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Original Research Article

Effect of *Shisha* (Water-Pipe) Smoking on Serum Lipid Profile and Antioxidant Vitamins among Smokers in Kano Metropolis

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Abstract: *Shisha* Smoking is a risk factor for coronary heart diseases. The smoke contains large amount of chemicals which are capable of generating reactive oxygen species which play an important role in oxidative stress which in turn leads to the development and progression of many disorders such as hypertension, cancer, diabetes mellitus and cardiovascular diseases. Monitoring lipid profile (HDL-cholesterol, LDL-cholesterol and triacylgycerides) and antioxidant vitamins (A, C and E) levels is very important to give an insight on the effect of *shisha* smoking on lipid profile and antioxidant vitamins. This study aimed to determine the effect of *shisha* smoking on serum lipid profile, and antioxidant vitamins in fifty (50) apparently healthy *shisha* smokers (exposed group) in Kano metropolis. A corresponding fifty (50) apparently healthy non-smokers were used as controls (non exposed group). The *Shisha* smoking was significantly associated with increased levels of total cholesterol (TC), triglyceride, LDL-cholesterol ($p<0.05$) in smokers compared to control group. However, there was a significant ($p>0.05$) decrease in HDL-cholesterol and serum antioxidant vitamins (A, C and E) in exposed group compared to non-exposed group. The results of this study also indicate that exposure of human being to *shisha* smoke over a period of time causes slight increase in lipid profile and antioxidant vitamins but the relation was statistically not significant ($p>0.05$). The findings suggest that *shisha* smoking causes dyslipidaemia and oxidative stress.

Keywords: *Shisha* (water-pipe), dyslipidaemia, oxidative stress.

ABBREVIATION

HDL-c: high density lipoprotein;
LDL-c: low density lipoprotein;
TC: total cholesterol;
TG: triglycerides

INTRODUCTION

Tobacco smoking is a global epidemic phenomenon especially in developing countries, and considered as the earliest example of a noninfectious disease that causes preventable deaths in the world. It has been estimated that tobacco smoking is responsible for the death of more than six million people in 2010 (WHO, 2014). Aden *et al.*,(2013) projected that it is expected that by 2030 the death will exceed million per year in developing countries. There are different methods to smoke tobacco including cigarettes, cigars, chew, pipes or water pipe. Water-pipe smoking and cigarette smoking are currently considered a fashionable way of tobacco leaves consumption, especially among young and middle aged males and females (Aden *et al.*, 2013).

Furthermore, smoking is considered the second leading cause of death and the fourth major risk factor for disease worldwide (WHO, 2005). It has been estimated that tobacco contains more than 400 chemical compounds of which many compounds are toxic and tumorigenic in nature (Shevchenko, 2012). It is believed that nicotine, the chemical agent of cigarette

smoking, is beyond the behavior of addiction, and with other chemicals participates to smoking related diseases (Shihadeh and Saleh, 2005). Even though *shisha* has been present for a millennium, far less study have examined its chemical constituents/ air quality relatives to cigarette. With tobacco being the main source of smoke in both *shisha* and cigarettes, *shisha* users are exposed to many of the same toxic compound/ by products as cigarette users but at dramatically higher levels, which might in fact produce worsened health effects in users (Eissenberg and Shihadeh, 2009).Moreover, the World Health Organization study group on tobacco product regulator (Tob Reg)(2006) stated that heat sources are commonly use to burn the tobacco, such as wood cylinders or charcoal, are likely to increase health risks since when such fuels combusted produce toxicants, including high levels of carbon monoxide, metals and cancer-causing chemicals (Pryor and Stone, 1993) . It has been reported that one puff of a cigarette exposes the smoker to more than 1015 free radicals and other oxidants. Free radicals are highly unstable molecules that are naturally formed during energy metabolism or sometimes, the biological system can be exposed to these radicals from avariety of environmental sources, such as Cigarette smoke, air pollution, and sunlight among others (Bensasson *et al.*, 1993; Droge, 2002; Valko *et al.*, 2007). Free radicals can cause "oxidative stress," a process that can trigger cell damage. Oxidative stress is thought to play a role in a variety of diseases including cancer, cardiovascular diseases, diabetes,Alzheimer's disease, Parkinson's disease, and eye diseases such as cataracts and age related muscular degeneration (Klein *et al.*, 1998; Gandhi *et al.*, 2009; Vardavas *et al.*, 2012).The defence system against radicals consists of enzymatic and non-enzymatic; essential and nonessential; endogenous and exogenous radical scavengers; Antioxidant vitamins with cigarette smoking (Eduardu *et al.*,1999).Vitamins A, C and E are among the essential free radical scavengers, hence there are called antioxidants (Burton *et al.*, 1982; Halvevy and Sklan,1987).The concentrations of these essential antioxidants, in contrast to those of other antioxidant defence systems, are determined mainly by dietary intake (Eduardu *et al.*,1999; Greg, 2003; Walter, 2010).Smokers usually have poor eating habits, consuming less fruits and vegetables, and thus, may ingest lower levels of essential antioxidants (Wallstrom *et al.*, 2001; Young and Woodside, 2001). The research aimed at determining the effect of shisha smoking on human serum lipid profile and antioxidant vitamins among smokers and non-smokers in Kano metropolis.

MATERIALS AND METHODS

Study Population

A total of one hundred subjects took part in this study consisting of exposed group (*Shisha*-Smokers) and non-exposed group (control group). Fifty (50) apparently healthy Smokers in Kano metropolis constituted the smokers group. Fifty (50) apparently healthy subjects who do not smoke *Shisha* constituted the non-smokers group and this group consisted largely of students from School of Health Technology, Kano and Shop attendants selected from Nasarawa, Kano municipal and Gwale local governments of Kano state metropolis.

Data Collection

A structured questionnaire eliciting information about the age, socio-economic status, health status, occupational history among others was issued to all the participants.

Ethical Consideration

The ethical committee of Kano State Ministry of Health approved the study protocol. (MOH/Off/797/T.1/1639). Ethical consideration and confidentiality were respected. An informed consent (consent form) (appendix V) was obtained from all participants of this study. The nature and purpose of the study was explained to the participants, following which they willingly consented to participation in the study.

Exclusion Criteria

- Subjects outside of the age group (15 – 30) years
- Involvement in any form of smoking other than shisha
- Volunteers under lipid lowering drugs
- Volunteers outside Kano state.
- Subjects that decline consent

Determination of Sample Size: The sample size was calculated according to the method described by Cochran (1975).

Experimental Design

Apparently healthy volunteers (n=100) from Kano metropolis between the ages of 15-30 years were recruited after collection of their respective data using Questionnaires and their serum were used for biochemical analysis. The one hundred (100) apparently healthy volunteers were divided into two groups; apparently (50) healthy smokers and apparently (50) healthy non-smokers. The smokers were also divided into three groups (>2year, 2-5 years and < 5years) depending on the period of *shisha* smoking. Blood samples were drawn in a fasting state (no food or drink, except water, for at least 12 hours) and allowed to coagulate. The coagulated blood was centrifuged to separate the serum from the whole blood at 4000 rpm for 5 minutes. The supernatant was pipetted into plain bottles and stored at 0^{o}C until required for analysis. The analyses include estimation of liver enzymes activities, lipid profile (TC, HDL-CH, and LDL;-CH) and antioxidant vitamins (Vitamins A, C and E) among shisha smokers and non-smokers

Lipid Profile Test

The lipid panel test system was used to measure total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides in whole blood, plasma or serum on a CardioChek PA or a CardioCliek Plus professional analyzer. The test system is used by Healthcare professionals and scientists to measure three blood analytes: total cholesterol, HDL-cholesterol, and triglyceride. The LDL-c can then be calculated from the results obtained for other parameters using the sformula, LDL= TC – HDL-c – (Trig/5). Cholesterol measurements are used in the diagnosis and treatment of disorders involving excess cholesterol in the blood and lipoprotein metabolism disorders. . PTS Panels test strips are designed for use with fresh capillary and fresh venous whole blood collected in "EDTA or heparin tubes. A memo Chip" is provided with each package of test strips and must be properly inserted into the analyzer before any test can be run. The *MEMo* Chip contains the test name, calibration curve, lot number, and test strip expiration date. After the test strip is inserted into the analyzer and blood applied to the test strip, test results are displayed before or after 90 seconds. This method is based on the earlier report by (John, 1991).

PROCEDURE

All instructions were carefully read before testing for best results. Participants were tested in a fasting state (no food or drink, except water, for at least 12 hours).

The *MEMO* Chip inserted that matches the lot number on the test strip vial and one of the buttons pressed to turn the analyzer ON. The test strip was held by the end marked "PTS" and the opposite end of the test strip was inserted into analyzer. The test strip was pushed in as far as it will go. When applying the sample, a pipette was used to apply 35-40 |uL of blood serum to the test strip blood application window. After 90 seconds, the results appeared on the display automatically on the analyzer: the button marked NEXT was pressed to view additional results. The test strip was removed and discarded.

Data Analysis

The Data collected and statistically analyzed using SPSS version 20.0. The results were expressed as mean ± standard deviation (SD). The parameters were analyzed statistically using student's t-test and One-way Analysis of Variance (ANOVA). Differences were considered statistically significant at $p<0.05$.

RESULTS

Table 1: Effect of Shisha Smoking on Serum Lipid Profile

Parameter	Smokers	Non-smokers
TC (mmol/L)	185.26±9.15[a]	166.54±7.25[b]
HDL (mmol/L)	49.94±8.56[a]	55.56±6.08[b]
TAG (mmol/L)	71.84±14.24[a]	66.44±6.67[b]
LDL (mmol/L)	83.78±9.17[a]	64.34±4.66[b]

Values are expressed as mean ± SD; values followed by different superscript letters in the same raw are considered significantly different at $p < 0.05$.

Table 1: Indicates the effect of *Shisha* Smoking on Serum Lipid Profile in Smokers and Non-Smokers. From the table it can be seen that the mean value of total cholesterol (TC), triacylgycerides (TAG), and low density lipoprotein (LDL) were significantly higher ($p<0.05$) in smokers than in non-smokers and the mean of high density lipoprotein (HDL) was decreased ($p<0.05$) in smokers than in non-smokers.

Table 2: Effect of Shisha smoking on Serum Antioxidant Vitamins

Parameter	Smokers	Non-smokers
Vitamin A(mg/dl)	11.98±3.04[a]	18.04±1.88[b]
Vitamin C(mg/dl)	51.81±15.35[a]	73.18±18.01[b]
Vitamin E(mg/dl)	50.61±8.83[a]	56.67±7.44[b]

Values are expressed as mean ± SD; values followed by different superscript letters in the same raw are considered significantly different at $p < 0.05$.

Table 2: shows the effect of *Shisha* smoking on human serum antioxidant vitamins among *Shisha* and Non-smokers. From the table it can be observed that the mean of vitamin A, C and E were significantly higher ($p<0.05$) in smokers than non-smokers.

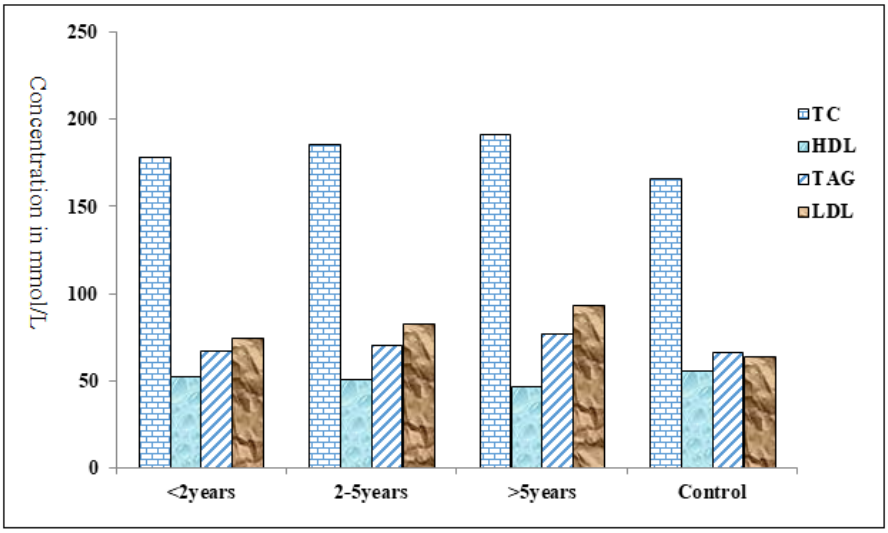

Figure 1: Effect of Shisha Smoking on Serum Lipid Profile in Smoker of different Period of Smoking

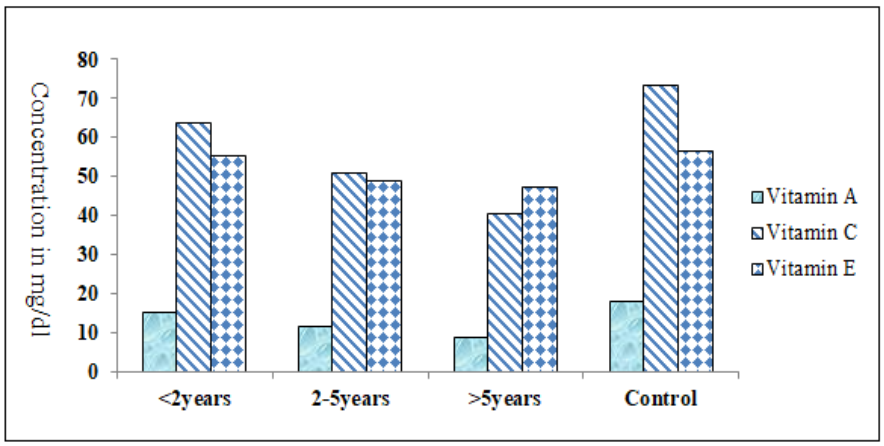

Figure 2: Effect of Shisha Smoking on Serum Antioxidant Vitamins in Smoker of different period of smoking

DISCUSSION

Shisha smokers are associated with coronary heart diseases compared to non-smokers. This may explain by various ways such as impairment in the integrity of arterial wall, derangement in the blood lipid and lipoproteins concentrations, alteration in blood coagulation.

This study revealed significantly ($p<0.05$) high concentration of total cholesterol, Triglycerides, LDL-cholesterol in *shisha* smokers compared non-smokers (Table 1).The increased in lipid levels may be explained by the following mechanism*: shisha* smoking causes absorption of nicotine into the body which leads to lypolysis and release of free fatty acids into the blood stream via action of adenyl cyclase in adipose tissue by nicotine stimulated secretion of catecholamine. These increased free fatty acids in liver give rise to increased hepatic triglycerides and VLDL-cholesterol synthesis, thus increasing the concentration of Triglycerides and VLDL-C in blood (Devaranavadgi *et al.*, 2012).

The present results agreed with many earlier reports (Kong, *et al.*, 2001; Zhu, *et al.*, 2011 and Devaranavadgi, 2012). Similar findings were found in the study done by (Trupti, *et al.*, 2014). The present study also showed significant decreased in the level of HDL-cholesterol ($P<0.05$) in smokers than in non-smoker (Table 1). Several studies reported high levels of plasma homocysteine in chronic smokers (O' Callaghan *et al.*, 2002). Plasma homocysteine is negatively correlated with HDL-C and Apo A-1. Decreased in HDL-Cholesterol in smokers may be due to smoking induced increase catecholamine release, causing increased in VLDL-cholesterol and decreased in HDL-cholesterol concentration. The present study findings are in line with the finding of Trupti *et al.*, (2014) who found that the mean of HDL-cholesterol was significantly lower and LDL-cholesterol was significantly higher among smokers compared control. Furthermore, the results of the current study with regard to the period of *Shisha* smoking, it was observed that there was an increased in the levels of serum total cholesterol (TC), triacylgycerides (TGs) and LDL-cholesterol with a slight decrease in HDL-cholesterol with regard to the increase in the period of *shisha* smoking. But the relation was statistically not significant (figure1).

Vitamin A is a strong antioxidant and is the best quencher of singlet oxygen. Its lipophilic nature allows it to pass across the membrane and scavenge free radicals (Wallstrom *et al.,* 2000). Several observations were reported on the effects of tobacco smoking and serum levels of vitamin A. Some reported a significant decrease (Chui *et al.,* 2009) while others observed a significant increase in the levels of Vitamin A (Biesalski *et al.,* 1986). This research however revealed that smokers had significantly lower levels of serum Vitamin A than non-smokers at $p<0.05$(Table 2) which might be due to destruction of this antioxidant vitamin during neutralization of free radicals present in *shisha* smoke which contains more free radicals than the biological system can handle (Abdulrahman *et al,.* 1997).

Vitamin C is a water-soluble vitamin that efficiently scavenges free radicals. It also promotes the regeneration of the active form of vitamin E (α-tocopherol) from α-tocopheroxyl radical produced during scavenging of ROS (Satyanarayana and Chakrapani, 2006).

This research showed that there was significantly ($p<0.05$) lower concentrations of vitamin C in the serum of *shisha* smokers compared to non-smokers (Table 2) which might be due to a combination of impaired vitamin C absorption and an increased turnover due to oxidative stresses affected by *shisha* smoking.

There are conflicting reports about the effects of tobacco smoking and serum Vitamin C levels. Some reports revealed that there was effect showing that smokers have statistically significant lower levels than non-smokers (Greg *et al.,* 2003) while others reported a reverse effect (Mezzetti *et al.,* 1995). A significant amount of research indicates that smokers may have higher requirements for vitamin C (Weber *et al.,* 1996; Burri and Jacob, 1997). These results confirmed that smoking is associated with decreased serum vitamin C concentrations.

Alberg (2002) reported that the average vitamin C concentrations were 27% lower in tobacco smokers compared with non-smokers.

The biological activity of vitamin E is almost entirely due to its antioxidant properties. In addition to its antioxidant role, vitamin E might also have a structural role in stabilizing membranes (Christine *et al.,* 2006).

In the same way, this study showed that the serum level of Vitamin E was found to be significantly lower in smokers than in non-smokers at $p<0.05$ (Table 2). This was in line with the findings of another study by Palanisamy *et al.,* (2009).

On the other hand, analyzing the results of the current study with regard to the period of smoking it was observed that there is decreased in the levels of serum A, C and E with regard to the increased in period of *shisha* smoking (figure3) and relation was not statistically significant (Figure 2).

CONCLUSION

The present study revealed increase in total cholesterol, Triglycerides and LDL- cholesterol with a decreased in serum HDL-cholesterol which reflect a great significance since the findings associated with coronary heart disease. Serum antioxidants vitamins (A, C, and E) decreased significantly compared to non-smokers.

This study also showed increase in the levels of total cholesterol, Triglycerides, LDL-cholesterol with a decreased in HDL- cholesterol and antioxidant vitamins as the period of *shisha* smoking increased.

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Conflict interest: There is no conflict of interest among authors.

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