



## **Analysis between Cigarette and Shisha Smokers for Early Atherogenesis: A Cardiovascular Disease**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors DI and MA designed the study. Authors DI and RKC performed experimental work. Authors DI and IWB performed statistical analyses and writing original draft. Authors DI, IWB and SAM performed review and editing. All authors, managed the literature searches, read, and approved the final version of the manuscript.*

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### **ABSTRACT**

**Aims:** Tobacco smoking is a major health issue in Saudi Arabia, particularly among the student population. Smoking is one of the major risk factors in the genesis of coronary atherosclerosis and the development of coronary heart disease. This study aimed to evaluate the effect of cigarette and shisha smoking on atherogenic indexes, lipid profile and hematological parameters of undergraduate smokers at Majmaah University.

**Methodology:** This cross-sectional study was conducted between November 2019 and March 2020, at the Department of Medical Laboratory Sciences, College of Applied Medical Sciences, Majmaah University, Al-Majmaah, enrolling 100 undergraduate students (35 cigarette smokers, 30 shisha smokers, 35 non-smokers). The subjects were asked to fast overnight and early morning blood samples were collected and analyzed to measure lipid parameters, complete blood cell count and LDH. Lipid parameters were used to calculate lipid indexes and atherogenic indexes.

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**Results:** Compared with non-smokers, cigarette and shisha smokers had significantly higher levels of TC, TG, LDL-C and VLDL-C, but significantly lower levels of HDL-C. The values of comprehensive lipid indexes, including non-HDL-C, TC/HDL-C, TG/HDL-C, LDL-C/HDL-C, atherogenic index (AI), lipoprotein combine index (LCI), and atherogenic index of plasma (AIP) were all significantly higher in cigarette and shisha smokers. Hematological parameters were found to be within normal reference range, however when compared with non-smokers, cigarette and shisha smokers has significantly higher values of complete blood cell count, except for lymphocytes and mean corpuscular hemoglobin concentration (MCHC) which were significantly lower. LDH was also found to be elevated in cigarette and shisha smokers.

**Conclusion:** The findings of this study shows that, both cigarette and shisha smokers have abnormal lipid profile suggesting dyslipidemia. Prediction of early atherosclerosis through the atherogenic indexes was observed to be significantly higher in shisha smokers than in cigarette smokers. Therefore, shisha smoking has more adverse effects on the health status of adult smokers. This study adds to the accumulating evidence on the harmful effects of shisha smoking, which is a growing epidemic among young smokers, and calls for the awareness of the possible consequences of developing early atherogenesis.

*Keywords: Atherogenic index; cigarette smoking; hematological parameters; lipid profile; shisha smoking.*

## 1. INTRODUCTION

Tobacco smoking is increasing exponentially. Approximately 1.1 billion people are estimated to consume tobacco, causing nearly 6.4 million deaths annually [1]. Projections indicate that if the current tobacco consumption trend persists, annual deaths will increase to 8 million people by 2030 [2]. The Tobacco Atlas report of 2018 reported that the majority of the world's current smokers developed the habit during their adolescence [3]. Thus, it is not surprising that the prevalence of cigarette and shisha (also known as waterpipe) smoking appears to be alarmingly high among university students in Arab countries for ages 15 to 37 years, with highest prevalence of tobacco smokers in Egypt (46.7%), Kuwait (46%) and Kingdom of Saudi Arabia (KSA) (42.3%) [4]. In Arab countries the prevalence of smoking among males is higher than in females. In KSA smoking was also observed to be more prevalent among males (32.7%) than females (5.9%) [4]. Recent studies from KSA have also reported alarmingly high prevalence of smoking among students at Majmaah University (30.4%) [5], King Saud University (27.8%) [6] and King Fahad Medical City (17.6%) [7].

Tobacco smoking either in the form of cigarette or shisha has been shown to increase concentrations of cholesterol and free fatty acid in the blood by increasing the levels of plasma total cholesterol (TC), triglycerides (TG), and low-density lipoprotein-cholesterol (LDL-C), including Apo-B, and decreasing the level of high-density lipoprotein-cholesterol (HDL-C) [8,9]. The

nicotine and other toxic substances from tobacco smoke is able to cause tissue oxidative damage at various levels and contributes significantly to the appearance of endothelial dysfunctions, which induces the arteriosclerotic process [8]. The underlying pathophysiology of atherosclerosis is attributed to endothelial injury, induced by oxidative stress which subsequently promotes atherogenesis via oxidation of LDL-C [8]. Various studies have also suggested that the continuous cigarette smoking has a serious negative impact on hematological parameters such as complete blood cell count, differential count, and endothelial dysfunction [10-12]. These effects may be linked to higher risks of developing early atherogenesis, chronic obstructive pulmonary disease, and/or cardiovascular diseases (CVDs) [12,13]. Moreover, non-communicable diseases are the major cause of mortality (73%) in Saudi Arabia, where 37% of deaths are accounted by CVDs. Among the risk factors for CVDs in Saudi Arabia, tobacco smoking contributes approximately 26% for male and 2% for female [14].

Generally, many young smokers consider shisha smoking as a safer alternative. However, Husain et al., suggested that shisha smoking is not safer than cigarette smoking [15]. Husain et al. also suggested that people who smoke both shisha and cigarettes experience worse health effects, including frequent respiratory infections, persistent cough, increased heart rate, and sleep disturbances. Moreover, the plasma levels of nicotine after one sitting of shisha smoking are equivalent to smoking two to three cigarettes

[16]. Shisha tobacco is also a key source of particulate matter that promotes oxidative stress and inflammation, indicating that smoking shisha is as harmful, if not more, as cigarettes [17,18]. Study conducted in Iran and Bangladesh reported that shisha smoking is associated with heart disease prevalence, and that the death due to ischemic heart disease was almost 2-fold higher in shisha smokers when compared to non-smokers [17].

Evidence have shown that compared to single lipid parameters, comprehensive lipid indexes (or atherogenic indexes) such as non-HDL-C, TC/HDL-C, TG/HDL-C, LDL-C/HDL-C, atherogenic index (AI), lipoprotein combine index (LCI), and atherogenic index of plasma (AIP) are considered as better predictors for CVDs [19,20]. Although many studies have reported the effect of cigarette smoking on lipid profile in Arab population, data on comparative analyses of comprehensive lipid indexes, lipid profile and hematological parameters of cigarette and shisha smokers in undergraduate students is scanty. Hence, this study aimed to evaluate the effects of cigarette and shisha smoking on the comprehensive lipid indexes, lipid profile, and hematological parameters of undergraduate smokers at Majmaah University. These findings will be useful in the development of necessary policies to control the prevalence of smoking and to understand the comparative impact of cigarette and shisha smoking on the atherogenic index and health status of young smokers.

## 2. MATERIALS AND METHODS

### 2.1 Study Design

The study was conducted at the Majmaah University College of Applied Medical Sciences. A total of 100 healthy male undergraduate students aged 18–30 years were enrolled, of which 35 were cigarette smokers, 30 were shisha smokers, and 35 were non-smokers. Informed consent was obtained from all participants who each declared that their participation was voluntary.

Participants were asked to provide the following information in consent form: demographic information (age and marital status), smoking status, duration and frequency of smoking, medical history including any medication and/or treatment they had received. Participants were further interviewed to corroborate the details about medical history in consent form. Inclusion

criteria was that the participants should be male students of Majmaah University, aged 18 to 30 years. The non-smokers were defined as participants who had never smoked. The cigarette smokers were defined as participants who regularly smoked 10–20 cigarettes per day for at least 1 year. Shisha smokers were defined as participants who regularly smoked 4–5 sessions of shisha per week for at least 1 year.

Subjects who reported to have any of the following medical conditions: diabetes and endocrine disorder, hypertension, renal disorder, CVD, and history of drug intake:  $\beta$ -blockers, lipid-lowering drugs, and steroids were excluded from the study to eliminate the effect of confounding variables. Subjects who drink alcohol and who underwent recent surgery were also excluded. Due to cultural reasons, smoking among women is extremely rare in this area; hence, women were not included in this study.

#### 2.1.1 Blood pressure and anthropometric measurements

Blood pressure and anthropometric measurements (weight and height) were taken on same day as when blood samples were taken by well-trained medical staff. Blood pressure was measured using a professional sphygmomanometer according to the Eight Joint National Committee guidelines [21]. Two blood pressure measurements were taken in a seated position at the upper arm at 1–2 min intervals. As per Eight Joint National Committee guidelines, the blood pressure values were classified as follows: normal (less than 120/80 mmHg), pre-hypertension (120-139/80-89 mmHg) and hypertension (greater than 140/90 mmHg) [21].

Weight and height measurements were taken using the Detecto® Physicians Scale. Body mass index (BMI) was calculated using the weight (in kilograms) divided by the square of the height (in meters) and classified according to the European Society of Cardiology and the European Atherosclerosis Society 2011 guidelines and WHO guidelines as follows: underweight (< 18.5), normal (18.5–24.9), overweight (25.0–29.9), and obese ( $\geq$  30.0) [22,23].

#### 2.1.2 Collection of blood

Participants were instructed to abstain from smoking for at least 12 hours before blood collection to minimize the acute effect of smoking on blood parameters. Blood samples were drawn from the antecubital vein after overnight fasting.

3 mL of whole blood were collected in EDTA tubes for hematological analysis. Another 4 mL were collected in plain red top tubes, incubated at room temperature for 30 minutes and centrifuged at 3000 g for 10 minutes. The separated serum samples were stored at -80°C for further analysis of lipid parameters and lactate dehydrogenase (LDH).

### 2.1.3 Estimation of lipid parameters

Serum TC, TG, and HDL-C were measured by enzymatic-colorimetric method using commercially available kits, Cholesterol liquicolor, Triglyceride liquicolor<sup>mono</sup> and HDL Cholesterol liquicolor (HUMAN Diagnostics Worldwide, Germany) respectively. All values were expressed in mmol/L.

The concentration of very low-density lipoprotein cholesterol (VLDL-C) was calculated as TG/2.2, and the concentration of LDL-C was determined using the Friedewald equation ( $LDL-C = TC - (HDL-C + TG/2.2)$ ) for participants with a TG < 4.52 mmol/L [24].

Atherogenic indexes were calculated as follows: AI = non-HDL-C/HDL-C, AIP =  $\log_{10}(TG/HDL-C)$ , and LCI =  $TC * TG * (LDL-C/HDL-C)$ , while other lipid indexes were expressed through simple ratios of lipid components as: non-HDL-C = TC minus HDL-C, TC/HDL-C, TG/HDL-C and LDL-C/HDL-C [19,20].

Plasma lipid abnormality or dyslipidemia was based on the expert panel of the National Cholesterol Education Program (NCEP) cutoff values of TC > 5.2 mmol/L, LDL > 3.4 mmol/L, HDL < 1.03 mmol/L, or TG > 1.7 mmol/L, or a combination thereof [25]. AIP value < 0.11 is associated with low risk of CVD; the values between 0.11-0.21 and upper than 0.21 are associated with intermediate and increased risks, respectively [19].

### 2.1.4 Estimation of hematological parameters and oxidative stress

Whole blood samples were analyzed for hematological parameters, total leucocyte count (TLC), polymorphs, lymphocytes, eosinophils, monocytes, total red blood cell (TRBC), platelet count, hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), using HA-22 CLINDIAG fully automated hematological

analyzer. Normal reference values for complete blood cell count parameters were based on study from Adeli et al [26].

To measure the oxidative stress, serum sample were analyzed for LDH using commercially available kit "LDH SCE mod. liquiUV" from HUMAN Diagnostics Worldwide (Germany).

## 2.2 Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 5.01 (GraphPad Software Inc., La Jolla, CA, USA). One-way ANOVA followed by Tukey's post hoc test was performed to detect any statistical difference. All data were expressed as mean  $\pm$  standard deviation, and P values < 0.05 was considered statistically significant.

## 3. RESULTS

### 3.1 Impact of Smoking on Blood Pressure and Anthropometric Measurements

The comparison of the baseline characteristics of all the participants are shown in Table 1. The mean age of non-smokers (22.9 year), cigarette smokers (23.2 years), and shisha smokers (23.1 years) was not significantly different between the groups.

The mean BMI of non-smokers, cigarette smokers, and shisha smokers was not significantly different. However, 20% of cigarette smokers and 20% of shisha smokers were found to be overweight compared to only 8.6% of non-smokers being overweight. The systolic/diastolic blood pressure and fasting blood glucose measurements in non-smokers, cigarette smokers, and shisha smokers was within the normal reference range (less than 120/80 mmHg for blood pressure and less than 99mg/dL for fasting blood glucose). Blood pressure measurements show that all the participants were normotensive.

### 3.2 Impact of Smoking on Lipid Profile

The lipid profiles of non-smokers, cigarette smokers, and shisha smokers are compared in Table 2. Compared to non-smokers, levels of TC, LDL-C, TG, and VLDL-C in cigarette smokers and shisha smokers were significantly higher. However, HDL-C levels in cigarette smokers and shisha smokers were significantly lower than non-smokers.

**Table 1. Baseline characteristics of non-smoker, cigarette smoker and shisha smoker participants**

	<b>Nonsmokers (n=35)</b>	<b>Cigarette smokers (n=35)</b>	<b>Shisha smokers (n=30)</b>
Age (years)	22.9 ± 3.3	23.2 ± 2.4	23.1 ± 2.2
Height (cm)	174.1 ± 3.3	173.4 ± 4.1	174.2 ± 4.2
Weight (Kg)	68.5 ± 5.9	68.9 ± 6.0	70.6 ± 6.7
BMI (Kg/m <sup>2</sup> )	22.6 ± 1.6	22.9 ± 2.2	23.3 ± 2.3
Systolic blood pressure (mmHg)	110.7 ± 6.1	108.2 ± 8.4	109.7 ± 7.5
Diastolic blood pressure (mmHg)	70.4 ± 4.2	69.9 ± 3.1	71.8 ± 5.1
Fasting Blood Glucose (mg/dL)	86.3 ± 4.9	85.7 ± 3.8	86.6 ± 5.6

BMI, body mass Index; Data are shown as mean ± standard deviation. The differences between groups were calculated using one-way ANOVA, followed by Tukey post-test. P value <0.05 was considered to be significant

**Table 2. Comparison of lipid parameters between non-smoker, cigarette smoker, and shisha smoker participants**

	<b>Nonsmokers (n=35)</b>	<b>Cigarette smokers (n=35)</b>	<b>Shisha smokers (n=30)</b>
<b>Lipid profile</b>			
TC (mmol/L)	4.27 ± 0.2	5.3 ± 0.5***	5.3 ± 0.3***
HDL-C (mmol/L)	1.3 ± 0.1	1.1 ± 0.2***	0.8 ± 0.1***†††
LDL-C (mmol/L)	2.36 ± 0.2	3.3 ± 0.5***	3.6 ± 0.3***††
TG (mmol/L)	1.38 ± 0.2	2.0 ± 0.1***	2.1 ± 0.1***
VLDL (mmol/L)	0.63 ± 0.1	0.9 ± 0.1***	0.9 ± 0.1***
<b>Comprehensive lipid indexes</b>			
Non-HDL-C	2.99 ± 0.18	4.2 ± 0.6***	4.5 ± 0.4***††
TC/HDL-C	3.35 ± 0.26	4.8 ± 1.2***	6.6 ± 1.0***†††
TG/HDL-C	1.07 ± 0.08	1.8 ± 0.4***	2.5 ± 0.5***†††
LDL-C/HDL-C	1.86 ± 0.27	3.0 ± 1.0***	4.4 ± 0.8***†††
AI	2.35 ± 0.26	3.82 ± 1.21***	5.59 ± 1.03***††††
AIP	0.03 ± 0.03	0.25 ± 0.08***	0.40 ± 0.08***††††
LCI	10.84 ± 1.53	32.85 ± 17.28***	48.82 ± 13.37***††††

TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglycerides; VLDL, very low-density lipoproteins; AI, atherogenic index; AIP, atherogenic index of plasma; LCI, lipoprotein combine index; Data are shown as mean ± standard deviation. The difference between groups were calculated using one-way ANOVA, followed by Tukey post-test. P value <0.05 was considered to be significant;

P-values: compared with non-smokers: \*\*\*P <0.001; compared to cigarette smokers: ††P <0.01, †††P <0.001

Furthermore, when compared to cigarette smokers, shisha smokers had significantly lower HDL-C levels, significantly higher LDL-C levels and elevated but not significant levels of TC, TG, and VLDL-C.

### 3.2.1 Impact of smoking on comprehensive lipid indexes

The comprehensive lipid indexes, such as non-HDL-C, TC/HDL-C, TG/HDL-C, and LDL-C/HDL-C, in both cigarette smokers and shisha smokers were significantly higher than those in non-smokers (Table 2). Moreover, non-HDL-C, TC/HDL-C, TG/HDL-C, and LDL-C/HDL-C values in shisha smokers were also significantly higher than those in cigarette smokers.

Comprehensive lipid indexes AI, AIP, and LCI were also found to be significantly higher in both cigarette smokers and shisha smokers in comparison with those of non-smokers. Compared with non-smokers, cigarette smokers showed a significant increase of 1.62-fold in AI, 8.18-fold in AIP, and 3.03-fold in LCI, whereas shisha smokers also showed a significant increase of 2.38-fold in AI, 13.25-fold in AIP, and 4.51-fold in LCI. Moreover, the AI, AIP, and LCI values were also significantly higher among shisha smokers compared with those of cigarette smokers.

Overall, both cigarette smokers and shisha smokers had a proatherogenic profile (higher TC,

TG, non-HDL-C, AI, AIP, and LCI values and a lower HDL-C value) with later exacerbation.

### 3.2.2 Impact of smoking hematological parameters and oxidative stress

Oxidative stress in the form of lactate dehydrogenase (LDH) increased in both cigarette smokers (2.17-fold) and shisha smokers (1.98-fold) compared with that in non-smokers as shown in Table 3. The LDH value in the shisha smokers was also significantly lower than that in cigarette smokers.

All hematological parameters in non-smokers, cigarette smokers, and shisha smokers were found to be within the normal reference range. Among the analyzed hematological parameters, levels of TLC, eosinophils, monocytes, platelets, Hb, HCT, MCV, and MCH in both cigarette smokers and shisha smokers were significantly higher than in non-smokers. Polymorphs also showed a significant increase in cigarette smokers but a non-significant increase in shisha smokers. In contrast, levels of lymphocytes and MCHC significantly decreased in cigarette smokers and shisha smokers.

## 4. DISCUSSION

### 4.1 Impact of Smoking on Blood Pressure and Anthropometric Measurements

Shisha smoking is increasingly popular especially among adolescents, mainly because of the myth that shisha smoking causes little, or no harm compared to that of cigarette smoking [27]. Therefore, we planned to study the impact of shisha and cigarette smoking on the lipid and hematological parameters on university undergraduate students aged 18–30 years. As reported in Table 1, we found that the mean blood pressure and mean BMI of the participants who smoked shisha or cigarettes was not significantly different to those of the non-smoking participants. Similar results were reported by Alomari et al. [28], showing no significant difference in blood pressure between the smoking groups (cigarette, shisha, and those who smoked both). Al-Numair et al. [29] has also reported that there was no significant difference between the BMI of shisha smokers and non-smokers. Al-Ajlan conducted a study on healthy college students between the ages of 18 and 35

**Table 3. Comparison of oxidative stress and hematological parameters between non-smoker, cigarette smoker and shisha smoker participants**

	<b>Nonsmokers (n=35)</b>	<b>Cigarette smokers (n=35)</b>	<b>Shisha smokers (n=30)</b>
LDH (IU/L)	77.5 ± 12.4	168.03 ± 22.4***	153.2 ± 17.9***††
Hematological profile			
TLC (10 <sup>9</sup> /L)	7.4 ± 1.3	8.6 ± 1.1**	8.4 ± 1.9*
Polymorphs (%)	52.4 ± 4.4	56.9 ± 5.3**	54.8 ± 7.7
Lymphocytes (%)	42.2 ± 4.4	36.1 ± 6.5***	37.3 ± 7.8**
Eosinophils (%)	2.5 ± 0.7	3.5 ± 1.2***	4.2 ± 0.7***††
Monocytes (%)	2.9 ± 0.6	3.4 ± 0.7**	3.7 ± 0.7***
TRBC (10 <sup>12</sup> /L)	4.95 ± 0.4	5.3 ± 0.5**	4.9 ± 0.4††
Platelets (10 <sup>9</sup> /L)	305.3 ± 50.3	367.5 ± 24.2***	356.5 ± 28.8***
Hb (g/dL)	15.3 ± 1.3	16.4 ± 0.6***	16.1 ± 0.8**
HCT (%)	44.6 ± 3.6	51.5 ± 2.7***	49.7 ± 2.6***
MCV (fL)	88.2 ± 5.8	95.1 ± 5.4***	93.0 ± 5.2**
MCH (pg)	29.5 ± 1.9	32.1 ± 1.6***	31.2 ± 1.7**
MCHC (g/dL)	34.3 ± 0.9	31.8 ± 1.5***	32.4 ± 0.9**

LDH, lactate dehydrogenase; TLC, total leucocyte count; TRBC, total red blood cells; Hb, Hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; Data are shown as mean ± standard deviation. The difference between groups were calculated using one-way ANOVA, followed by Tukey post-test. P value <0.05 was considered to be significant;

P-values: compared with non-smokers: \*P <0.05, \*\*P <0.01, \*\*\*P <0.001; compared to cigarette smokers:

††P <0.01

years in Riyadh, also reported a lack of significant difference between smokers and non-smokers for blood pressure and BMI [30]. Possible reason for such results may be because these studies were conducted on healthy individuals of similar age groups. Additionally, the students were in their early years of smoking, whereas the negative effects mainly emerge after long-term use.

#### 4.2 Impact of Smoking on Lipid Profiles

Several studies have reported that shisha and/or cigarette smoking have substantial negative effects on the lipid profiles of healthy individuals and can lead to cardiovascular diseases [9,10,17]. Our study (Table 2) revealed significantly higher levels of TC, TG, LDL-C, and VLDL-C in cigarette smokers and shisha smokers when compared with those of non-smokers. However, significantly lower levels of HDL-C were found in cigarette smokers and shisha smokers than in non-smokers. We also observed that the shisha smokers had significantly lower HDL-C and significantly higher LDL-C levels compared to cigarette smokers. TC, TG and VLDL-C level were not significantly different between cigarette and shisha smokers. Overall, our finding revealed the abnormal lipid profile or dyslipidemia in cigarette and shisha smokers.

Al-Numair et al. reported similar findings where he demonstrated that shisha smokers have significantly higher level of LDL-C ( $3.61 \pm 0.69$  mmol/L), TG ( $1.84 \pm 0.22$  mmol/L) and decline in HDL-C level ( $1.05 \pm 0.09$  mmol/L) in comparison with non-smokers ( $3.06 \pm 0.61$ ,  $1.56 \pm 0.19$  and  $1.17 \pm 0.10$  mmol/L) [29]. Study conducted by Al-Ajlan on university students also reported the significant increase in TG, but non-significant variations for other lipid parameters among smokers than non-smokers which were contradict to our results [30]. Previous study on college students has also reported significantly higher levels of TC, TG, LDL-C, VLDL-C and significantly lower level of HDL-C in smokers ( $197.76+19.37$ ,  $166.84+27.00$ ,  $127.13+20.68$ ,  $34.25+4.42$  and,  $35.91+4.51$  mg/dl, respectively) compared to non-smokers ( $155.38+24.09$ ,  $121.2+32.7$ ,  $84.08+24.42$ ,  $24.24+6.54$  and,  $46.9+6.71$ , respectively) [31]. Certain subspecies of HDL-C are known to act as natural antioxidants, preventing the oxidation of lipids and biological membranes [32]. Therefore, the decrease in HDL-C among the cigarette and shisha smokers may be due to high levels of

oxidative damage, which reduces these antioxidant levels [17]. Smoking also increases the levels of nicotine and carbon monoxide in blood plasma, resulting in increased amounts of TG and non-HDL-C [8,16,17].

#### 4.2.1 Impact of smoking on comprehensive lipid indexes

Comprehensive lipid indexes were measured as they are believed to be better predictor of CVD compared to the single lipid parameter [19,20]. Our analysis of comprehensive lipid indexes revealed that non-HDL-C, TC/HDL-C, TG/HDL-C, LDL-C/HDL-C, AI, AIP, and LCI in cigarette smokers and shisha smokers were significantly higher than those in non-smokers (Table 2). Moreover, these indices in shisha smokers were even significantly higher than those in cigarette smokers. These higher lipid index values suggest that the risk of developing dyslipidemia is higher in smokers.

Among all the lipid indexes, AIP has been reported as the strongest marker to forecast the risk of atherosclerosis and CVDs, which reflects the true relationship between protective and atherogenic lipoprotein [19,20]. AIP value  $< 0.11$  is associated with low risk of CVD; the values between  $0.11-0.21$  and upper than  $0.21$  are associated with intermediate and increased risks, respectively [19]. Our results show that cigarette smokers had an AIP value of  $0.25$ , indicating they had a higher risk of developing CVDs compared with non-smokers with much a lower AIP value of  $0.03$ . Interestingly, the AIP value in shisha smokers increased by  $60\%$  compared to that of cigarette smokers, suggesting that the chances of developing atherosclerosis-mediated CVDs are higher in shisha smokers than in cigarette smokers.

#### 4.2.2 Impact of smoking on oxidative stress and hematological parameters

LDH is an intracellular cytoplasmic enzyme that acts as a biomarker for oxidative stress and cell damage and is used as a diagnostic tool for various illnesses. The results of our findings (Table 3) have shown significantly higher levels of LDH in cigarette smokers (2.16-fold) and shisha smokers (1.97-fold) than those in non-smokers ( $77.51 \pm 12.44$  IU/L), indicating increased levels of oxidative stress. Our findings are in line with previous mentioned reports [33,34] suggesting that LDH was significantly increased in smokers. Rezaq et al. also reported

significant increase in the level of LDH in shisha smokers ( $138.34 \pm 2.7$  U/L) compared to control group ( $92.2 \pm 1.8$  U/L) [34]. High levels of LDH indicate disease progression, resulting in cell death; this is mainly due to an increase in oxidative stress, which is regarded as an early predictor of tissue damage and has been associated with acute myocardial infarction a critical cardiovascular event which can proceed to atherosclerosis [35]. Tobacco smokers inhale high levels of free radicals that alter the antioxidant defenses of healthy tissue/cell like RBC or pulmonary tissues and lead to increased amounts of LDH enzyme [36].

The results of our study showed that both shisha and cigarette smoking have adverse effects on hematological parameters. In our study (Table 3), the values of TLC, eosinophils, monocytes, platelets, Hb, HCT, MCV, and MCH were significantly higher for the smoking groups, whereas the MCHC and lymphocyte values were significantly lower in both cigarette smokers and shisha smokers than those in non-smokers.

The significant increase in Hb in smokers is correlated with previous studies [10,13]. An increase in hemoglobin concentration is believed to be mediated by exposure to carbon monoxide, and that the increase in Hb in smokers is a compensatory mechanism. Tobacco smoking increases levels of carbon monoxide, which binds to hemoglobin to form carboxyhemoglobin, an inactive form of hemoglobin with no oxygen carrying capacity. To compensate for the decreased oxygen carrying capacity, smokers maintain a higher level of Hb and hematocrit than non-smokers and may increase the risk of cardiovascular and cerebrovascular events [16,17,18,37]

A significant increase in the RBC count and hematocrit of smokers has also been reported in an earlier study [31]. Increased RBC and HCT in smokers can be explained by the fact that increased carboxyhemoglobin causes tissue hypoxia, which leads to increased secretion of erythropoietin, thus increasing erythropoiesis. Additionally, carbon monoxide from smoke increases the permeability of capillaries, which in turn decreases the level of plasma, leading to polycythemia, thus increasing the RBC and HCT values in blood.

Our study showed significantly higher levels of MCV and MCH and significantly lower MCHC levels in cigarette smokers and shisha smokers

compared with those of non-smokers. Higher values of MCV and MCH in smokers were also confirmed by Khan et al. [11] and Kung et al. [38]. Pankaj et al. [39] did not find any significant changes in MCV and MCH levels, which is contrary to our findings. However, the same study reported significantly lower MCHC values among smokers, as shown in our study. Our study also showed that lymphocytes were significantly lower in both cigarette smokers and shisha smokers than in non-smokers, as reported by other studies [40,41]

Furthermore, our study showed a significantly higher value of TLC in cigarette and shisha smokers compared with that in non-smokers. The increase in TLC is in line with earlier studies [31]. The exact mechanism of how smoking increases TLC is not fully understood; however, nicotine-induced release of catecholamine and steroid hormones from adrenal glands have been shown to increase the level of leucocytes [42].

The atherogenic effect of smoking is assumed to be partially mediated by leucocytes and that this may be a useful biomarker of endothelium damage. Several studies have confirmed that the number of leucocytes is a predictor of atherosclerosis and CVD [43,44]. A higher number of leucocytes in the cigarette and shisha smokers in our study suggests that they were at higher risk of atherosclerosis and CVD in relation to non-smokers. There are various natural products and nanoparticles synthesized from them having great antioxidant potential, which might reverse early atherogenesis and smoking induced cardiovascular diseases [45-53].

Our study has some limitations. This study was cross-sectional, thus preventing the assertion of a causal relationship between smoking and subsequent disease. The data were sampled from only one university, so there was a possibility of selection bias and some limitation in generalization of results. This study was limited by the relatively small sample size and confined to males and specific age group belonging to same geographical area. The frequency/duration of smoking is subjective and could not be measured, which can lead to an information bias. The main limitation of this study is that important factors which may contribute to the cardiovascular risk factors among youth such as dietary habits, physical activity and genetics were not included.



## 5. CONCLUSION

Dyslipidemia, oxidative stress, and adverse hematological parameters due to cigarette or shisha smoking lead to early occurrence and progression of atherosclerosis. We conclude that tobacco smoking, whether in cigarette or shisha forms, has severe adverse effects on lipid profiles, oxidative stress, and hematological parameters; however, shisha smoking has a more adverse impact on human health, especially those of university undergraduates. Although young smokers, especially shisha smokers, may have good health status with no signs of disease, they are also prone to developing early atherogenesis. While there are many policies and awareness programs conducted by the government to increase awareness of the negative health impacts of cigarette smoking, very few address the health impacts of shisha smoking. We recommend conducting a similar study on a larger scale to further investigate the biochemical changes at the molecular level and better understand the pathophysiological differences between cigarette and shisha smoking on health.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## CONSENT

"All authors declare that 'written informed consent was obtained from the participants for publication of this study and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal."

## ETHICAL APPROVAL

Ethical approval was obtained from the Majmaah University Research Ethical Committee prior to the study (Approval No.: MUREC- Apr.23/COM-2019/26).

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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