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Water-pipe (shisha) smoking influences total antioxidant capacity and oxidative stress of healthy Saudi males

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Abstract

Water-pipe smoking has been practiced extensively for about 400 years. Water-pipe smoking is common in the Arabian Peninsula, Turkey, India, Pakistan and other countries. Previous studies have confirmed water-pipe smoking has negative health effects similar to those of cigarette smoking. The aim of the present research was to study the effect of water-pipe smoking on the risk factors for cardiovascular diseases as well as oxidative stress and total antioxidant capacity of healthy Saudi males. Two hundred healthy Saudi males (100 non-smokers and 100 smokers) ranging in age 19-50 years old from Riyadh, the capital of Saudi Arabia were selected for this study. Subjects in the smoker group smoked tobacco (Ma'ssel) using a water-pipe (shisha) at least two times per day (two heads of Ma'ssel per day). Each subject was interviewed and asked to provide demographic information and their typical smoking habits. Blood pressure and anthropometric measurements (weight and height) were measured by well-trained staff. Fasting blood samples were collected, and serum samples were analyzed for low density lipoprotein cholesterol (LDL-cholesterol), high density lipoprotein cholesterol (HDL-cholesterol), triglycerides (TG), apolipoprotein A-1 and B (apo A-1 and apo B), malondialdehyde, vitamin C and total antioxidant capacity. The statistical method of t-test was used to compare the mean values obtained between the smoker and non-smoker groups. The chi-square statistical test was used to compare marital and education status of study subjects by groups. Serum concentrations of HDL-cholesterol and apo A-1 were significantly ($p<0.05$) lower in smokers than in non-smokers. However, LDL-cholesterol, apo B, triglycerides and malondialdehyde were significantly ($p<0.05$) higher in smokers than in non-smokers. Total antioxidant capacity and vitamin C were significantly ($p<0.05$) lower in smokers than in non-smokers. This study provided limited data to suggest that water-pipe smoke is at least as toxic as cigarette smoke.

Key words: Water-pipe smoking, total antioxidant capacity, oxidative stress, lipid profile, Saudi Arabia.

Introduction

Tobacco use is the second major leading cause of death in the world. Tobacco use is currently responsible for the death of one in ten adults worldwide, about 5 million deaths each year. If current smoking patterns continue, tobacco use is projected to cause approximately 10 million deaths annually by the year 2020 [1]. Of these tobacco-related deaths, 70% are likely to occur in developing countries, making tobacco use a global epidemic [1, 2]. Unfortunately, despite the fact that developing countries bear the burden of this epidemic, most research addresses developed countries. Moreover, research tends to focus on tobacco use methods that are prevalent in developed countries, such as cigarettes and often does not consider methods common in developing countries, such as the water-pipe [3].

Water-pipe smoking has been practiced extensively for approximately 400 years with more than 100 million people worldwide who smoke water-pipes daily [4, 5]. Water-pipe use is a common practice in the Arabian Peninsula, Turkey, India, Pakistan, Bangladesh and some regions of China [6-9]. A sharp increase in the popularity of the water-pipe use has been noted in recent years [10]. It has been found in national and local surveys in Saudi Arabia [11], Kuwait [12], Egypt [13], Syria [14] and Lebanon [15, 16] that 20–70% have used a water-pipe and 22–43% are currently using a water-pipe.

Water-pipe terminology can depend upon the region and includes names such as "shisha", "boory" or "goza" in Egypt and Saudi Arabia [17-19]; "narghile", "nargile" or "arghile" in Jordan, Lebanon and Syria [20-21] and "hookah" in Africa and India [22, 23]. Besides terminology, there is also regional variation in shape, size and appearance of tobacco smoked [5, 18]. In this study the term "water-pipe" refers to tobacco use methods in which smoke passes through water. Generally, water-pipes consist of a head, body, water bowl and hose [24]. Tobacco is placed in the head and often covered with perforated aluminium foil; burning charcoal is placed on top of the foil. Water half-fills the bowl, submerging a tube through which smoke enters but not the hose-connected tube through which smoke leaves. Thus, an inhalation at one end of the hose produces a vacuum in the air-filled space of the water bowl, causing smoke to pass through the water (producing bubbles and the onomatopoetic moniker "hubble bubble") into the hose-connected tube and to the smoker. Disposable plastic mouthpieces can be added to limit the spread of disease [24]. The most common type of tobacco used in the water-pipe is called Ma'ssel [14, 21], which is sweetened and flavoured (for example, apple, mint and cappuccino). Other forms of tobacco may contain less sweeteners or flavours and are called Ajami, Tumbâk or Jurâk [2, 17, 21].

According to the size of the heads of the water-pipe, there are two kinds: large or small. The large head holds nearly 20 g of tobacco, the small head contains about 10 g of tobacco. Smoking 1 g of Ajami produces 35.65 mg (range of 30.0-413 mg) of nicotine, but 1 g of Ma'ssal produces 3.35 mg (range of 1.8-6.3 mg) of nicotine [25]. Ma'ssal use contains small but not negligible amounts of the addictive substance nicotine [21, 26]. Moreover, the World Health Organization study group on tobacco product regulator (Tob Reg) [24] states that "commonly used heat sources to burn the tobacco, such as wood cylinders or charcoal, are likely to increase health risks since when such fuels when combusted produce toxicants, including high levels of carbon monoxide, metals and cancer-causing chemicals.

The actual working temperatures that can be measured during the water-pipe smoking process are below or about 100°C, a temperature quite different from the temperature measured at the tip of a cigarette (850-900°C). At these temperature ranges chemical reactions of the Maillard-type occur between the aldehyde component of sugars, especially between the molasses and nitrogenous compounds such as ammonia (NH_4OH) which is used by tobacco manufacturers to produce various aromatic compounds [6, 27]. Scientists recognize the effect of tar from cigarette smoke is hazardous and carcinogenic but this effect is not associated with the quantity but with the quality of the type of tobacco. Thus, tar production from water-pipe smoking has completely different health effects from tar produced by cigarette smoking [3].

Studies of water-pipe smoking have shown that approximately 5 g of charcoal were consumed in the course of a single smoking session, suggesting the possibility of large quantities of carbon monoxide being delivered to the smoker [28]. Knishkowy and Amitai documented in their examination of narghile smokers the aerosol of narghile smoke has concentrations of carbon monoxide, nicotine (tar) and heavy metals [29]. These concentrations were as high or higher than those of cigarette smokers. The limited scientific data regarding the adverse health consequences of water-pipe smoking suggest dangers similar to those associated with cigarette smoking which include malignancy, impaired pulmonary function. Other health consequences not associated with cigarette smoking include infectious diseases resulting from pipe sharing and the frequent addition of alcohol or pscychoactive drugs to the tobacco. Wolfram *et al.* [5] found water-pipe smoking causes a significant increase of *in-vitro* oxidative stress. Relatively little research has been devoted to the health effects of water-pipe smoking. Thus, there is a need for research to clarify the harmful effects of water-pipe smoking on human health. This study was designed to investigate the effect of water-pipe smoking on cardiovascular disease risk factors such as elevated blood pressure, elevated LDL-cholesterol, low HDL-cholesterol, elevated TG, elevated apo A-1 and elevated apo B. In addition, this study evaluated the effect of water-pipe smoking on oxidative stress by measuring serum concentration of malondialdehyde, serum vitamin C and total antioxidant capacity in healthy Saudi males.

Methodology

Selection of subjects: Two hundred (100 non-smokers and 100 smokers) healthy Saudi males ranging in age from 19-50 years old, from Riyadh, the capital of Saudi Arabia, were selected for this study. A smoker was determined if he smoked tobacco (Ma'ssel) using only a water-pipe (Shisha) at least two times per day (i.e., smoked two heads of tobacco (Ma'ssel) per day) for at least one year. Exclusion criteria included individuals with a history of cardiovascular, endocrine or gastrointestinal disorders and did not take medication or nutritional supplements. A written and signed informed consent was obtained from all subjects. All components of the study were approved by research committee, College of Food Science and Agriculture, King Saud University.

Demographic and smoking habits information: Each subject was interviewed and asked to provide demographic and smoking habit information. The demographics included age, marital status (married and not married) and education status (low - illiterate or elementary, medium - intermediate or secondary and high - college or higher). Smoking habits included smoking period (years) and number of Ma'ssel heads smoked daily using a water-pipe.

Blood pressure and anthropometric measurements: Blood pressure and anthropometric measurements (weight and height) were measured by well-trained staff. Blood pressure (mm Hg) was measured on the same arm with a standard cuff while the participant was sitting and in a relaxed position. Three separate measurements were taken and the average recorded. All anthropometric measurements were taken with the participant wearing light clothing, standing relaxed and looking straight ahead, with arms at the sides, feet together, and with weight equally distributed over both legs [30]. The weighing scale was zeroed before and after every measurement and standardized with a certified weight every day. Weight measurements were taken using the Clinical Detecto Balance-Beam scale (Detecto Scale Inc., Brooklyn, NY). Body Mass Index was calculated using weight (in kilograms) divided by height (in meters squared).

Collection of blood: Subjects were asked not to smoke 12 hours before sampling to exclude the effects of acute smoking on the blood parameters studied. Two overnight fasting blood samples were collected from all subjects. The first blood samples were centrifuged at 3000*g* for ten minutes at room temperature and then serum was analyzed for serum lipids. The second blood sample was centrifuged at 2000*g* for fifteen minutes at 4°C, then the serum samples were stored at -80°C until analyzed for serum vitamin C, total antioxidant capacity and malondialdehyde. Hemolyzed samples were excluded from the analysis.

Analytical methods: Serum concentrations of LDL-cholesterol, HDL-cholesterol and triglycerides determinations were performed by standard procedures with the Cobas Integra Analyzer (Roche Diagnostic Systems, Switzerland) [31-33]. Apo A-1 and apo B were determined using the immunoturbidimetric assay method [34]. Serum concentrations of malondialdehyde were determined colorimetrically using the malondialdehyde assay kit according to the method of Ohkawa *et al.* [35] which is based on the reaction between malondialdehyde and thiobarbituric acid. Analysis of serum concentrations for vitamin C were determined by high-performance liquid chromatography according to the method described by Kenneth and Trevithick [36]. Serum concentrations of total antioxidant capacity were determined colorimetrically using the total antioxidant status assay kit according to the method of Miller *et al.* [37]. This assay relies on the ability of antioxidants in the sample to inhibit the oxidation of 2,2'-azino-di-[3-ethylbenzthiazoline sulphonate] (ABTS) to ABT^+ by metmyoglobin. The amount of $ABTS^+$ produced can be monitored by reading the absorbance at 600 nm.

Data analysis: Analysis of data was performed using the Statistical Package for the Social Sciences, version 11.0 (SPSS) computer software. Descriptive statistics were adapted to display data in means ± SD and percentages. The statistical method of t-test was used to compare the mean values obtained between the smoker and non-smoker groups. Chi-square statistical test was used to compare marital and education status of study subjects by groups. Differences and correlations were considered significant whenever the p-value was ($p<0.05$).

Results

General characteristics of male water-pipe smokers and non-smokers are shown in Table 1. The mean age, body weight, height and Body Mass Index of the subjects were statistically similar between smokers and non-smokers. No significant differences in marital and education status were identified between smokers and non-smokers. Diastolic and systolic blood pressure was significantly ($p<0.05$) higher in smokers than in non-smokers. The mean smoking period of male water-pipe smokers was approximately nine years. The mean number of tobacco (Ma'ssel) heads smoked by male water-pipe smokers was about 2.5 per day.

Comparisons of serum lipid profiles of male water-pipe smokers and non-smokers are shown in Table 2. Serum concentrations of HDL-cholesterol and apo A-1 were significantly ($p<0.05$) lower in smokers than in non-smokers. However, LDL-cholesterol, apo B and triglycerides were significantly ($p<0.05$)) higher in smokers than in non-smokers. Malondialdehyde was significantly ($p<0.05$) higher in smokers than in non-smokers.

Table 1. General characteristics of male water-pipe smokers and non-smokers †.

Parameter	Smokers n=100	Non-smokers n=100
Age (year)	36.33± 5.61 [a]	38.63± 4.98 [a]
Marital status (%) *		
Currently married	84	82
Not married	16	18
Education status (%)*		
Low education	0	0
Medium education	38	36
High education	62	64
Height (m)	1.75 ± 0.10 [a]	1.74 ± 0.13 [a]
Body weight (kg)	81.50 ± 11.00 [a]	80.00 ± 10.5 [a]
Body Mass Index (BMI)	26.27 ± 3.0 [a]	25.98 ± 3.2 [a]
Blood pressure (mmHg)		
Diastolic	88.10 ± 3.24 [a]	80.99 ± 2.98 [b]
Systolic	134.22 ± 3.36 [a]	120.20 ± 3.10 [b]
Smoking period (year)	8.89 ± 1.28	-
Number of heads smoked per day §	2.42 ± 0.50	-

† Values are expressed as mean ± SD.
* Values expressed as percent with no significant difference between smokers and non- smokers (chi-square).
[ab] Values with different letters in the same row are significantly different at $p<0.05$ (t-test)
§ Number of tobacco (Ma'ssel) heads smoked using a water-pipe per day

Table 2. Serum concentrations of lipid profiles and malondialdehyde of male water-pipe smokers and non-smokers†.

Parameter	Smokers n=100	Non-smokers n=100
HDL-cholesterol (mmol/L)	1.05 ± 0.09 [a]	1.17 ± 0.10 [b]
LDL-cholesterol (mmol/L)	3.61 ± 0.69 [a]	3.06 ± 0.61 [b]
Triglyceride (mmol/L)	1.84 ± 0.22 [a]	1.56 ± 0.19 [b]
Apolipoprotein A-1(mmol/L)	42 ± 1.92 [a]	46 ± 1.10 [b]
Apolipoprotein B (mmol/L)	2.38 ± 0.40 [a]	2.02 ± 0.61 [b]
Malondialdehyde (nmol/mL)	2.93 ± 0.41 [a]	2.26 ± 0.59 [b]

† Values are mean ± SD
[ab] Values with different letters in the same row are significantly different at $p<0.05$

Table 3. Serum concentrations of total antioxidant capacity and vitamin C of male water-pipe smokers and non-smokers†.

Parameter	Smokers n=100	Non-smokers n=100
Total antioxidant capacity (mM)	1.08 ± 0.24 [a]	1.47 ± 0.42 [b]
Vitamin C (mg/L)	5.20 ± 0.33 [a]	9.01 ± 0.51 [b]

† Values are mean ± SD
[ab] Values with different letters in the same row are significantly different at $p<0.05$

Serum concentrations of total antioxidant capacity and vitamin C of male water-pipe smokers and non-smokers are shown in Table 3. Serum concentration of total antioxidant capacity and vitamin C were significantly ($p<0.05$) lower in smokers than in non-smokers.

Discussion

The composition of the tobacco used in water-pipe smoking is variable and not well standardized. The nicotine contents of water-pipe tobacco have been reported to be 2 to 4%, in comparison to 1 to 3% for cigarettes [38]. The carbon monoxide concentrations in water-pipe smoke were significantly greater than that of cigarette smoke [39]. The mean carboxyhemoglobin concentrations were higher among water-pipe smokers than among cigarette smokers or non-smokers [17].

According to another report, urinary cotinine concentrations were similar for water-pipe smokers and cigarette smokers [5]. Water-pipe smokers may absorb higher concentrations of nicotine and heavy metals because of higher concentrations in the smoke itself or because of the mode of smoking, including frequency of puffing, depth of inhalation and length of smoking session [29]. Carbon monoxide concentration may also be elevated because of the charcoal used to burn the narghile tobaccos [29]. Contrary to popular opinion, the water in the pipe probably filters out only a small portion of noxious substances [29]. Therefore, this study was designed to evaluate the influence of water-pipe smoking on some cardiovascular risk factors (i.e., blood pressure, serum concentration of LDL-cholesterol, HDL-cholesterol, TG, apo A-1 and apo B). In addition, to study the effect of water-pipe smoking on *in-vivo* oxidative stress by determining serum concentrations of malondialdehyde, total antioxidant capacity and vitamin C. This study provided limited data to suggest that water-pipe smoke is at least as toxic as cigarette smoke.

Water-pipe smoking and anthropometric measurements and blood pressure: The data in Table 1 shows that there was no significant difference in height, weight and Body Mass Index between water-pipe smokers and non-smokers. These results are similar to our previous results on cigarette smoking [40]. When we compared the effects of water-pipe smoking with cigarette smoking on blood pressure of healthy men, the results revealed that water-pipe smoking was associated with a significant increase of systolic and diastolic blood pressure compared with non-smoking [40]. These results are similar to the results of Al-Kubati *et al.* [41] who mentioned that water-pipe smoking increases the activity of the autonomic nervous system, increases heart rate and blood pressure and impairs the baroreflex more than cigarette smoking. They suggested this is attributed to a higher quantity of nicotine in water-pipe smoking [21, 41]. The long duration of smoke inhalation during puffs should lead to more absorption of nicotine in the lungs, so it is not surprising that the plasma nicotine level is about three time as high for water-pipe (60 mg/mL) [42] smoking compared to light and ultra-light cigarette (16.33 mg/mL) [43] smoking. The mechanism of increasing blood pressure and heart rate by nicotine is believed to be by activation of the sympathetic nervous system with a release of norepiephrine and epinephrine[44], vasopressin release [45] or by effects on endothelial function [46].

Water-pipe smoking and lipoproteins and apolipoproteins A-1 and B: The data in Table 1 show that water-pipe smoking significantly ($p<0.05$) increased serum LDL-cholesterol and its specific apolipoprotein B concentrations but significantly decreased HDL-cholesterol and its specific apolipoprotein A-1 concentrations. These results may be due to the lowering effects of water-pipe smoking on serum vitamin C concentration (Table 2) which lead to significant increase in LDL-cholesterol and its specific apolipoprotein B concentration and decreases in HDL-cholesterol and its specific apolipoprotein A-1 concentrations. These results are confirmed with results of other investigators who mentioned that vitamin C status has a significant positive association with HDL-cholesterol and apolipoprotein A-1 levels and an inverse association with LDL-cholesterol and apolipoprotein B levels [29, 47, 49].

Sorci-Thomas *et al*. mentioned that vitamin C deficiency in Guinea pigs lowered serum apolipoprotein A-1 concentration by lowering its mRNA level and suppression of its synthesis in the liver [50]. Ginter proposed that vitamin C influences HDL-cholesterol concentration through regulation of lipoprotein lipase activity [51]. Apolipoproteins were suggested as better indicators of cholesterol metabolism than lipoproteins. The apolipoproteins are more stable than their respective lipoproteins during acute changes and also play an active role in lipoprotein metabolism [52]. Apolipoprotein A-1 is an important structural protein of HDL-cholesterol [52, 53], which has a major role in reverse transport of cholesterol from peripheral tissues to the liver. Apolipoprotein A-1 functions as an activator of lecithin-cholesterol acetyltransferase, a key enzyme in reverse transport of cholesterol from peripheral tissues to the liver [53]. Apolipoprotein B facilitates cholesterol delivery to tissues and is an essential structural component of LDL-cholesterol. Apolipoprotein B is bound to specific receptors on the surface of cells, especially liver cells [54, 55-57]. Activity of hepatic LDL apolipoprotein B and its receptors was a major determinant for hepatic cholesterol uptake and plasma cholesterol levels. The risk of water-pipe smoking increased due to greater synthesis of LDL apolipoprotein B and its receptors. Consequently the result was significant increases of LDL-cholesterol and total cholesterol.

In the same respect, Al-Numair [40] and Hughes *et al* [58] found that cigarette smoking is related to higher LDL-cholesterol and lower HDL-cholesterol, though there is a dose response relationship. Hughes [58] and Fievet and Fruchart [59] mentioned that the lowering effect of cigarette smoking on HDL-cholesterol was related to increased lipase activity (lipoprotein lipase and hepatic lipase) which is associated with HDL-cholesterol metabolism. A unifying mechanism of smoking-induced higher serum LDL-cholesterol concentrations might be due to the effect of smoking on copper metabolism which increases LDL-cholesterol peroxidation (ox LDL-C) [60]. The peroxidation of LDL-cholesterol prevents its uptake by the body cells causing an elevated concentration in serum [40]. Another explanation for the effect of smoking on reducing HDL-cholesterol and increasing triglyceride levels (Table 2) may be through insulin resistance [61, 62]. HDL-cholesterol and triglyceride are markers for the insulin resistance syndrome (syndrome X) [58]. Tobacco smoking has been reported to increase insulin resistance.

Water-pipe smoking and serum concentrations of total antioxidant capacity, vitamin C and malondialdehyde: The serum concentration of total antioxidant capacity of water-pipe smokers was significantly lower than that of non-smokers. These results suggest evidence for oxidative stress and an impaired oxidant defense system of water-pipe smokers. The oxidative damage of water-pipe smokers was associated with significant increases of serum malonaldihyde concentration as shown in Table 2. The higher concentration of malondialdehyde in the serum of water-pipe smokers indicates the presence of higher lipid peroxidation process. These results are in accordance with the results of Wolfram *et al.* who reported that a single water-pipe smoking session increased oxidative injury (malondialdehyde) significantly [5]. On the other hand, the significantly lower concentrations of vitamin C in the serum of water-pipe smokers compared to non-smokers (Table 2) may be due to several factors including, decreased vitamin C consumption, impaired vitamin C absorption or an increased turnover of vitamin C [63, 64]. The low serum vitamin C concentrations in the present study were due to a combination of impaired vitamin C absorption and an increased turnover due to oxidative stress affected by water-pipe smoking. These results confirm that smoking is associated with decreased plasma vitamin C concentrations [65-70].

Alberg reported the evidence consistently shows that the average vitamin C concentrations were 27% lower in tobacco smokers compared with non-smokers [71]. Significant increases of malondialdehyde and significant decreases of vitamin C in the serum of water-pipe smokers are in accordance with previous studies [65, 70-74]. The imbalance between peroxidants and antioxidants, linked to decreased smoke-related antioxidant capacity and increased free radical generation, especially in the arterial tissues, might render smokers more prone to peroxidative stress. In the same respect, Elsayed and Bendich mentioned that, smoking was shown to deplete the body of endogenous antioxidants such as vitamin C and E, ß-carotene, ubiquinole glutathione and α -lipoic acid [75].

Conclusions

Water-pipe smoking has been show to produce oxidative stress (high serum malonaldehyde) and an impaired oxidant defense system (low serum total antioxidant capacity and low vitamin C concentrations). Contrary to popular opinion, the water in the pipe probably filters out only small portions of noxious substances, therefore, the blood pressure of water-pipe smokers was higher than that of cigarette smokers and non-smokers.

Alterations observed in LDL-cholesterol and HDL-cholesterol levels and their specific apolipoprotein B and A-1 levels in the serum of water pipe smokers are probably secondary to the lower concentrations of serum vitamin C. Decreased serum vitamin C concentrations has been shown to increase apolipoprotein B and decrease apolipoprotein A-1 levels and consequently increased LDL-cholesterol and decreased HDL-cholesterol concentrations.

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