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EFFECT OF SHISHA SMOKING ON SOME BIOCHEMICAL VARIABLES IN MALES IN SALAH AL-DIN GOVERNORATE

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ABSTRACT

The aim of this study was to evaluate the effects hookah smoking on homocysteine, vitamin B12, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), glutathione, and gamma-glutamyl transferase and to compare zinc and copper levels using atomic absorption spectroscopy. Cigarette smoke contains a lot of toxic chemicals that pose a health risk. This research was conducted in Tikrit, where the blood of 30 hookah smokers and 25 non-smokers (control group) between the ages of 19-40 years was drawn. According to the results, it was observed that the level of homocysteine increased significantly with gamma-glutamyl transferase, zinc and very low-density lipoprotein at a significant level of $P < 0.05$. On the other hand, there was a decrease in the levels of cholesterol, LDL, vitamin B12, copper, and glutathione with a variance of $P < 0.01$. Participants who smoked hookah also had higher concentrations of HDL in the blood compared to the control group with a P value < 0.05.

INTRODUCTION

The use of shisha has become popular all over the world, especially in Arab societies, where it is spreading among young people as a natural phenomenon available daily in homes, cafes and public places[1]. Shisha consists of soaked and fermented tobacco with fruit flavors such as grape, strawberry, apple, lemon, and raspberry. According to doctors, hookah is more dangerous than smoking other types of tobacco, but the misconception that hookah is less harmful than other types of tobacco has contributed to its spread. Burning charcoal in a hookah releases carbon monoxide and other toxic substances. Sharing hookahs between people in public places increases the risk of transmitting infectious diseases and microbes of respiratory infections. The health effects of hookah include sudden and premature death among young people, which increases the risk of heart disease, blood vessel blockage, atherosclerosis, and cancerous diseases such as lung, mouth, esophagus, bladder, and gum cancer, in addition to addiction to inhaled nicotine[2] .

Homocysteine

A compound suggested several decades ago as a primary risk factor in cardiovascular disease[3]. High levels of homocysteine (>15 μmol/L) represent substantiated risk indicator for cardiovascular disease [4]. Evidence suggests that high concentration of homocysteine are a strong predictor of increased mortality in these groups [5]. Studies have also revealed a strong relationship with other diseases with high homocysteine levels, such as diabetes [6], Alzheimer's disease, osteoporosis[7], kidney failure[8].There are several factors that lead to high levels of homocysteine in the body, Genetic factors include deficiency of vitamins such as B12 and B6, which are essential cofactors in the metabolism of homocysteine and the enzymes involved [9].

Gamma-glutamyl transferase (GGT)

Also known as EC: 2.3.2.2, is enzyme an aminotransferase. That is consists of a glycoprotein, has a molecular weight is about 68,000 Daltons ,comprises of two subunits [10]. Gamma-glutamyl transferase performs a critical part in glutathione metabolism, which acts as an antioxidant in amino acid metabolism. In addition, it contributes to maintaining glutathione levels inside cells [11][12], and it also has a role in the oxidation process and stimulating several types of free radicals and metal ions [13].

Glutathione

Is a tripeptide found in animals, plants, and bacteria. Its main function is to maintain enzymes in their effective reduced form, as glutathione contains a sulfhydryl group (SH), and when enzymes are oxidized and lose their reduced form, they become inactive[14].

Cyanocobalamin

Commonly referred to as vitamin B12, is a vitamin that soluble in water that is necessary for every cell in the body to grow and operate. Vitamin B12 deficiency is the cause of a condition characterized by the absence of hydrochloric acid in gastric juice, so the daily human need is about 5 micrograms of this vitamin[15] .

Zinc and Copper

Zinc is an important trace element in small amounts, as too much of it can cause harmful effects such as stomach cramps and then nausea and vomiting[16]. There are many health problems resulting from zinc disturbance[17] ,and its relationship to many physiological conditions such as general weakness, bone necrosis, anemia, diarrhea, and incompetence.

Copper is a necessary element for body, because it is able to attach to several enzymes and proteins that are essential to cells. Free copper takes part in oxidation processes that produce in free radicals, which impair the efficiency and functionality of the cell. Because it affects copper, which has antioxidant properties, it also has an impact on the equilibrium of copper within the cell [18].

The objectives of this study include:

1. Studying the relationship between some biochemical variables in shisha smokers.

2. Measuring the gamma-glutamyl transferase activity in blood serum shisha smokers compared to non-smokers.

3. Estimating the concentrations of metals via means atomic absorption spectroscopy, such as zinc & copper.

METHODOLOGY

The used in this study included the following:

1. All blood samples were collected in the city of Tikrit from smokers and non-smokers of shisha. The ages for samples ranged between (19-40) years and included a total of (55) samples (30 smokers and 25 non-smokers). Smokers were separated into two age-related groups. Their ages ranged between (19-30) years and (31-40) years. Samples were collected during the period from January 1, 2024 to May 1, 2024.

2. The samples were centrifuged at 5000×g five minutes to acquire serum blood.

3. Biochemical tests were performed using a ready-made standard kit from the French company BIOLABO to measure cholesterol variables, HDL-Cholesterol, LDL-Cholesterol, and VLDL.

4. A ready-made standard kit from the American company Crystaichcm was used to measure homocysteine (tHcy) according to the researcher's method[19].

5. A vitamin analysis kit was used for B12 provided by Sigma-Aldrich[20], Gamma-Glutamyl Transferase from Human-Germany[21][22], and Glutathione from England, in addition to measuring the concentrations of zinc and copper according to the method used by the researcher.

Analytical Statistics

The statistical Package for the Social Sciences, or SPSS (version 24), was used to statistically evaluate the data. In order to compare two variables and find differences between values that produced probability values (P values), significant levels of variables were determinant using a T test.

RESULTS AND DISCUSSION

Countries on the Mediterranean coast strongly indicate the need for effective anti-smoking strategies and focus on the factors that distinguish waterpipe use from cigarette use[23]. There is evidence indicating a relationship between hookah use and nicotine addiction, as well as between smoking and health risks, which are not limited to cancer, cardiovascular disease, respiratory disease, and its negative effects on pregnancy outcomes[24][25][26].

Biochemical variables

Table (3-1): Shows the measurement of homocysteine level and the concentration of some variables in smokers and non-smokers.

Parameters	Smoking n=30 Mean ± SD	Nonsmoking=25 Mean ± SD	P-Val
Hb (gdl^{-1})	13.09±3.57	14.35±4.29	< 0.05
WBC ($10^{-9}l^{-1}$)	6970±445.66	6455±439.59	< 0.05
Homocysteine (µmol/L)	18.99±7.00	7.98±2.01	< 0.05
Vitamin 12 (pg/ml)	307.25±119.75	485.33±153.50	< 0.05
Total Cholesterol(mg/dl)	201.81±23.47	97.61±16.21	< 0.01
LDL-C (mg/dl)	142.61±19.01	82.30±14.27	< 0.01
VLDL-C (mg/dl)	21.31±8.51	19.75±6.25	< 0.05
HDL-C (mg/dl)	42.87±9.87	52.06±12.66	< 0.05

Statistical analysis showed a significant increase in homocysteine levels in when comparing smokers to non-smokers at ($P < 0.05$) as shown in table (3-1). This increase in homocysteine levels is due to an increased possibility of blood clots, so the function of this compound as a potential danger cardiovascular disease has been suggested[27][28]. In addition, a relationship has been shown between the amount of homocysteine and the general health status of individuals, including healthy and unhealthy factors such as a healthy lifestyle and healthy nutrition[29], which leads to increased oxidative stress [30].Homocysteine levels should not exceed the maximum (9µmol/L), and although previous studies indicated different normal levels (10-15µmol/L), recent research has confirmed that the best level of homocysteine should be (9µmol/L)[31]. It is also noted that homocysteine levels decrease with age, which contradicts previously known mechanisms, as shown in Table (3-2).

Table 3.2 Level of homocysteine

Ages	**years of age (19-30)**	**years of age (31-40)**	**P-Val**
Parameters	**Mean ±SD**	**Mean ±SD**	
Hb	14.00±3.98	11.20±2.09	0.05*
WBC	72.03±4.45	6899±404.01	0.001**
Homocysteine	19.51±7.55	17.67±7.00	0.05*
V_{12}	324.26±156.11	307.25±119.75	0.05*
Total Cholesterol	187.19±19.99	200.90±22.98	0.05*
HDL	43.01±9.11	37.89±8.77	0.01*
LDL	135.58±17.00	141.22±19.33	0.001**
Copper	91.38±13.99	78.59±13.11	0.01*
Zinc	95.12±16.33	90.09±16.09	0.05*
GSH	6.29±3.00	5.41±2.99	0.01*
GGT	51.31±11.98	49.29±10.93	0.05*

Studies have found that increasing cholesterol levels may lead to heart and arterial diseases. High cholesterol is due to an imbalance in the distribution of fats in the blood. It was the probability value ($P<0.05$), indicating the significance of height[32]. High low-density lipoproteins are associated with increased cholesterol from food intake, as low-density lipoproteins transport cholesterol from the liver to the bloodstream. When receptors on the surface of cells hinder binding, the concentration of LDL increases. Low-density lipoproteins (LDLs) can filter through the walls of blood vessels and are transformed into oxidized lipoproteins (Oxi-LDL) due to the deposition of LDL on the artery wall, and this can lead to atherosclerosis or clumping together [33]. In addition, low HDL might result from elevated fatty acids levels not being converted into esters, which increases triglyceride levels and reduces HDL as a result of their association with high fat intake. The factors that reduce HDL concentration are the same factors that reduce proteolysis. The fatty type is very low in VLDL density and depletes HDL particles by preventing both lipoprotein transport[34].

Studies have found that vitamin B12 deficiency is more likely in smokers than in non-smokers ($P < 0.05$) due to factors such as ulcers in the stomach cells or certain medical procedures on the stomach. In addition, vitamin B12 deficiency may be caused by intestinal worms, which leads to its loss and excretion in the stool. Symptoms of vitamin B12 deficiency appear in neurological & digestive problems[35].

In Table (3-3), we observed a substantial rise in of gamma-glutamyl transferase concentration (GGT) at the probability level ($P < 0.05$). GGT employs glutathione as a constant source of cysteine in the cell, where it is produced within the cytosol and then transported outside the cell. GGT is responsible for the destruction of glutathione and other antioxidants such as the major

thiol in mammalian cells[36]. Researchers have found that high GGT activity indicates susceptibility to atherosclerosis and the evolution of other heart diseases like myocardial infarction, heart failure, stroke, diabetes, in addition to increased triglyceride levels, liver disease, and excessive alcohol consumption[37][38]. Serum GGT concentration is considered a sensitive indicator of oxidative stress[39] .

Table (3-3): Show some parameters.

Parameter	**Smoking n=30**	**Nonsmoking =25**	**P-Val**
Statistics	**Mean ± SD**	**Mean ± SD**	
Copper (mg/dl)	89.66±14.31	177.25±22.11	< 0.05
Zinc (mg/dl)	95.67±16.23	76.34±13.00	< 0.05
GSH (µM)	6.51±3.71	11.99±4.98	< 0.05
GGT (mM)	50.11±11.34	30.68±9.85	< 0.05

Although the kidneys contain a popular version of GGT, the activator found in serum is produced by the liver. GGT is considered an accurate marker of liver disease, but its clinical use is limited due to lack of clarity as ALP. For example, a high GGT level shows great importance in cases of liver damage or bile obstruction, where the GGT concentration can be up to 5-30 times higher. In adults, studies show that the highest GGT level (55 IU/L) is for men[40] .

The validity of GGT increases with the severity of the disease and with age. These results align with previous research indicating that elevated GGT levels could be useful in estimating older persons risk of cardiovascular death [41][42] .

Glutathione

Table (3-3) shows an important result, which is the presence of a reduction in of glutathione levels at a probability level ($P < 0.05$). Glutathione is considered an effective cell booster and is found in the tissues of living organisms and plants[43]. It plays a role in removing damage caused by metabolic activities and free radicals, thus regulating the capacity of the immune system[44][45]. Furthermore, reducing glutathione levels has a significant impact on maintaining red blood cell homeostasis. Glutathione works together with vitamin C as antioxidants for cells, responding to free radicals and converting to dehydroascorbate[46]. When glutathione is reduced in the cell strengthening mixture, glutathione reductase (GSH-RD) oxidized it is to GSSG and then converts it to the oxidzed structure GSH[47].

Zinc and Copper

The results presented in Table No. (3-3) show a significant reduction in level copper in blood, with a probability value ($P<0.05$), whereas the finding show that smokers had higher zinc levels than those in the reference group. This could be due to copper's role as a co-catalyst for the enzyme superoxide dismutase (SOD), which degrades oxidized molecules. Zinc and copper are minerals that contribute to eliminating free radicals and oxidizing compounds. By converting them into hydrogen peroxide, they can contribute to controlling and eliminating oxidation[48][49]. According to certain research blood serum concentration of zinc & copper may be elevated in atherosclerosis patients, which may increase lipid binding[50].

CONCLUSION

Hookah smoking is considered a health risk factor, as it is associated with a higher risk of heart disease, atherosclerosis, and lung cancer. Data analysis shows that the impact of hookah smoking on health is no less dangerous than smoking cigarettes. There exists a close link between shisha smoking

and modifications in biochemical variables. Effect of hookah smoking is attributed to increasing oxidative stress and reducing the number of antioxidants.

Recommendations

It is recommended to assess the levels of some enzymatic and non-enzymatic antioxidants in hookah smokers. It is recommended to measure heavy metal levels among smokers of shisha opposed to non-smokers. It is recommended to conduct a comparative study that includes cigarette smokers, shisha smokers, and electronic cigarette (vape) users, and compare them with non-smokers.

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