human gesellschaft für biochemica und diagnostica mbh, Germany. Serum LDL test were performed using LDL cholesterol liquicolor kit, Ref#10094 by human gesellschaft für biochemica und diagnostica mbh, Germany.

Malondialdehyde tests were performed using Glorybios human MDA ELISA kit, catalogue #:12148, China. Homocysteine test were performed using Glorybios human homocysteine ELISA kit, catalog #:95434, China.

The findings of the study were assessed for statistical analysis using IBM SPSS 21.0. Descriptive statistics were computed for each of the variables analyzed. Quantitative variables such as lipid profile, MDA, and homocysteine were tested for normality using the Shapiro-Wilk test. Data with p value less than 0.05 was considered non-normally distributed data. Results were presented as the median, interquartile range (IQR) for non-normal data. Groups were compared by the Mann-Whitney U test. The observed difference was assumed significant with a p-value<0.05.

RESULTS

Our findings showed that the mean±SD of age among the waterpipe smokers and non-smokers group was 40.30±6.44 and 39.74±6.29, respectively. All the biochemical markers had non-normal distribution. Comparison of medians of triglyceride (p=0.001), total cholesterol (p≤ 0.001), LDL (p≤0.001), and MDA (p≤0.001) of the two groups showed significant differences. HDL level was significantly lower in group II compared to group I (p=0.003), as shown in Table-I.

Serum levels of Hcy were found to be significantly higher in group II as compared to group I, and the difference were statistically significant (p≤0.001), as shown in Table-I. In group I, all subjects had homocysteine levels of less than 15 µmol/L, while in group II, 6.6% of waterpipe smokers had homocysteine levels of more than 15 µmol/L, as shown in table-II.

Table-I: Comparison of biochemical parameters between groups.

Variables	Group I (Non-smokers: n=30) Median (IQR)	Group II (Waterpipe smokers: n=30) Median (IQR)	p-value
Triglycerides (mg/dl)	126.50(63.75)	173.50(60.00)	0.001**
Total cholesterol (mg/dl)	185.50(30.50)	214.50(99.25)	<0.001***
HDL (mg/dl)	41.46(9.52)	33.83±8.22	0.003**
LDL (mg/dl)	129.50(34.00)	161.00(77.00)	<0.001***
MDA (mmol/L)	0.30(0.20)	0.70(0.80)	0.001**
Homocysteine (µmol/L)	6(1.27)	9.95(3.10)	<0.001***

HDL= High density lipoprotein, LDL=Low density lipoprotein, MDA = Malondialdehyde, **: very significant, ***: Highly significant p-value.

Table-II: Distribution of homocysteine in group I and group II.

Homocysteine (μmol/L)	Group-I(Nonsmokers) n=30(%)	Group-II(Waterpipe smokers) n=30(%)
<15	30(100)	28(93.3)
>15	0	2(6.6)

DISCUSSION

In this study, we compared the lipid profile, MDA, and homocysteine levels between waterpipe smokers and non-smokers. Triglyceride, total cholesterol, and LDL were higher, whereas HDL levels were lower in waterpipe smokers than non-smokers. Javed et al. concluded in their study that tobacco smokers are at high risk of developing dyslipidemias. They conducted a study at the Cardiology department at Punjab Institute of Cardiology Lahore. They calculated mean lipid profiles in smokers for total cholesterol, triglycerides, HDL, LDL, and LDL and showed similar results as in our study [10].

Another study was conducted in Hasilpur, Pakistan, to find out the effect of tobacco smoking on the lipid profile of inhabitants of this region. The outcomes of their study showed a non-significant increase in serum triglyceride, HDL, LDL, and VLDL levels in smokers compared to non-smokers. However, the mean serum total cholesterol and triglycerides level of smokers was significantly higher [11].

Our results are consistent with the findings of a study conducted on males in Salahaddin province, Iraq, to know the effects of waterpipe smoking on levels of biochemical variables [12]. They found a significant rise in cholesterol and LDL in waterpipe smokers. HDL was found significantly decreased. LDL is transformed into oxidized LDL after filtration through the walls of blood vessels and is deposited on the arterial walls. This exposes the body to atherosclerosis. Significantly lower levels of HDL while higher levels of LDL and triglyceride were found in waterpipe smokers, although the difference was insignificant.

Our results were consistent with the findings of a case-control study to discover the outcomes of cigarette and waterpipe smoking on lipid profiles among adult males in Rafah Governorate, Palestine. Waterpipe smokers were reported to have high serum cholesterol, triglycerides, LDL, and low HDL levels compared to non-smokers [13].

In a study that was conducted in Iraq on water pipe smokers, cigarette smokers, and controls, twenty male subjects within the age range of 25–40 years were included in each group. A relative increase in total cholesterol, T.G., and LDL while a decrease in HDL concentrations in waterpipe and cigarette smokers as compared to controls was observed [14].

Another study was conducted on adult males in the age range of 30–60 in Iraq. They aimed to show the effects of hookah smoking on liver functions, lipid profile, and adult blood

count. They found total cholesterol and T.G. levels high in hookah smokers, although the results were not statistically significant. Unlike our findings, HDL levels were high in their study [15].

Adenylyl cyclase in adipose tissue is activated by nicotine which causes the breakdown of fats. Excess amount of free fatty acids (FFA) in the liver leads to the accumulation of T.G. in it as well as the synthesis of LDL, which increases the level of T.G. and LDL in the blood. Smoking may cause a rise in total cholesterol, LDL, and T.G. due to the inhibition of Lipoprotein Lipase activation [16].

The molecular structure of lipids contains abundant reactive double bonds, which make it an easy target for oxidation. Free radicals in waterpipe smoke attack the lipids of cell membranes which leads to the release of MDA, leading to the damage of blood vessels and tissues [17]. We found significantly higher MDA levels ($p=0.001$) in hookah smokers.

A cross-sectional comparative study was carried out at Isra University Hyderabad, Pakistan. They recruited 60 smokers and non-smokers from Jaindal Kot, a small village located midway between Hyderabad and Matari. They found significantly raised MDA levels in healthy smokers than in non-smokers, consistent with our study [18].

Our results are consistent with a study on male smokers in Sudan [19]. The results of their study showed a significant rise in MDA levels in heavy waterpipe smokers who were smoking about 1–2 times per day. Light smokers who were smoking one time per week showed no significant difference. They associated the oxidative damage of waterpipe smokers with an increase in serum MDA concentration because it shows a higher lipid peroxidation process.

A significant increase in serum MDA in waterpipe and cigarette smokers was found as compared to non-smokers in a study conducted at the University of A.L. Iraqi, with 14 participants in each group. Our results are consistent with their findings [5]. Tobacco smokers with high plasma homocysteine levels are more susceptible to developing cardiovascular disease. They exert similar effects, such as endothelial dysfunction, cholesterol and triglyceride synthesis dysregulation, and thrombosis. They also stimulate the activation of monocytes and the proliferation of vascular smooth muscle. This ultimately leads to the development of atherosclerosis and ischemic injury [20].

In our study, serum levels of Hcy were higher in waterpipe smokers as compared to non-smokers and the difference was statistically significant ($p<0.001$). A study was conducted by Iqbal et al. on healthy adults in the age range of 18–60 years from Sultanabad, an area in the East region of Karachi, Pakistan. Their study showed a positive association between smokeless tobacco consumption and hyperhomocysteinemia [21].

A prospective study was carried out at Baqai Institute of Diabetology and Endocrinology, Baqai Medical University Karachi, Pakistan. They concluded that serum homocysteine level was significantly correlated with BMI, male gender, and smoking [22]. Homocysteine levels were also measured in a study on 200 males of Bangladesh in the age range of 20-40 years. In this study, mean serum Hcy levels were significantly higher ($p < 0.001$) in adult male tobacco smokers than in non-smokers [23].

The cross-sectional design of this study makes it unable to institute the cause-and-effect relationship between waterpipe smoking and the development of cardiovascular diseases, only an association can be determined. Tobacco smoke exposure may be assessed objectively through the measurements of nicotine and cotinine levels in body fluids (urine, plasma, saliva).

CONCLUSION

Waterpipe smoking is associated with changes in lipid profile, homocysteine and MDA levels that may increase the risk of cardiovascular diseases in the study participants.

ACKNOWLEDGEMENT: We thank all participants of the study, library staff and lab assistants of PGMI for their valuable contribution.

CONFLICT OF INTEREST: None.

GRANT SUPPORT AND FINANCIAL DISCLOSURE: None.

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Author's Contribution:

Sidra Latif: Substantial contributions to the conception or design of the work.

Muniza Saeed: Final approval of the version to be published.

Submitted for publication: 10-10-2022

Accepted after revision: 14-02-2023