Effect of Active Cigarettes Smoking, Water-Pipe Smoking and Snuff (Naffa) Inhalation on Bmi, Lipid Profile, And Plasma Glucose. A Case Control Study of Libyan Males

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Abstract:
Tobacco use in all its forms represents a very well-known preventable risk factor for cardiovascular diseases (CVD). Alteration of plasma lipids levels is one of the mechanisms by which it causes CVD. In addition, there are controversial reports linking tobacco use with diabetes. This case-control study is aiming to investigate the interrelationship between tobacco use and BMI, lipid profile, and plasma glucose in Libyans residing in Tripoli region. The study was conducted on 200 healthy male subjects, including, 50 non-smokers aged 40.98±8.07, 50 cigarette smokers aged 41.32±7.39, 50 water-pipe users aged 42.04±7.39, and 50 snuff inhalers aged 39.36±7.00. BMI was estimated as (kg/m²), lipid profile and fasting plasma glucose were estimated in triplicate by enzymatic colorimetric method and expressed as (mg/dL). Our results showed that BMI is significantly higher in tobacco users than non-smokers (P < 0.0001). Total cholesterol and triacylglycerols are significantly higher in tobacco users (P < 0.0001). HDL is significantly higher in non-smokers (P < 0.0001). LDL not affected by tobacco use (P < 0.32). Fasting plasma glucose significantly higher in tobacco users (P < 0.0001). In conclusion, tobacco use affects the BMI, lipid profile, and glucose which are components of the metabolic syndrome in the Libyan male's population.

Keywords: Dyslipidemia, CVD, Tobacco, Total cholesterol, Triacylglycerols, LDL, HDL

Introduction
Cardiovascular disease (CVD) is a class of diseases that involve the heart, and/or blood vessels (arteries, capillaries, and veins). It involves diseases that affect the cardiovascular system, primarily cardiac diseases, vascular diseases of the brain and kidney, and peripheral arterial disease[1]. Currently, coronary heart disease and stroke are the leading causes of morbidity and a leading contributor to mortality worldwide[2]. CVD prevalence is stable in the developed countries and rapidly raising in the developing countries[3]. According to World Health Organization statistics, approximately 80% of deaths caused by CVD occurred in developing countries[4]. Dyslipidemia, hypertension, obesity, insulin resistance, and hyperhomocysteinemia are the major CVD risk factors[5]. Dyslipidemia, which is a very well-established risk factor of CVD, is characterized by elevation of plasma total cholesterol (TC), low density lipoproteins (LDL), and triacylglycerides (TAGS); and decrease of plasma high density lipoprotein(HDL) concentrations[6]. Several studies reported a relationship between tobacco use and dyslipidemia[7,8]. According to the WHO Report on the Global Tobacco Epidemic, 2013, Country profile- Libya, Tobacco consumption is very common among males, in spite of the legislation that approves rulings on the control of smoking, tobacco and all tobacco products[9]. To the best of our knowledge there are no studies of in-
terrelationship between tobacco use and dyslipidemia in Libyans. Hence, the current study aimed to assess the interrelationship between cigarettes smoking, water-pipe smoking and Snuff(Neffa) inhalation, body mass index, lipid profile and glucose levels in Libyans from Tripoli region.

Methods
The study was conducted in the period from September to December 2012 in the Biotechnology Research Center- BTRC (Twesha, Libya).

Two hundred Libyans, male subjects were enrolled in this study. They were 50 cigarettes smokers, 50 water-pipe (Shisha) smokers, 50 Snuff (Neffa) inhalers and 50 age matched non-smoker controls. All tobacco users are on their habit for over 5 years. All tobacco users are on their habit for over 5 years. The study subjects aged 25-59 years old, were recruited randomly from the workers at Biotechnology Research Center (Twesha, Libya), Medical Research Center, and Officers from the Libyan Navy Studies Academy. All participants signed a consent form. Five ml fasting whole blood were collected in a tube containing heparin as an anticoagulant. They were centrifuged and the clear plasma was stored in 1.5ml tubes at 5oC until use within two days of collection as recommended by the manufacturer. Plasma lipid profile and glucose were estimated in triplicate for each sample using the DIALAB kits (DIALAB Produktion, Wiener Neudorf, Austria) according to the manufacturer's instructions.

Statistical analysis was carried out using MS excel software. All results are reported as mean ± standard deviations. ANOVAs was used to compare mean differences in studied parameters between all four groups and it was followed by the post-hoc Bonferroni adjustment test to inter-comparison of the means of studied parameters between each tobacco use status (cigarette smoking, water-pipe smoking, and snuff inhalation) and the non-smoker controls. Categorical variables were compared by the Fisher’s exact test.

Results
Anthropometric measurements (Mean±SD) of the study groups are shown in table (1). These include age(years), body weight (kg), height (meters), and BMI weight (kg)/ square height (square meter) of the study subjects in non-smokers (NS), cigarette smokers(CS), water pipe smokers (WPS), and snuff inhalers(SI). The last column of the table shows the P-value of ANOVA.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NS (N= 50)</th>
<th>CS (N=50)</th>
<th>WPS (N=50)</th>
<th>SI (N=50)</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>40.98±8.07</td>
<td>41.32±7.39</td>
<td>42.04±7.39</td>
<td>39.36±7.00</td>
<td>P= 0.24</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>70.06±7.60</td>
<td>86.58±10.23</td>
<td>80.84±8.12</td>
<td>83.94±9.64</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.74±0.08</td>
<td>1.75±0.08</td>
<td>1.73±0.10</td>
<td>1.75±0.07</td>
<td>P= 0.79</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>23.18±1.61</td>
<td>28.28±2.75</td>
<td>26.96±3.18</td>
<td>27.52±2.16</td>
<td>P &lt; 0.0001</td>
</tr>
</tbody>
</table>

BMI= body mass index, CS= cigarette smokers, NS= non-smokers, SI= Snuff Inhaler, WPS= Water-pipe smoker P < .05 was considered statistically significant.

Our results showed that comparing tobacco users with non-smokers group using analysis of variance revealed significant differences (P < 0.0001) for the Body weight and the BMI and no significant differences for Age (P= 0.24) and Height (P= 0.79). Biochemical parameters of the study groups are shown it table (2). Comparison of total cholesterol (TC), triacylglycerols (TAGs), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and glucose in non-
smokers (NS), cigarette smoking (CS), water-pipe smoking (WPS) and snuff inhalation (SI) groups. The last column shows the P-value of ANOVA analysis.

Table (2) Showing lipid profile and glucose (Mean± SD) and mean comparison in non-smokers and tobacco users.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NS</th>
<th>CS</th>
<th>WPS</th>
<th>SI</th>
<th>ANOVA P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dL)</td>
<td>177.04±12.98</td>
<td>214.74±10.20</td>
<td>213.44±11.91</td>
<td>218.12±10.05</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>TAGS (mg/dL)</td>
<td>167.86±11.70</td>
<td>213.98±10.78</td>
<td>221.00±10.73</td>
<td>219.72±10.73</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>141.32±6.29</td>
<td>142.80±5.84</td>
<td>140.50±6.70</td>
<td>141.32±6.21</td>
<td>0.32</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>41.86±4.02</td>
<td>39.00±6.43</td>
<td>36.08±5.51</td>
<td>36.48±4.92</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>86.98±11.48</td>
<td>101.22±11.50</td>
<td>91.30±12.96</td>
<td>99.10±10.77</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

HDL-C= HDL cholesterol, LDL-C= LDL cholesterol, TAGS= Triacylglycerol, TC= total cholesterol.
P < 0.05 was considered statistically significant.

The results of ANOVA post-hoc t-test for the biochemical parameters is presented in table (3). It shows the p-value of t-test analysis applied on the study biochemical parameters between every two tobaccos use status.

Table (3) Shows comparison of the p-value of post-hoc t-test between different tobacco use status.

<table>
<thead>
<tr>
<th>Non-smoker</th>
<th>Cigarette</th>
<th>Water-Pipe</th>
<th>Snuff (Naffa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>1.00</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>TC</td>
<td>1.00</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>TAGS</td>
<td>1.00</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>LDL-C</td>
<td>1.00</td>
<td>0.23</td>
<td>0.53</td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.00</td>
<td>0.01</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.00</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

P < 0.0125 was considered as statistically significant after Bonferroni adjustment.

Association of tobacco use with pre-diabetes (plasma glucose > 110 mg/dL) was tested using the Fisher's exact test table (4). It showed that cigarette smoking and
snuff inhalation are in significantly higher association with pre-diabetes (p< 0.0001) in comparison with non-smokers. However, water-pipe smoking is not associated with pre-diabetes (p=0.056).

Table (4) Shows association of tobacco use with pre-diabetes.

<table>
<thead>
<tr>
<th>Plasma Glucose</th>
<th>NS</th>
<th>CS</th>
<th>WP</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;110 mg/dL</td>
<td>49</td>
<td>36</td>
<td>45</td>
<td>37</td>
</tr>
<tr>
<td>&gt;110 mg/dL</td>
<td>1</td>
<td>14</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.0001</td>
<td>0.056</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Tobacco use is largely considered as a major risk factor for the development of cardiovascular diseases. More than 10% of deaths worldwide from CVD 2000 are attributed to tobacco use[10]. Worldwide, tobacco use causes more than 6 million deaths annually, and it is projected that it will cause more than 8 million deaths annually by 2030[9].

Tobacco use is thought to cause cardiovascular disease through several mechanisms including lipid abnormalities; thrombogenic effects; endothelial injury and dysfunction; and inflammation[11]. The current study investigated the effect of tobacco use (cigarette smoking, water-pipe smoking and snuff inhalation) on BMI, lipid profile and plasma glucose in a sample of Libyan population residing in Tripoli region. All groups of the subjects (non-smokers, cigarette smokers, water-pipe smokers and snuff inhalers) were males of comparable age. They were normotensive, and non-diabetic. Regarding the effect of tobacco use on BMI, our results table (1) showed that neither nonsmokers nor tobacco users were obese according to[12]. However, non-smokers had normal BMI while tobacco users were overweight, the body mass index (BMI) being 23.18±1.61 for non-smokers, 28.28±2.75 for cigarette smokers, 26.96±3.18 for water-pipe smokers, and 27.52±2.16 for snuff inhalers. Analysis of variance showed that the BMI of tobacco users is significantly higher than that of the non-smokers control (P < 0.0001).

In the literature there are several conflicting reports regarding this matter. Several previous studies showed that cigarette smoking reduces the BMI in comparison with non-users[13,14]. However, some reports showed that cigarette smoking has no effect on BMI in normal subjects[15], while others are in agreement with our results for cigarette smokers[16] and for water-pipe smokers[17].

Application of ANOVA post-hoc t-test for the BMI table (3) showed that there is significant difference between the non-smoker controls and the cigarette smoking (P < 0.0001), water-pipe smoking (P <0.0001) and snuff inhalation (P < 0.0001). This holds even after Bonferroni adjustment, i.e. p value of <0.0125 is considered to be significant. BMI is significantly higher in the cigarette smoking compared with the non-smokers control (P < 0.0001) and water-pipe smoking (p= 0.05), but it became not significant with water-pipe smoking after Bonferroni adjustment.

However, there was no significant difference in the BMI between cigarette smoking and snuff inhalation (p= 0.13). BMI is not significantly different between water-pipe smoking and snuff inhalation (p= 0.30). In a sample of US men, Shimokata et al, (1989) showed that, BMI of the light smokers is less than that of non-smokers whereas heavy smokers have higher BMI than non-smokers[18]. Chiolero et al, 2007, found that cigarette smoking was associated with BMI in a dose dependent manner, resulting in increased prevalence for obesity in heavy smokers, particularly in men[19].
Yun and colleagues (2012), found that there is association between smoking and abdominal obesity but not overall obesity, in men with Type 2 Diabetes[20]. Most recently, Fischer et al, (2014) reported that nasal dry snuff use was positively associated with higher BMI in young Swiss men[21].

Regarding the effect of tobacco use on total cholesterol (TC) and triacylglycerols (TAGs), our results showed that the non-smokers have significantly lower TC and TAGs than tobacco users table (2). This is in line with previous studies[22,23]. Post-hoc t-test table (3) showed that there is significant difference in TC and TAGs between nonsmokers and cigarette smoking (p< 0.0001), waterpipe smoking (p<0.0001), and snuff use (p< 0.0001). There is no significant difference in TC between cigarette smoking and water-pipe smoking (p= 0.56) nor with snuff inhalation (p= 0.1). This contradicts the previous observation[24].

Regarding TAGs, there is significant difference between cigarette smoking with water-pipe smoking (p= 0.005), which is in line with[24], and snuff inhalation (p= 0.03) The later does not hold after Bonferroni adjustment TC and TAGs levels are not significantly different between snuff inhalers and water-pipe use.

Regarding the effect of tobacco use on LDL levels, our results showed that there is no significant difference (p= 0.32) in LDL levels between nonsmokers and tobacco users.

This result is in agreement with the result of studies conduct on Saudi Arabians[25], Tunisians[24], Nigerians[15], Singaporeans[26], and on Turkish populations[27]. But it contradicts results of several other studies[28,29] that showed increase in LDL levels in tobacco users.

Regarding the effect of tobacco use on HDL levels, results of the current study showed that serum HDL levels are significantly lower in tobacco users compared with non-smokers (p< 0.0001). This is in agreement with the results of several studies conducted worldwide [15,28,29,24,26]. But it contradicts results of other studies that showed no significant difference in HDL levels between smokers and non-smokers[27,30].

Post-hoc t-test showed that serum HDL levels are significantly higher in cigarette smokers than water-pipe users (p=0.02) and snuff inhalers (p= 0.03).

But after Bonferroni adjustment it becomes non-significant Regarding the effect of tobacco use on glucose levels, taking into account the definition of pre-diabetes as fasting plasma glucose levels of >_ 110 mg/dL[31].

The results of this study revealed that there is a significant association between cigarette smoking and snuff inhalation with plasma glucose levels (p-0.0001). This is in accordance with the results of some studies that found a strong association between tobacco smoke exposure and subsequent development of pre-diabetes [15,19,23,32,33] or type2 diabetes[34].

Results of some other studies showed no consistent association between smoking and fasting blood glucose[28,26]. However, other studies reported association between smoking and diabetes in men, but not in women[28,35].

Recently, in a large population based sample of young and healthy individuals, Aeschbacher et al, (2014) reported strong association between smoking and pre-diabetes in young adults with a low burden of smoking exposure, and suggested that nicotine dependence as a potential mechanism of this relationship[36].

These results clearly demonstrate that all forms of tobacco use have an effect on the components of dyslipidemia and pre-diabetes. Hence, clear effective strategies to control the spread of tobacco use among Libyans are urgently needed.
Conclusion
In conclusion, results of the current study showed that tobacco use either in the form of cigarette smoking, water-pipe smoking or snuff inhalation, would affect some components of dyslipidemia and pre-diabetes in among the studied Libyan male population, and hence contribute to the development of metabolic syndrome that leads to cardiovascular diseases and diabetes type 2.

References
14) Pednekar MS, Gupta PC, Shukla HC, Hebert JR. Association between tobacco use and body mass index in urban Indian population: implications for public health in India. BMC Public Health. 2006; 6:70.


