DOI : 10.35124/bca.2019.19.2.3605

Biochem. Cell. Arch. Vol. 19, No. 2, pp. 3605-3611, 2019 | www.connectjournals.com/bca | *ISSN 0972-5075*

DETERMINATION OF MYELOPEROXIDASE, HOMOCYSTEINE AND RELATED OTHER PARAMETERS WITH YOUNG SMOKERS OF CIGARETTE AND NARGHILE IN BAGHDAD CITY

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(Received 30 April 2019, Revised 27 July 2019, Accepted 12 August 2019)

ABSTRACT : Smoking is the inhalation of smoke tobacco burning that cover with narghile and cigarettes. There are a many problems result smoking such as, deterioration of health in general, undesirable social phenomenon, waste of money and time and stress.This study design to identify the effects of smoking narghile and cigarettes on levels of MPO, HCYand lipidprofil via determination of those parameters in cohort younger of Baghdad city. So to found the correlation coefficient of MPO and those parameters in groups that smoking cigarette and narghiledaily.As well as, to consider the MPO as biomarker to predictor coronary heart diseases. Increased levels of MPO, HCY total cholesterol (TC), triglyceride (TG), very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) were significant in all groups of smokers compared with nonsmokers. The risk of narghile impact on human health may be similar or worse than cigarette smoking and that risk due to complication of coronary heart disease.

Key words : Myeloperoxidase (MPO), Homocysteine (HCY), smoking.

INTRODUCTION

Smoking is the inhalation of smoke tobacco burning that cover with pipes, narghile and cigarettes. There are a many problems result smoking such as, Deterioration of health in general, undesirable social phenomenon, waste of money and time and stress (Yousif, 2016). So thought smoking habit a physical addiction to tobacco products, smoke composed 2.94 mg Nicotine, 802 mg tar, 145 mg CO and approximately to a smoke of single cigarette, larger quantities of chrysene, phenanthrene and fluoranthene.In fact, the number of puffs and their volume by narghile are about 10 times higher than cigarettes and the concentration of metals is higher. The burning temperature for narghile is 450°C compared to 900°C for cigarette (Yousif, 2016). The peak concentration of nicotine in cigarettes and narghile is the same, but coronary heart disease and lung cancer are the main risk factor for smoking and inflammation is strongly involved in the pathogenesis of atherosclerosis and there are numerous indications that cigarette smoking is associated with conditions of chronic inflammation and oxidative stress (Jacob *et al*, 2013). A potential risk factor for many diseases including cardiovascular disease, coagulation and Alzheimer's disease (Rosenbaum, 2018). Myeloperoxidase (MPO) (EC 1.11.1.7) is a one from the peroxidase group and always expressed in immune cells, for example lymphocytes, neutrophils, cells, plaques and in other body cells (Khan *et al*, 2018). MPO level in smokers are exist significantly higher than nonsmokers. Result of the lack of clarity of the complete biochemical mechanism for neutrophilia's, oxidative stress has emerged as a major player in the release of the MPO enzyme from these cells. Neutrophils are called white blood cells (WBCs), which play a major role in defense against microbial seizures as well as in innate immunity. There are many proteins and enzymes to side the MPO in neutrophils that note as antimicrobial properties such as alkali phosphatase, lysozyme, NXPH, oxides, gelatinase, lactoferrin, collagenase etc. Mpo is the most abundant and important antimicrobial agent mentioned previously, where its 5% of the dry weight of the neutrophils and 25% of the granule azurophilic proteins (Khan *et al*, 2018; Aratani, 2018; Aratani, 2018). MPO is released in both the pharynx compartment and the extracellular environment when neutrophils in the blood and peripheral tissues are activated. It is therefore part of the innate immune system of host defense against invasive microorganisms (Aratani, 2018; Mankhi, 2015).

Homocysteine (HCY) is a non-protein sulfur containing á-amino acid. It is similar to acidic amino acids,

but differs by exist the methylene bridge. Biologically, the pathway of methionine (a basic amino acid abundant in meat, seafood, dairy products and eggs) produced by removing the terminal methyl group. In addition, homocysteine synthesis can be recycled to methionine or converted to cysteine with folate acid and vitamin B (Rosenbaum, 2018).

Aim of the study : The study aims to identify the effects of smoking Narghile and cigarettes on levels of MPO, HCY and lipidprofil, via determine of those parameters in cohort younger in Baghdad city. Where we aim to find the correlation coefficient of MPO and those parameters in groups that smoking cigarette and Narghile daily as well as to consider the MPO as a biomarker to predict or coronary heart diseases.

MATERIAL AND METHODS

Sampling

This study was conducted between January and April (2019) in Baghdad, Iraq. All samples were randomly selected. Subjects were divided into three groups, cigarette smokers (n = 30), Narghile smokers (n = 30) and non-smoking groups (n = 30). The age ranges between (16-20) years. The volume of 5 ml of venous blood samples was made with dry tubes. After centrifugation, the serum was frozen at -8°C until analysis. Total cholesterol (TC), triglyceride (TGs), high-density lipoprotein (HDL), the measurement was done in all subjects after fasting 12 hours. All male subjects were randomly selected, evaluated and selected by detailed medical history, physical examination, comprehensive screening, and routine investigations to exclude any underlying diseases and exclude any diseases that affect lipid levels.

Determine of MPO and HCV

The quantitative sandwich enzyme immunoassay (ELISA) technique was employed for evaluation of MPO and HCY.

Lipidrofil determine

Enzymatic method was utilized to determination TC and TG, while HDL use participationmethod, but LDL, VLDL evaluate according calculate equation LDL mg/dl = TC mg/dl – [HDL mg/dl + VLDL mg/dl]. VLDL mg/dl = TG mg/dl /5.

Statistical analysis

Mean values and standard deviations (SD) were utilized for continuous variables.Student t-test was used to compare the significance of the difference in the mean values of control and cohort groups. ($p<0.001$) was considered high significance, as well as we found the relationship between MPO and parameters for narghile andcigarette smoker group by use the correlation coefficient.

RESULTS

From Table 1 note, levels of MPO, HCY, TC, TG, HDL, LDL and VLDL concentration in sera of G1 for Control and G2 how taken cigarette and G3, who smoke narghile. A results shows highly significant increase levels formyeloperoxidase ($P<0.0001$) in sera of group 3 (G3) (32.10±7.29)U/L and group 2 (G2) (26.30±5.48U/L) compare with group 1 (G1) (21.60±5.07), respectively. In addition, there are highly significant increases ($P< 0.0001$) when compared MPO levels in both cohort groups (G2 and G3). However, we noticed level of MPO is higherin G3 than that in G2.

From Table 1, levels of HCY in sera for (G1), (G2) and (G3) there area highly significant ($p \leq 0.001$) increase in levels of both group 2(5.97±1.59) µ mol/L and group 3(4.01±1.11) µ mol/L (when compared with group 1 (G1) (4.68±1.36) µ mol/L. In addition, exist non-significant ($p > 0.05$) different in HCY level between coherent group2 howtaken cigaretteand cohort group 3 how taken narghile when compared between them.

Nevertheless, we observed non-significant for CH levels in sera of group 1 (G1) (156.77±15.61) mg/dl when compared with group2 (G2) (159.13±17.75)mg/dl, but shows significant increase ($p< 0.05$) when compared between group 1 and group 3 (G3) (164 ±12.18) mg/dl.

On the other hand,showssignificant increase ($P< 0.05$) in G3)(79.91 ±13.99) mg/dl and G2 (73.8±13.77) mg/dl when compared with 1 (G1) (65 ±15.61) mg/dl for TG levels.

While we shows non-significant in HDL for group 2 (G2) (43.33±3.30)mg/dl when compared with group 1 (G1) (44.53±3.10)mg/dl but, we shows highly significant decrease for G3 (41.47± 2.92) mg/dl when compared with group 1.

In addition, we demonstrate non-significant in for HDL levels in sera of group 2 (G2) (43.33±3.30) mg/dl. When compared with group 1 (G1) (44.53±3.10) mg/dl, but we shows highly significant decrease for G3 (41.47± 2.92) mg/dl when compared with group 1.

Commencing this study, we show non-significant in LDL levels for group 2 (G2) (101.04±19.11) mg/dl when compared with G1 (99.23±16.29)mg/dl, but we shows significant increase ($p<0.05$) when compared G3 (106.56±12.24) md/dl. In addition,we show non-significant deferred when compared between G3 Vs G2. Ultimatly we confirm significant increase ($p<0.05$) when compared

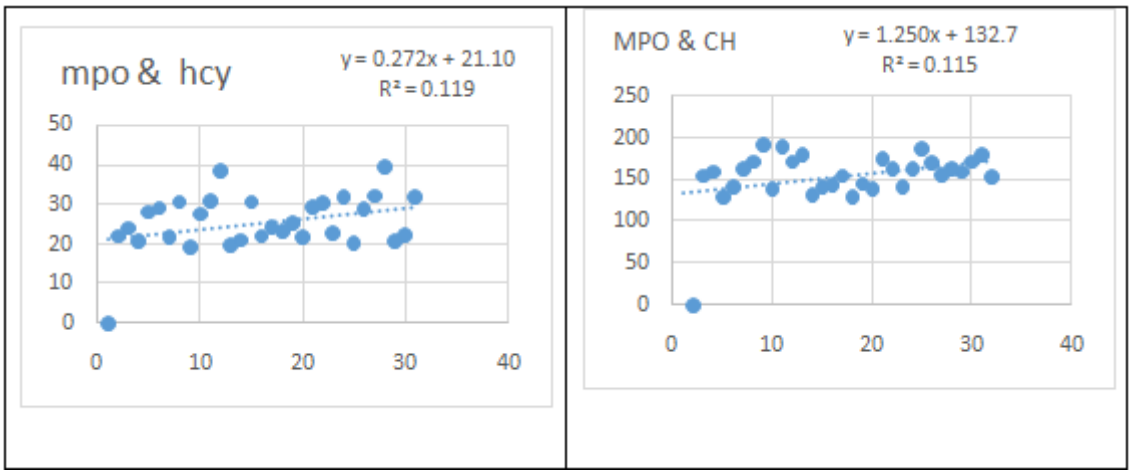

Fig. 1 : Illustrate Link relationship between MPO and HCY.

Fig. 2 : Illustrate Link relationship between MPO and CH.

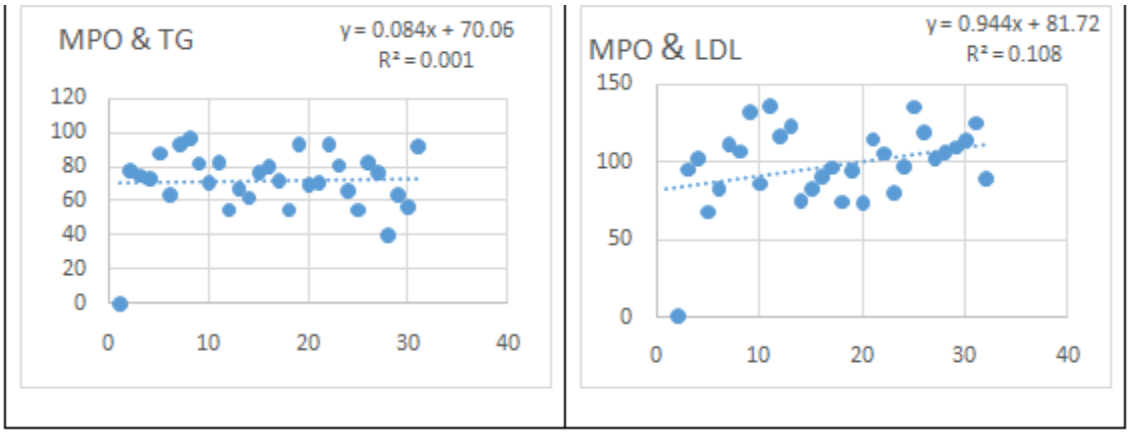

Fig. 3 : Illustrate Link relationship between MPO and TG.

Fig. 4 : Illustrate Link relationship between MPO and LDL.

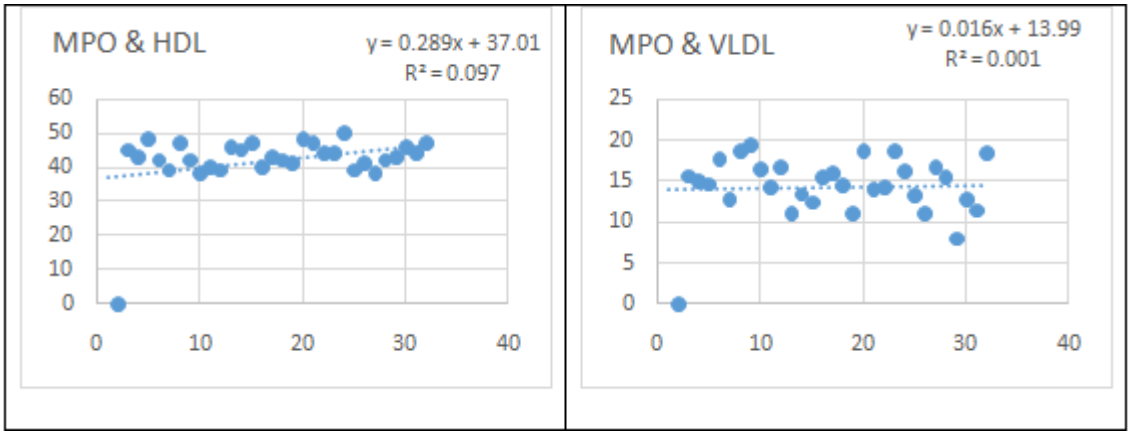

Fig. 5 : Illustrate Link relationship between MPO and HDL.

Fig. 6: Illustrate Link relationship between MPO andVLDL.

Those figures illustrated the correlation between MPO and parameters in Narghile group.

G2(14.76 ±2.75)mg/dl with group 1 G1 (13±3.12) mg/dl. We show highly significant increase (P< 0.001) for VLDL levels in sera ofgroup 3 (G3) (15.98±2.80) mg/dl when compared with group. We note non-significant between G3 Vs G2 all this noticed in Table 1. Beside that we show highly significant positive correlation when compared between MPO with HCY and HDL for G2 and G3 was illustrated that in Table (2) and Figs. 1, 4, 7, 10; respectively, but we observed highly significant negative correlation when compared between MPO with TG, LDL and VLDL for G2 and G3 was demonstrated in Table 2 and Figs. 2, 3, 5, 6, 8, 9, 11, 12.

DISCUSSION

A recent study shows that smoking is a risk factor leads to coronary atherosclerosis and this study agrees

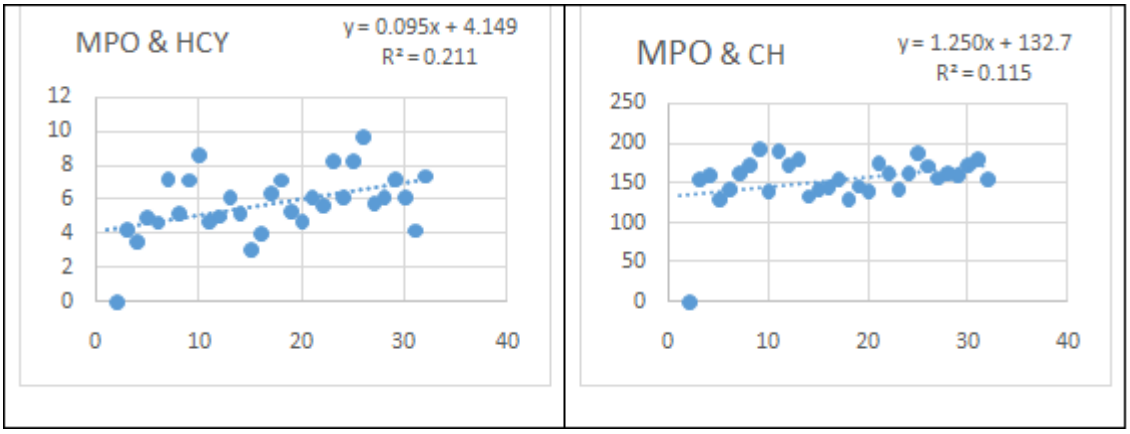

Fig. 7 : Illustrate Link relationship between MPO and HCY.

Fig. 8 : Illustrate Link relationship between MPO and CH.

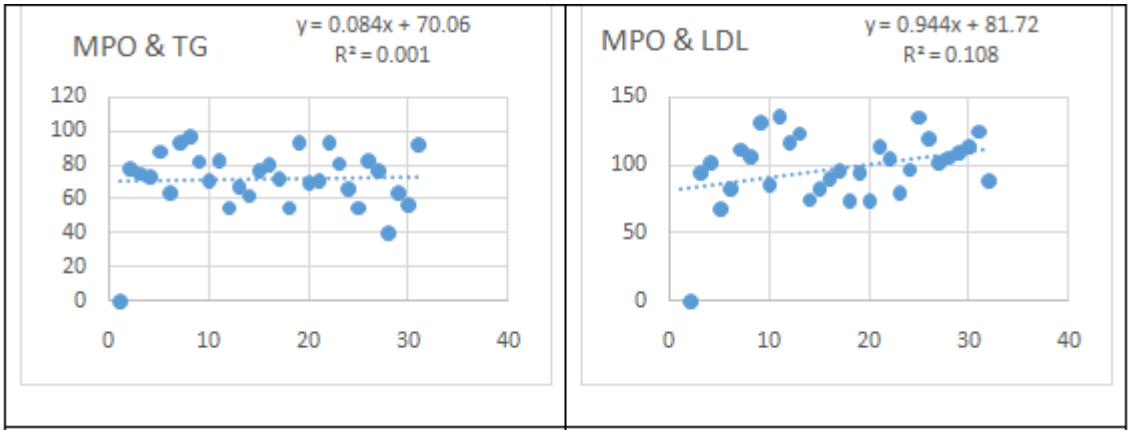

Fig. 9 : Illustrate Link relationship between MPO and TG.

Fig 10 : Illustrate Link relationship between MPO and LDL.

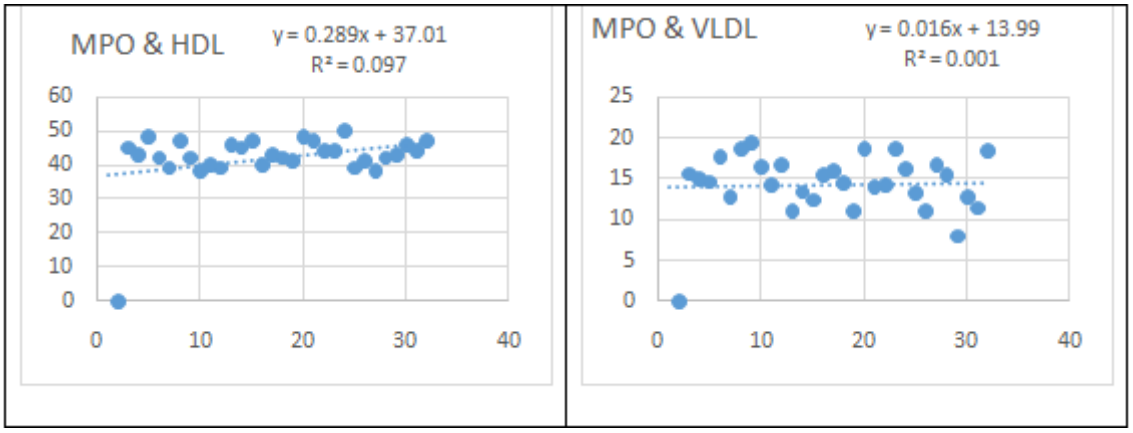

Fig. 11 : Illustrate Link relationship between MPO and HDL.

Fig. 12 : Illustrate Link relationship between MPO and VLDL.

Those figures illustrated the correlation between MPO and parameters in cigarette group

with another study showing that smokers are at risk for complications of coronary artery disease, vasodilatation, peripheral artery disease or stroke.

The mechanism of smoking which may effect on cardiovascular disease is not quite clear. Smoking leads to increases blood pressure, levels of low-density lipoprotein and cholesterol. In addition, further facilitates oxidation, promotes plaque buildup and increases adhesion of cells in the wall of the vessel, leading to adhesion to monocytes and rectangles. Smoking reduces the bioavailability of nitric oxide and contributes to nicotine dysfunction (Rudolph *et al*, 2008). The lining of the blood vessels and the weakness of the function of the lining of the blood vessels is one of the important reasons for the increase of vascular diseases and heart diseases so other studies have shown that nicotine stimulate nucleotide neutrophils. In addition, done nicotine activation adenine phosphate in the nucleotide that leads to reduced oxidase

Table 1 : The levels of MPO, HCY, TC, TG, LDL, VLDL, and HDL concentration in sera of enrolled groups.

Parameters	Control G1No.(30) Mean±SD	Cigarette G2 No.(30) Mean±SD	NarghileG3 No.(30) Mean±SD	G2 vs. G1	G3 vs. G1	G3 vs. G2
MPO(U/L)	21.60 ± 5.07	26.30±5.48	32.10±7.29	HS	HS	HS
HCY(μ mol/L)	4.01 ±1.11	5.97±1.59	4.68± 1.36	HS	HS	NS
CH(mg/dl)	156.77±15.61	159.13 ±17.75	164 ±12.18	NS	S	NS
TG(mg/dl)	65 ±15.61	73.8±13.77	79.91 ±13.99	S	HS	NS
HDL(mg/dl)	44.53± 3.10	43.33±3.30	41.47±2.92	NS	HS	S
LDL(mg/dl)	99.23±16.29	101.04 ±19.11	106.56 ±12.24	NS	S	NS
VLDL	13 ±3.12	14.76 ±2.75	15.98 ±2.80	S	HS	NS

Table 2 : The correlation relation between MPO and parameters in narghile and cigarette group.

Narghile parameter	MPO		Cigarette parameter	MPO	
	r	value		r	value
HCY	0.04	HS	HCY	0.18	HS
CH	-0.02	HS	CH	0.31	HS
TG	-0.12	HS	TG	0.07	HS
HDL	0.12	HS	HDL	0.25	HS
LDL	-0.02	HS	LDL	0.34	HS
VLDL	-0.12	HS	VLDL	0.07	HS

production.

This study compatible with other studies, where shows levels of MPO derived from neutrophils are higher in smokers compared to nonsmokers, moreover, this study suggest PMN is stimulated within the circulatory system due toreleased MPO in the blood stream with its substrates. In addition, clinical tests in previous years have shown that MPO not only plays a key role in pathophysiology of hemorrhagic vascular disease, but also is a useful diagnostic biologic marker in patients with acute coronary syndrome (Teng *et al*, 2017). MPO is widely associated with many human cardiovascular diseases, as well as works to start and spread heart disease (Rudolph *et al*, 2008).

The information that MPO plasma levels are already high when plaque is ruptured and thus happened before myocardial necrosis - supportthe hypothesis that MPO would be suitable as an early sign of acute coronary syndrome. In fact, a set of evidence indicates that MPO is produced from highly activated PMNs due to the initiation and development of smoking dependent blood vessels in atherosclerosis, therefore, atherosclerosis result fromsmoking via bioavailability of NO ventricular due to increased LDL while oxidation of HDL, Arterial plaque development and instability. However, MPO is one of the main causes of biomarkers in the progression of coronary artery disease in smokers and it can be considered a new biological indicator in patients with cardiovascular disease (Schabath *et al*, 2002). While other study demonstrated the relationship between Asbestos Exposure and cigarette smoking and highly increasing in MPOconsecration due to lung cancer (Schabath *et al*, 2002). Finally, the production of reactive oxidative types through MPO stimulating pathways may have a significant impact on the promotion of inflammatory events that are not only caused by immunological defenses but also result in tissue damage resulting from the arrangement of inflammatory conditions, consist atherosclerosis. MPO appears to be associated with a range of procedures involved in reproduction, subsequent reproduction and subsequent complications of atherosclerotic plaque. This result represents the components of the MPO pathway acceptable targets for promoting prognostic biomarkers and therapeutic interventions to stop cardiovascular disease and arteriosclerosis (Nicholls and Hazen, 2005). This study is very consistent with another study, where the group of patients with coronary artery disease in the Indian patients and the control group were observed to increase significantly, so found not significant correlation between homocysteine plasma level and other traditional risk factors for cardiovascular disease and diabetes consumers smoking, smoking and alcohol (Ranjith and Devika, 2017; Nicholls and Hazen, 2005). The reason for the high levels of plasma in Hcy is the lack of vitamin B12 or folate. HHcy is responsible for various systemic and neurological diseases. HHcy is a risk factors that leads to systemic atherosclerosis, cardiovascular disease (CVD) and stroke, regardless of known causes such as high blood pressure, diabetes, smoking and hyperlipidemia.

So that elevated levels of HCY are implicate in the development and progression of vascular disease. It is known that high HCY led to endothelial dysfunction and has been refer to be due to impaired bioavailability of NO, first mechanism for reduced bioavailability of NO is mediated via asymmetricdimethyl arginine (ADMA), this endogenous inhibitor of endothelial nitric oxide synthase (eNOS) was competes with the natural substrate, L-arginine thus preventive the formation of NO (Moretti

and Caruso, 2019). Elevated plasma levels of ADMA have been related with HHCY and endothelial dysfunction in both animals and humans, Apart from inhibiting the production of NO, ADMA may also encourage the "uncoupling" of eNOS, in that way increasing the synthesis of superoxide and other reactive oxygen species, which is causes, decrease NO bioavailability (Kumar *et al*, 2017). A study suggested that cigarette smoking increases the amino acid, which in turn is strongly associated with cotinine and plasma both thiocysteine and thiosulfate can be used by the enzyme thiocysteine to incorporate sulfur into cyanide, thereby detoxifying the cyanide to thiocyanate. Most smokers have a tendency to develop vitamin B12 and blood thinners after 20 years of smoking (Singh, Effect of Cigarette Smoking on Serum Homocysteine and Vitamin B12 Level in Male Population of Udaipur, 2016). Accordingly, homocysteine levels were higher in mothers exposed to smoking. Exposure to smoking during pregnancy is usually associated with lower levels of folate, vitamin B12 and higher levels of amino acid. This leads to increased awareness of the disadvantages and disadvantages of smoking as well as encouraging the cessation of smoking in all people, especially pregnant women (Tuenter *et al*, 2018). It is worthwhile this study consider the first studyin Iraqi exactly in Baghdad city that determination homocysteine level in cohort groups and compare this parameters in group that taken cigarette and group three taken narghile we show homocysteine levels was in border line with normal range we thought this fact depend. The nature of the group, for example, the number of times taken cigarette ornarghile was taken daily as well as depend on small size group was selected also period of smoking because the sampling was younger.

As well as, the risk factor of coronary heart disease among smokers is more than non-smokers. This can be explained by various associations such as impaired arterial wall integrity and deviations of blood lipid concentration, lipid protein and changes in blood clotting. The study noticed a high concentration for triglyceride, VLDL-C in group 2 and group 3 compared with group 1 as shown in Table 1. The fact of this study agreement with other study who thought the high levels of fat in smokers can be explain by the suggested mechanism: Smoking and nicotine absorption in the body, leading to fat degeneration then free fatty acid secretion in the bloodstream by activating the adrenal cyclase in the adipose tissue via nicotine-induced catecholamine's. These excess free fatty acids in the liver due to increased produce of triglyceride and sulfur, thus increasing the concentration of triglyceride and VLDL-C in the blood. As well as, we show non-significant total cholesterol, HDL and LDL-C in smokers for group II compared to non-smokers (group 1) but we showed a decrease in HDLC in smokers for narghile group compared to nonsmokers (Table 1). This result for current study disagreement with other studyand this difference due to the lack of smoking period compared to other studies that took the group of smokers for long periods duration (Singh, Effect of Cigarette Smoking on Serum Lipid Profile in Male Population of Udaipur, 2016).

In addition, another study report that MPO enzyme has also been shown to be implicated in lipid peroxidation and nitrate by generating a product of oxidation, nitrogen dioxide. MPO also have ability to oxidize LDL and HDL, An important step at the beginning of atherosclerotic bile plateau, indication that oxidative thiocyanate oxidation by MPO, significantly increased in chronic smokers, in the presence of hydrogen dioxide, so that modifies LDL cholesterol, due to elevated LDL cholesterol, which leads to elevated cholesterol in cholesterol-overloaded proteins. As well as other study noticed the relationship between monocyte to high density lipoprotein cholesterol ratio (MHR) in smoker and nonsmoker group they suggested elevated MHR is relationship with cigarette smoking and may be a useful biomarker of systemic inflammatory response in smokers. Smoker clan who has high MHR levels can easily be recognized during routine complete blood count (CBC) analysis and could possibly assistance from preventive treatment (Yýlmaz and Kayançiçek, 2018) by the way other study demonstrated that cigarette smoking group produce substantial amount of oxidative stress noticedsmoke inhaled by cigarette or narghile lead to increase effect of nicotine compound lead to same risk of alteration and inflammation via these two kinds of smoking methods as well as smoking significantly increase clinical biomarker of oxidative damage to proteins, nucleic acid like DNA and lipids (Bonnie *et al*, 2015).

Cigarette smoking generates substantial quantities of oxidative stress; increased levels of the amino acid may lead to lower HDL-C levels by several mechanisms. Further deficiency of HDL-C in chronic smokers can also be explained by increased catecholamine-induced excretion of smoking, causing increased VLDL-C and decreased concentrations of HDL-C. Thus, smoking promotes coronary artery disease and arteriosclerosis by reducing the anti-atherosclerosis factor HDL-C and increasing lipoproteins that can harden the arteries LDL-C that may lead to further damage to the lining of the vascular.

Depend on the results of this study we conclude elevated levels of MPO, HCY and LDL-C in sera of enrolled smoking group with cigarette or narghile due to

development coronary heart disease lead to utilize these parameters to predictor heart diseases in these group.

CONCLUSION

The risk of narghile impact on human health may be similar or worse than cigarette smoking and that risk due to complication of coronary heart disease.

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