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## Hematological and biochemical changes associated with water-pipe (Shisha) smoking for some volunteers in Missan province

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### Abstract

**Aim of the study:** Clinical and experimental studies detected that waterpipe smoking is more harmful than a cigarette with can induce oxidative stress and inflammation. The current study was performed to investigate the effect of water pipe smoking on hematological parameters and evaluation the biochemical parameters including a lipid profile, live function enzymes, alkaline phosphatase, total protein, creatinine, blood urea nitrogen, and blood glucose.

**Method:** The study was performed on (150) volunteers who agreed to participate in this study divided into water pipe smokers and nonsmokers aged between (20-60) years. Five (5ml) of venous blood samples were collected, each blood sample was separated into two tubes, the first tube with EDTA for hematological assessment and the second was centrifugation and the serum was stored in a -20°C freezer till handled for biochemical analysis for determining lipid profile, liver function enzymes, alkaline phosphatase, creatinine, blood urea nitrogen and blood glucose. Complete blood picture for collecting blood samples was performed by automatic methods (System X kx-21n automated hematology analyzer; JAPAN CARE CO., LTD) including hemoglobin (Hb), white blood cells (WBCs), red blood cells (RBCs), Platelets and Haematocrit or packed cell volume (PCV). Biochemical tests and lipid profile analysis were performed in laboratories of Al-Sadder Teaching Hospital in Amarha City according to the standard methods described in the Analysis Kits used in this study were products of Spanish Company Spinreact.

**The results:** The results observed that the water pipe smokers were in ages between 31-40 years with a percentage of 43%, followed by the aged 20-30 years with a percentage of 25%. Hematological analysis for the blood samples collected from water pipe smokers and non-smoking (control) observed a significant increase in RBCs, WBCs, HCT, Hb, and Plt in water pipe smokers as compared with the non-smoker group ( $p < 0.05$ ). Lipid profile values observed a significant increase in the total cholesterol levels, (LDL), vLDL) and Triglyceride with a significant decrease in HDL ( $P > 0.05$ ) in water pipe smokers as compared with the non-smoker group. Significant increase in the levels of AST, ALT, and alkaline phosphatase enzyme, also the creatinine, blood urea nitrogen, and blood glucose observed a significant increase in ( $P > 0.05$ ) as compared with non-smokers. On other hand, there was a significant decrease in total proteins in water pipe smokers.

**Conclusions:** Water pipe smoking caused abnormal changes in complete blood picture (CBC) and serum lipids such as the total cholesterol and Triglyceride levels. Also harmed the liver functions and kidney functions.

**Keywords:** Water pipe smokers, hematological, biochemical parameters, liver functions, kidney functions tests.

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## Introduction

Water pipe smoking (WPS), also known as the *hookah* (Arab and Britain), *shisha* (German), *narghile* (Armenia and Bulgaria), *hubble-bubble* (India), *goza* (Egypt), *chichi* (France), *qalyan* (Persia), *madaa* (Yemen) and *gaya* (Ethiopia), is a form of tobacco use engaged in by men and women in the Middle East, Africa, and Asia. Since the early 1990s, there has been a significant increase in its use around the world and it is estimated that there are more than 100 million peoples worldwide who smoke water pipe daily (Maziak *et al.*, 2018).

Despite the common perception that water pipe is less harmful than cigarettes because the smoke is purified as it passes through the water, the medical assessments of the health impacts of water pipe smoking are still in an early form, the literature to date agrees in considering waterpipe smoking to be at least as dangerous as cigarette smoking (Jawad *et al.*, 2018).

Tobacco smoke contains more than 4000 compounds that harm human health, such as free radicals, nicotine, and carbon monoxide responsible for many health problems as nicotine defeats the formation of a clot in the coronary arteries and weakens the activity of blood vessels and increases the imbalance of functions and alters vascular permeability and lipid accumulation (El-Nachef & Hammond, 2008).

The free radicals and peroxides resulting from hookah smoke with the physiological phenomenon such as the manufacture of prostaglandin and thromoxan associated causing various diseases, including atherosclerosis cancers, gastroenteritis, gum disease, and some autoimmune diseases, and a decrease in the activity of the lipoprotein enzyme, which causes a state of hyperinsulinemia, and thus an increase in the proportion of both cholesterol and triglycerides and decrease in the level of HDL (Salloum *et al.*, 2016).

Tobacco smoking in general is associated with many dangerous chemicals and toxins, not the least of which are carbon monoxide (CO) and nicotine.

(Knishkowsky *et al.*, 2005) Cigarette and waterpipe smoking, therefore, share the same toxins and waterpipe smokers are likely to be exposed to higher levels because the average length of time spent smoking is longer than for cigarettes (Eissenberg *et al.*, 2009).

Waterpipe sessions are commonly 30 - 60 minutes and involve roughly 100 inhalations of 500ml of smoke each (Shihadeh *et al.*, 2005), resulting in 50 000ml of smoke inhalations in total compared to 500ml – 600ml per cigarette (Shihadeh *et al.*, 2005).

The study was conducted in by Shihadeh *et al.* (2003. specifically looked at the chemical composition of waterpipe smoke and found that while nicotine levels in a single waterpipe session were like those of a single cigarette, the tar content was almost 20 times greater than that of a low tar cigarette. In addition, they found higher levels of arsenic, chromium, and lead and higher levels of carboxyhemoglobin in the blood than with cigarette smoking.

The research studies also indicate that water pipe has led to an increase in the risk of infectious diseases, cardiovascular disease, pulmonary illness, cancers, and low fetal birth weight in pregnant women. The hematologic index alterations are used as physiological markers of organ and tissue damage. Therefore, the various pharmacological actions of nicotine and other materials led to a change in the status of hematologic and hemostatic parameters (Knishkowsky and Amitai, 2005).

Few studies dealt with the effect of water pipe smoking on hematological parameters in both humans and animals. This study aimed to investigate the effect of WP smoking on hematological parameters such as red blood cells (RBCs), hemoglobin (Hb), hematocrit (Hct), white blood cells (WBCs), and platelet counts in Wistar rats between four and twelve weeks of exposure.

This study aimed to investigate the effect of water pipe smoking on hematological parameters such as red blood cells (RBCs), hemoglobin (Hb), hematocrit (Hct), white blood cells (WBCs), and

platelet counts, also evaluate the biochemical parameters include lipid profile including total cholesterol, LDL-ch, HDL-ch, Triglyceride and VLDL-ch, live function enzymes, alkaline phosphatase, total protein, creatinine, blood urea nitrogen and blood glucose.

**Material and methods**

The study was performed in the period between (July - 2019 and first February -2021) and conducted on (150) volunteers who agreed to participate in this study divided into water pipe smokers and nonsmokers aged between (20-60) years with an average age (of 40) year. Five (5ml) of venous blood samples were collected from each volunteer, each sample of the blood was separated into two tubes. The first tube with EDTA for hematological assessment and the second blood tube was centrifugation for 10 minutes at 3000rpm, the serum was stored in a -20°C freezer till handled for biochemical analysis for determining lipid profile, live function enzymes, alkaline phosphatase, creatinine, blood urea nitrogen and blood glucose. Complete blood picture was shown from the collected blood samples by automatic methods

(System X kx-21n automated hematology analyzer; JAPAN CARE CO., LTD) including hemoglobin (Hb), white blood cells (WBCs), red blood cells (RBCs), Platelets and Haematocrit or packed cell volume (PCV).

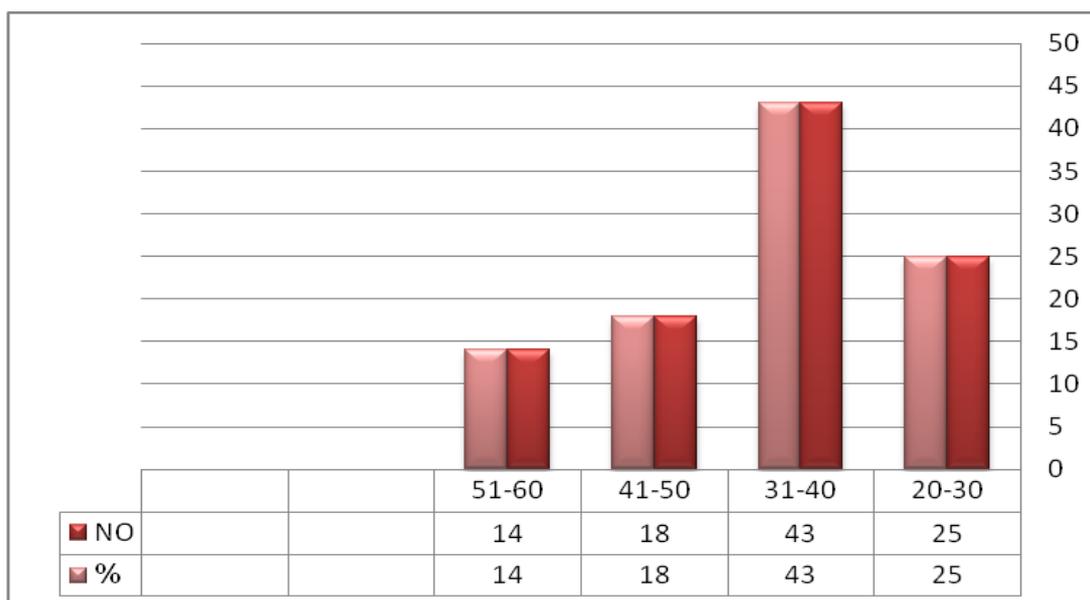
Biochemical tests and lipid profile analysis for all these parameters were performed in laboratories of Al-Sadder Teaching Hospital – Amara City according to the standard methods described in the Analysis Kits used in this study were products of Spanish Company Spinreact.

**3.2: Statistical analysis.**

The results were expressed as mean ± standard error (SE). Statistical analyses were made with a one-way analysis of variance (ANOVA) using SPSS 17. The criterion for statistical significance was ( $P < 0.05$ ).

**The results**

The results of this study observed that the water pipe smokers were in ages between 31-40 years with a percentage of 43%, followed by the aged 20-30 years with a percentage of 25%, fig (1)



**Fig (1) show the ages of volunteers that use water pipe smoking (NO: 100).**

The results of hematological analysis for the blood samples collected from water pipe smokers and nonsmoking (control) observed a significant increase in red blood cells (RBCs) in the water pipe smokers group as compared with the nonsmoker group ( $p < 0.05$ ), also observed a significant increase in white blood cells (WBCs), hematocrit (HCT), hemoglobin (Hb), Platelets (Plt), neutrophils and lymphocytes percentages in water pipe smokers as compared with the control group ( $p < 0.05$ ), Tab(1) and Fig(2).

Lipid profile values were detected in blood samples obtained from water pipe smokers which include total cholesterol, LDL-ch, HDL-ch, Triglyceride, and vLDL-ch were observed significant increase in total cholesterol, Low-density

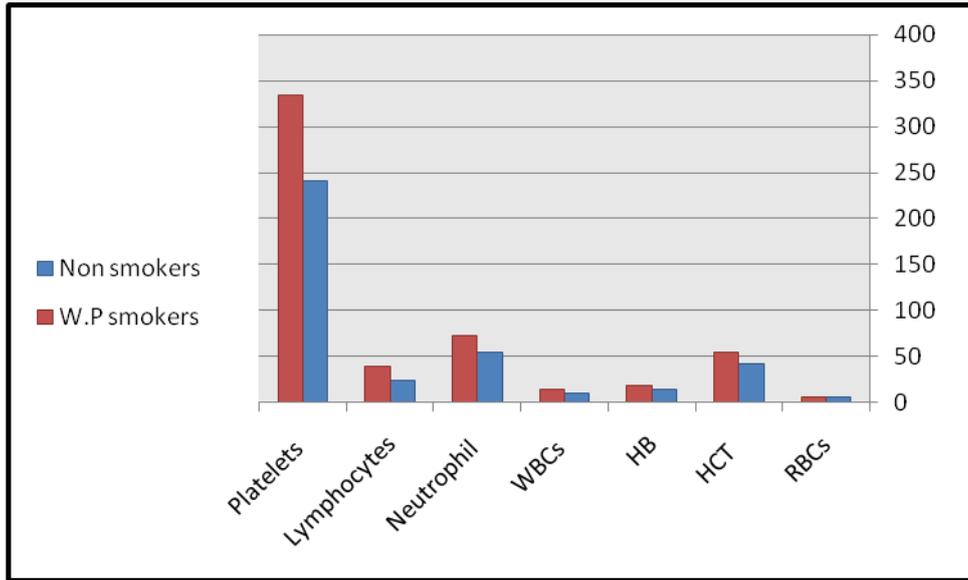
lipoprotein (LDL), very Low-density lipoprotein (vLDL) and Triglyceride, with significant decrease HDL-ch in ( $P > 0.05$ ) as compared with nonsmoker group, Table (2) and Fig (3).

The biochemical analysis of the blood serum obtained from volunteer's water pipe smokers observed a significant increase in levels of Aspartate aminotransferase (AST), Alanin aminotransferase (ALT), and alkaline phosphatase enzyme in ( $P > 0.05$ ) as compared with non-smokers. Creatinine, blood urea nitrogen, and blood glucose also observed a significant increase in ( $P > 0.05$ ) as compared with non-smokers. On other hand, there was a significant decrease in total proteins, Table (3) and Fig (4) and Fig (5).

**Table-1: Comparison of some of the hematological parameters of water pipe smokers and nonsmokers. (N:150 volunteers).**

| Parameters                                         | Groups           |                  |
|----------------------------------------------------|------------------|------------------|
|                                                    | Nonsmokers       | *W. P smokers    |
| RBCs (100000 cells/ $\mu$ l)                       | 5.13 $\pm$ 0.35  | 6.22 $\pm$ 0.29  |
| HCT (%) )                                          | 41.29 $\pm$ 1.93 | 53.83 $\pm$ 1.65 |
| HB (g/dl)                                          | 13.8 $\pm$ 0.55  | 17.6 $\pm$ 0.49  |
| WBCs (*10 <sup>3</sup> Cells/mm <sup>3</sup> )     | 10.2 $\pm$ 0.75  | 14.4 $\pm$ 0.56  |
| Neutrophil %                                       | 54.14 $\pm$ 3.74 | 72.45 $\pm$ 3.84 |
| Lymphocytes%                                       | 24.2 $\pm$ 4.13  | 39.4 $\pm$ 4.69  |
| Platelets (Plt *10 <sup>3</sup> /mm <sup>3</sup> ) | 240.6 $\pm$ 14.2 | 334.5 $\pm$ 22.4 |

\* W. P=Water pipe, WBC=White blood cells, LYM=Lymphocytes, NEU=Neutrophils, RBC=Red blood cells, HGB=Hemoglobin, HCT=Hematocrit, PLT=Platelets. Values are expressed as means  $\pm$  SD; n=150.



**Fig(2):** Show the changes in hematological parameters for water pipe smokers and nonsmokers. (N:150 volunteers).

**Table-2:** Show lipid profile parameters for water pipe smokers and nonsmokers (N:150 volunteers).

| Parameters                     | Groups       |               |
|--------------------------------|--------------|---------------|
|                                | Nonsmokers   | *W. P smokers |
| <b>T.Cholesterol (mg/dl)</b>   | 141.39±41.8  | 208.89±80.8   |
| <b>HDL-cholesterol(mg/dl)</b>  | 53.85±14.54  | 33.98±80.8    |
| <b>LDL-cholesterol(mg/dl)</b>  | 130.19±28.4  | 172.67±42.3   |
| <b>Triglycerides (mg/dl)</b>   | 143.76±32.23 | 285±103.16    |
| <b>VLDL-cholesterol(mg/dl)</b> | 39.75±35.5   | 87.63±55.8    |

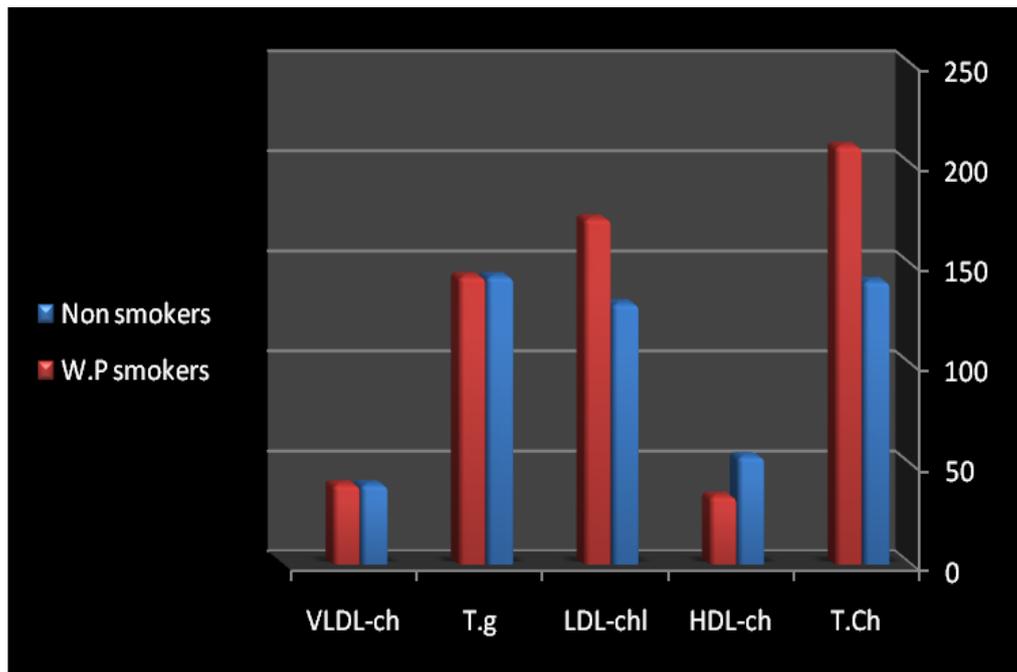


Figure (3): Observed lipid profile for the water pipe smoker and nonsmokers (N:150 volunteers).

Table-3: Show serum biochemical parameters of water pipe smokers and non-smokers (N:150 volunteers). ALP=Alkaline phosphatase, AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, B.Glucose=Blood glucose.

| Parameters                            | Groups      |               |
|---------------------------------------|-------------|---------------|
|                                       | Non-smokers | *W. P smokers |
| Alkaline phosphatase (IU/l)           | 118.7 ±7.51 | 144.8±3.98    |
| T.protein (gm/dl)                     | 7.87±1.15   | 5.37±0.35     |
| Creatinine (mg/dl)                    | 1.1±0.12    | 1.85±0.05     |
| Aspartate aminotransferase AST (IU/l) | 239±37.8    | 370 ±94       |
| Alanin anino tranferase ALT (IU/l)    | 234 ± 54    | 293 ± 45      |
| Blood urea nitrogen (mg/dl)           | 13.9 ±1.85  | 14.7±3.55     |
| B. Glucose (mg/dl)                    | 95.8 ± 5.3  | 135.3±8.2     |

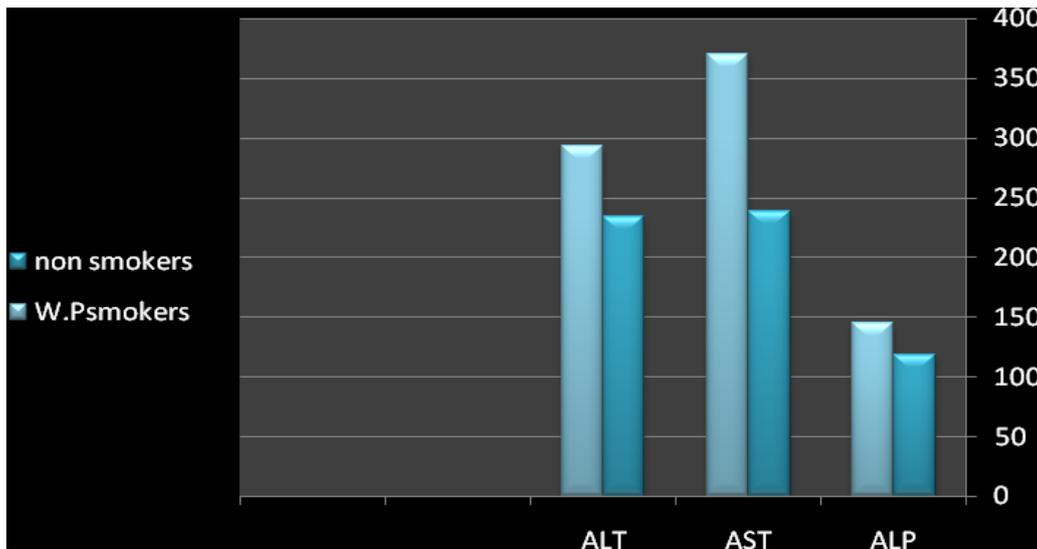


Figure (4): Observed the changes of some serum biochemical parameters for water pipe smokers and non-smokers (N:150 volunteers), ALP=Alkaline phosphatase, AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, B.Glucose=Blood glucose.

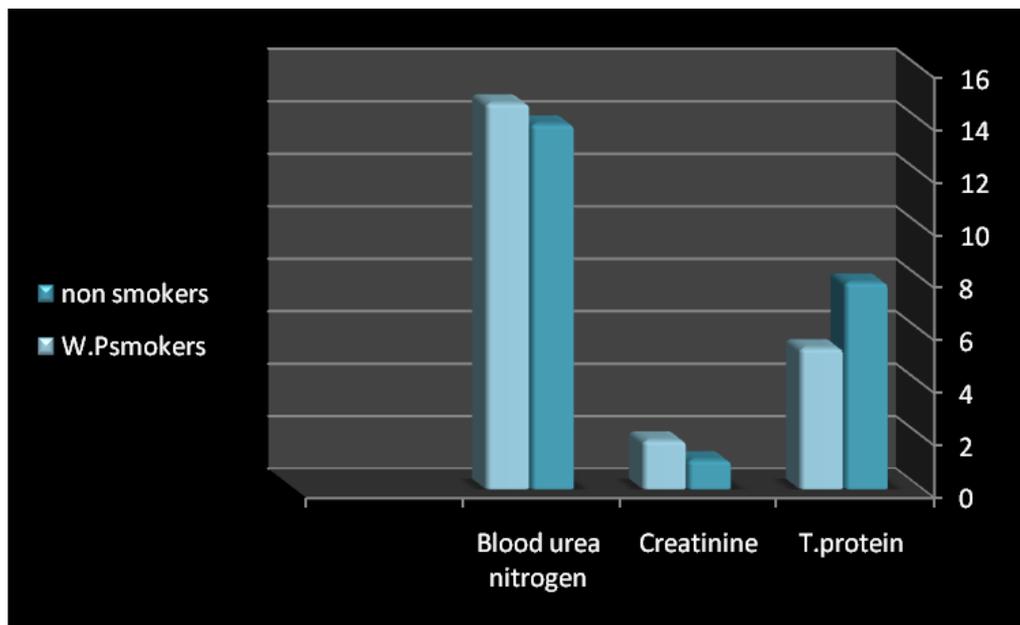


Figure (4): Observed the changes of some serum biochemical parameters for water pipe smokers and non-smokers (N:150 volunteers), T.protein= Total protein.

**Discussion**

Water pipe smoking was increased dramatically during the decade due to improved taste and social acceptability as a safer option than cigarette smoking, but recent clinical and experimental studies detected that waterpipe smoking more harmful than a cigarette with can induce oxidative stress and inflammation (Badran and Laher,2020).

The results of this study observed that most water pipe smokers the aged between (31-40) with a percentage of 43% followed by those aged (20-30) years with a percentage of 25%.

Maziak *et al* (2015) reported that the prevalence of water pipe smoking is more likely at the early age of 4.9 times between (25-40) years as compared with 45 years or older.

Hematological analysis of blood samples collected from water pipe smokers observed a significant increase in the red blood cells (RBCs) ( $P < 0.05$ ) as compared with non-smokers. Also, the result showed a significant increase in hemoglobin concentration (Hb), hematocrit (HCT), white blood cells (WBCs), and platelets count in water pipe smokers as compared to nonsmokers.

Water pipe smoking can increase the risk of cardiovascular diseases and increase heart rates and blood pressure which may cause death due to chronic heart disease two folds higher than compared non-smokers (Islami *et al*, 2013; FDA, 2020).

Moghaddam and Reza arab (2014). were found that waterpipe smoking caused an increase in RBCs count and elevated the levels of Hb and HCT which correlated with an increase in the numbers or size of RBCs.

Hakim *et al* (2011) reported that an increase in carboxyhemoglobin formed from a combination of carbon monoxide with hemoglobin leads to reduces the transfer of oxygen to tissues. Tobacco contains nicotine which leads to peroxidation of the erythrocytes membranes and disrupts oxygen delivery to the tissues and stimulates produce more RBCs with an increase in Hb and HCT.

Aghaji *et al* (1990) found elevation of the platelets in water pipe smokers as compared with non-smokers, while Hussain *et al* (2021) found no significant differences between the platelets in smokers and non-smokers. Moghaddam and Reza Arab (2014) reported an increase in WBCs in water pipe smokers and found about 20-25% higher than non-smokers.

Chang *et al* (2010) showed an increase in leukocytes which might be due to nicotine which induce the release of catecholamine releasing an increase in blood lymphocytes, also added that smoking can cause irritant to the respiratory tract with resulting inflammation which leads to an increase in leukocytes. Water pipe smoking can produce much

more tar (100-fold), nicotine (10-fold), CO(3fold), and polycyclic (Shihad and Saleh, 2005).

El-Zaatari *et al* (2016) observed a significant increase in WBCs, Hb, and PCV with nonsignificant changes in the RBCs in 30 people smoking hookah as compared with people without using any kind of smoking.

The results of this study observed a significant increase in packed cell volume (PCV) in water pipe smokers. Nakkash and Khalid (2010) reported there are significant increases in Hb concentration and PCV value in people smoking shisha may be due to an increase in the volume or mass of red blood cells and reduction in the binding of hemoglobin to oxygen and an increasing association with carbon monoxide (CO) with increase saturation of Hb molecules with CO (Hb-CO) which led to poisoning the blood.

The results of the current study observed a significant increase in total cholesterol, low-density lipoprotein (LDL), very low-density lipoprotein (vLDL), and triglyceride, with a significant decrease in high-density lipoprotein (HDL).

Chaonachi and Sajid (2010) reported that the danger of water pipe and cigarette smoking can promote free radicals' production which interacts with biological molecules to cause elevation of oxidative stress by increasing lipid peroxidation, also added the elevation of free radicals can damage the heart muscles and blood vessels and may react with cholesterol leading to build up of fatty materials on the wall of arteries.

The study was performed by Chweed (2018) on 75 males in Nasirriya city to investigate the effects of shisha smoking on the serum lipid profile and found highly significant changes in lipid profile (Triglyceride, total cholesterol, LDL-ch, and v LDL) were found to harmful effect on the healthy lifestyle. Al-Fayz *et al* (1988) found that shisha smoking can produce the same effect as cigarette smoking on the lipid profile which considers predisposing factors for developing coronary heart disease due to stimulation of the adrenal sympathetic system by

nicotine levels which stimulates the release of catecholamine and induce lipolysis of adipose tissues and lead to increase the levels of serum free fatty acids and impairment of lipoprotein metabolism. Zaid and Salloum *et al* (2016) found a significant decrease in levels of HDL-ch in tobacco smokers as compared with nonsmokers.

The result of this study also observed a significant increase in AST and ALT in water pipe smokers as compared with nonsmokers, on the other hand, increase in alkaline phosphatase in water pipe smokers. Ababneh *et al* (2018) reported that the smoking model can induce liver injury by increasing levels of AST and ALT in water pipe and cigarette smoking, where the smoking can cause oxidative damage in the hepatocytes and alteration in the liver enzymes, therefore cessation can reverse injury of the liver and restored liver enzymes to the normal level. Jacob *et al* (20117) found a significant increase in the levels of glutamic –oxalo acetic transaminase (GOT) and glutamate pyruvate transaminase (GPT) in heavy cigarette smoking. Zahraa (2021) showed an increase in the level of alkaline phosphatase in the blood of cigarette smoking which may be associated with some extrahepatic mechanisms, and high cigarette consumption correlated with low total protein and albumin levels.

The results also observed a significant increase in serum creatinine, urea and blood glucose in water pipe smokers as compared with non-smokers. renal function tests are important in determining renal dysfunction and renal failure and monitor response to the treatment. The concentration of creatinine and blood urea may be altered in cigarette smoking which indicates abnormal changes in renal function (Habib and O`Mahoney,2016; Noboriska *et al*,2017).

Ahmed *et al* (2015) reported elevation in creatinin and urea concentration in cigarette smokers as compared with non-smokers, where cigarette smoking can increase renovascular resistance and lead to significant changes in glomerular infiltration

rate and a decrease in the flow of distal convoluted tubules with a decrease in urea reabsorption.

Darawshy *et al* (2021) found a strong association between smoking and pre-diabetes where nicotine plays important role in increasing blood glucose levels among cigarette smokers as compared with non-smokers. **In conclusion**, currently showed that water pipe smoking caused abnormal changes in complete blood picture (CBC) and serum lipids such as the total cholesterol, LDL and Triglyceride levels, and HDL-ch which observed a significant decrease from the normal value as compared with non-smoking. Also harmed the liver functions which reflected on the liver enzymes secretion on the blood serum. water pipe smoking showed an effect on the kidney function testes through elevation of creatinine and urea in the serum, also found an association between the water pipe smoking and pre-diabetes where the nicotine may play role in increasing blood glucose levels.

So, we **recommend** that using water pipe smoking as an alternative to cigarette smoking has the same adverse effect and dangerous impact on human health, therefore need more information about the risks of water pipe smoking and other forms of tobacco including substitution and multiple product smoking.

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