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Comparison study of myeloperoxidase and homocysteine levels in Iraqi adolescent cigarette and narghile smokers

A thesis

Submitted to the College of Education for Pure Science / Ibn Al-Haitham / University of Baghdad in Partial Fulfillment of the Requirements for the Degree of Master of Science in chemistry

By

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بسم الله الرحمن الرحيم

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I hereby declare that this thesis has been performed under my Supervision in chemistry department, college of education for pure science Ibn Al-Haitham of Baghdad University in partial Fulfillment of the requirements for the degree of Master of Science in chemistry. Degree master in science Biochemistry.

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Certification

We, the examining committee, after reading this thesis **"Comparison study of myeloperoxidase and homocysteine levels in Iraqi adolescent cigarette and narghile smokers "** and examining the student **" Sarah Kareem Sahi"** in its contents, find that it is qualified for the degree of master of science in Biochemistry with grade of (Excellent) on (18/11/2019).

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Dedication

To the light of the heavens and the earth "God", Lord of the worlds

To, our Prophet, our Savior, our intercessor, and our Master Mohammed, peace be upon him

To who helped me and support me since my childhood to who brought me to this level of knowledge to the beloved (my dear father), I ask God to extend your life so that you will be happy with what your daughter has reached through your efforts continuous and great.

To the source of love and affection ... To the icon of mercy and beauty ... To the most beautiful women on my beautiful heart (my dear mother)

To the friends of life.... To who I rejoice and feel secure in their presence.... To the my loves and friends (Ahmad, Tota, Abode, Zahra)

For everyone who helped me from my family and friends.

To my angelic (Haneen)

For the house birds (Maryam, Ezel))

Sarah2019

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My sincere thanks and appreciation to all my friends

Thank you so much

Sarah 2019

ABSTRACT

This study was designed to identify the effects of cigarettes and narghile smoking on levels between MPO, Hcy and lipid profile via determination of those parameters in subject younger of Baghdad city so to find the correlation coefficient of Mpo ,Hcy and those parameters in groups that smoking cigarette and narghile daily, as well as, to consider the MPO as biomarker to predictor coronary heart diseases.

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 (G2= 30), narghile smokers (G3= 30) and non-smoking groups (G1= 30).

The age ranges for all subject between [16-20] years, five ml of venous blood samples was taken from subject that divided into two parts one of them utilized to measure hemoglobin (Hb) and packed cell volume (PCV).

The other part put in plain tube and centrifuged at 3000 rpm for10 min to obtain serum ,which it was divided in Eppendorf tube and freeze in -8 ° C until determination of MPO, Hcy ,TC,TG , HDL,LDL and VLDL.

Enzymatic method was utilized to determination TC and TG, while HDL use participation method, However LDL, VLDL evaluate according calculate equation while the method of sandwich enzyme immunoassay (ELISA) technique was employed for evaluation of MPO and Hcy.

The results showed highly significant increasing of levels of myeloperoxidase ($P \leq 0.001$) in sera of G3 and G2 when compared with G1.In G2 and G3 for MPO levels also was noticed from results that levels of MPO higher in G3 than that G2.

Results show a highly significant ($p \leq 0.001$) increase in levels of both G2 and G3 when compared with G1 and there was non-significant ($p > 0.05$) different in Hcy level between G2 and G3.

Nevertheless, non-significant was observed in TC levels in sera of G1,when compared with G2. as well as significant increase was found ($p< 0.05$), in G3 when compared with G1 .Result noticed significant increase ($P< 0.05$) inTG in G2 when compared wih G1 and there was a highly significant ($p\leq 0.001$) different in G3 when compared wih G1. By the other hand, result noticed a significant increase ($P< 0.05$) in G3 and G2 when compared wih G1 for TG levels , While show non-significant difference in HDL levels between G2 and G3 .Result noticed significant increase ($P< 0.05$) in LDL in G3 when compared wih G1 and there was non-significant ($p > 0.05$) different in G2 when compared wih G1.

Result noticed significant increase ($P< 0.05$) in VLDL in G2 when compared wih G1 and there was a highly significant ($p\leq 0.001$) different in G3 when compared wih G1. As well as, result noticed significant increase ($P< 0.05$) in AIP in G2 when compared wih G1 and there was a highly significant ($p\leq 0.001$) in G3 when compared wih G1. Results noticed significant increase ($P< 0.05$) in Hb concentration and Pcv percent in G3 compared with G1.

Conclusions the Conclusions of the current study were **:**

The levels of MPO in narghile smokers group is higher than that in cigarette smokers group .The study proved a highly significant elevation of Hcy levels in cigarette smokers group compared to narghile smokers group. From the present data showthat the levels of TC , TG , LDL . VLDL, AIP , Pcv and Hb in narghile group are higher than that of cigarette group while the levels of Hcy ,HDL in cigarette group are higher than that narghile group .Because of the increasing in MPO and Hcy levels in sera of cigarette and narghile smokers

when compared with control group , so these individuals are may be more prone to cardiovascular disease and atherosclerosis in the future .

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List of Abbreviations

Symbols	Terms
AA	Aortic Aneurysm
ASP	Aspartic Acid
APO	Apo Lipoprotein
AIP	Atherogenic Index of plasma
ADMA	Asymmetric Dimethylarginine
BC	Before Christ
B12	Vitamin B12
BMI	Body Mass Index
CBC	Complete Blood Count
CHD	Coronary Heart Disease
COPD	Chronic Obstructive Pulmonary Disease
TC	Cholesterol
CVD	Cardiovascular disease
CO	Carbon Monoxide
DNA	Deoxyribonucleic Acid
DM	Diabetes Mellitus
EDTA	Ethylenediaminetetraacetate
ELISA	Enzyme – linked immunosorbent assay
ENOS	Endothelial Nitric Oxide Synthase
FRS	Framingham Risk Score
G1	Group 1 non-smokers
G2	Group 2 cigarette smokers
G3	Group 3 narghile smokers
GOD	Glyceraldehyde -3-phosphate dehydrogenase
HB	Hemoglobin
HCY	Homocysteine
HIS	Histidine
HOCl	Hypochlorous Acid
HOBr	Hypobromous Acid

HOSEN	Hypothiocyanous acid
HDL	High Density Lipoprotein
HRP	Horseradish peroxidase
HCT	Hematocrit
IHD	Ischaemic heart disease
KDa	Kilodalton
LDL	low-density lipoprotein
MPO	Myeloperoxidase
NO	Nitrogen monoxide
NO_2	Nitrogen dioxide
OD	Optical density
OX-LDL	Oxidized Low-Density Lipoprotein
PVD	Peripheral vascular disease
PFT	Pulmonary function tests
PMN	Polymorphonuclear neutrophil
PCV	Packed cell volume
ROS	Reaction oxygen species
SMC	Smooth Muscle Cells
SD	Standard deviation
TG	Triglycerides
TC	Total cholesterol
VIIC	Factor VII coagulation
VLDL	very low-density lipoprotein Cholesterol

CHAPTER

ONE

INTERODUCTION

1.1 Smoking:

Smoking means the inhalation of the smoke of burning tobacco cover in cigarettes, pipes and narghile. Casual smoking is the act of smoking sometimes, in a social situation or to relieve stress. Smoking behavior is a physical addiction to tobacco products [1].

Risk factors of smoking is increasing coronary heart disease by 2 to 4 times, stroke by 2 to 4, developing lung cancer by 25 times for men while 25.7 for women, smoking led to lost overall health for smoker, increased did not go to work with increased health care utilization and cost, as well as cigarette smoking harms every organ of the body, due to many diseases, reduces the health of smoker[2].

General but when quitting smoking lowers the risk for smoking-related diseases then add years to life.

Tobacco is predicted to kill 10 million people annually over the next 20 to 30 years, most of these deaths occurring in developing countries. More than one billion men, women, and youths in the world utilized tobacco products, which approximately to about 35% of men in developed countries while for women, 22% in developed countries utilized tobacco products.

The second major cause of death in the world results from a taken tobacco product. It is presently responsible for the death of one in ten adults' worldwide. The other risk factors for smoking including low birth weight and asthma[3].

Nicotine is the active ingredient in tobacco. Tobacco use is divided into smoking tobacco burning such as cigarettes and narghile as well as smoking without tobacco burning such as electronic narghile[4].

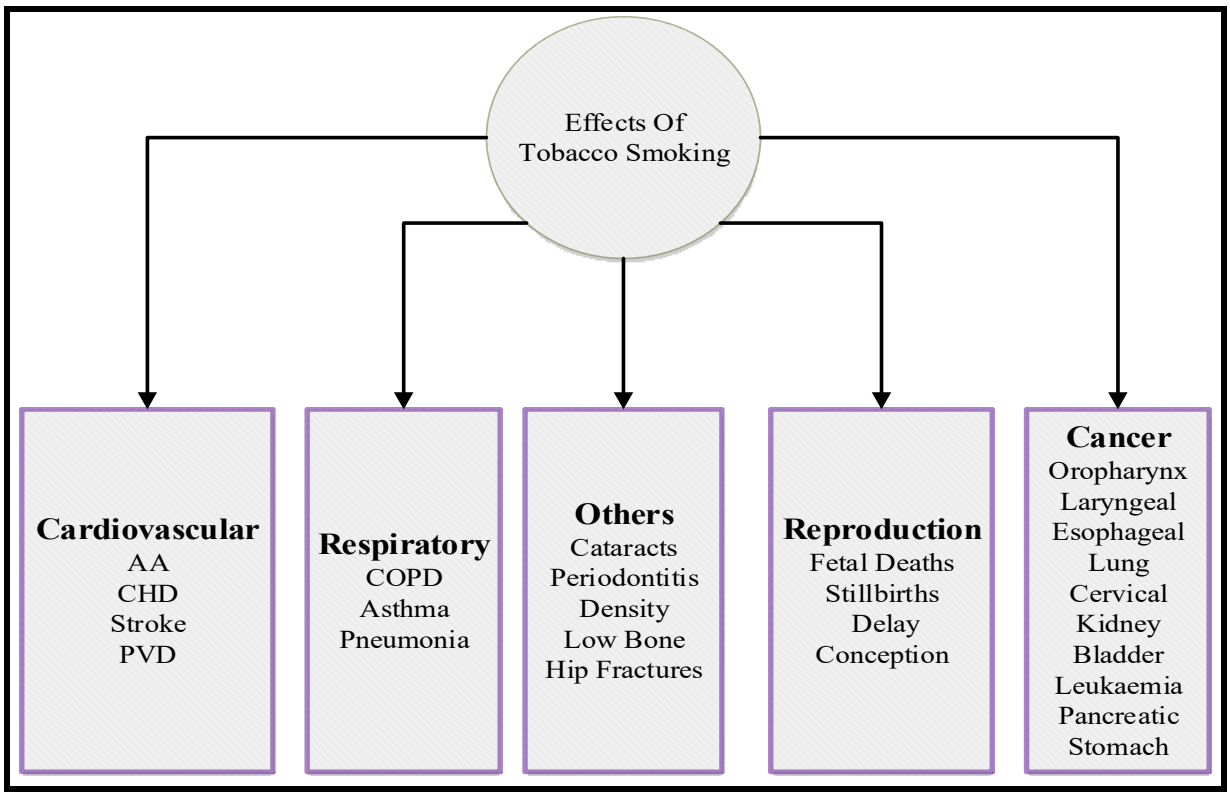


Figure (1-1): The effects of tobacco smoking [5].

1.1.1 History of smoking:

The information about the compound tobacco smoking from around 5000 BC when the Mayans used it during sacred ceremonies. Smoking transfer to "Western civilizations" in the sixteenth century after the colonization of south America. After that, prevalent during the first world war and raised peak frequency in the USA after the second world war. "Doll and Hill's" reports in the 1950s of the adverse effect of smoking. "James I" of England who in 1604 inscribed in his 'Counterblast To Tobacco' That demonstrated the side effect of smoking is harmful on eye, nose, brain and to the lungs[5].

1.2 Adverse effect of smoking and oxidative stress-mediated vascular dysfunction:

1.2.1 Complication of Smoking Cigarette Tobacco on Cardiovascular disease:

Cigarette smoking is a major cause of cardiovascular disease, and this is consistent with other epidemiological studies, which have observed a significant relationship between morbidity, mortality and cardiovascular disease , tobacco smoke caused by dysfunction of the endothelium, hemodynamic changes, insulin resistance, dyslipidemia, inflammation and hypercoagulability plays an important role in promoting the development of pustular plaque in smokers [6].

Metabolism of smoke cigarette caused to mixture of different toxic chemicals. Like, Nicotine, CO, and oxidizing gases [7].

Nicotine compound has a vital role, which induce the ganglia nervous system, central nervous system, increases heart rate, blood pressure, and cardiac output, all of those due to increased myocardial oxygen demand.

Other research noticed utilize carbon monoxide (CO), or other component of cigarette smoke, did not involve in the process of atherosclerosis or thrombosis related to cigarette smoking.

From previous study, unhealthy people have such as concentration to tobacco users, CO does not influence blood pressure, plasma catecholamine, platelet aggregation [8].

Also, other compound like oxidizing gases may be participate in found of a physio pathological state mean oxidative stress. Therefore the oxidative stress thought the major factor that mediated raised of ROS and plays bio vital role in the development of CVD[9].

Free radicals when smoking cigarette, can be synthesis by this pathway:

- Gas or molecules of smoking cigar.
- Activation" macrophages or neutrophils circulating "to produce ROS.
- Platelets activation.
- Production ROS from the endogenous compound for example uncoupled endothelial nitric oxide synthase, xanthine oxidase, and the mitochondrial electron transport chain chemicals oxidizing, such as NO and many free radicals are found at high concentrations in cigarette smoke produce mediate endothelial dysfunction.

There are a many of the metals in the smoke of cigarette and narghile such as cadmium , aluminum , copper ,lead ,nickel and zinc , which are implicated in oxidation of cellular proteins produce to structural damage and endothelial dysfunction[10].

The main cause that contribute to cigarette smoking is the weakening of blood vessels, thrombolytic state, oxidative stress and inflammation. Oxidizing chemicals affect the functions of endothelial vessels and oxidize low-density lipoproteins (LDL) with decrease the production of NO this is due to the loss of smooth muscle cell (SMC) role from the well.

As well as, when contacting smoking, endothelial cells release inflammatory and synthetic interleukins. These results lead to weakened endothelial vessels.

Cigarette smoke catalyze the modulation of the fibrin system by repress the plasminogen activator of tissues from the lining of blood vessels. Moreover, it is susceptible to activation of various stages; contribute to platelet activation and thrombus formation.

In fact, cigar smoking produces a large number of adhesion molecules on the surface of platelets leading to greater platelet activation of the platelets[11].

1.2.2 Smoking cigarette correlated with oxidative stress:

The component of free radicals produce from smoking cigarette have negative effects resulting in oxidative stress. So inside the cell imponderables between ROS and antioxidant systems led to oxidative stress . Reactive oxygen species (ROS) produce, via physiological situations, by metabolism of oxygen, which plays a major role in cellular signaling and safe. Produce the fragment of ROS that led to lipid peroxidation, DNA strand breaks [12].

Another destruction of structure for cell and functionality according to report smoking tobacco there are about 5,000 hazardous chemical compounds such as hydrocarbons, free radicals and oxidizing gases .So that the components of smoking cigar decrease intracellular antioxidant, result to oxidation stress mechanisms [11].

1.2.3 Smoking of cigarette relationship with inflammation:

Atherosclerosis usually produce from pathogenesis of inflammation and numerous lines of guide that connected smoking cigar with conditions of chronic inflammation. Anywise the oxidative stress with inflammation have two strongly correlated conditions. [13].

1.2.4 Smoking cigarette correlated with endothelial dysfunction:

The main leader in vascular function is endothelial cells that consider is control factor ,so that deficiency of regulatory mechanisms activated via these cells leads to "inflammation, vascular remodeling, and development of endothelial dysfunction".

The first event of atherosclerosis is produced by endothelial dysfunction diagnosis by an imbalance between vasodilatation and vasoconstriction, a pro-inflammatory endothelial cell status, an increased monocyte adhesion, and a deficiency bioavailability of NO[14].

Moreover, in a smoker low-density lipoprotein (LDL) was more susceptible to oxidation via increases ROS and NOS presence . Decreasing of bioactivity of NO led to oxidized LDL(ox-LDL) result in less bioactivity of NO deriving from the endothelium this damage of function is related to an increase of inflammatory cells that could cross the vascular wall. By the way foam cells formation from uptake of ox-LDL by macrophages via identification receptors, the other mechanism share with endothelial dysfunction is the increased ability of endothelial cells to hold to effector immunity cells such as monocytes, macrophages, T lymphocytes [15].

1.2.5 Smoking cigarette correlated with platelet activation:

Platelet activation and coagulation are two variables related to cardiovascular disease and the of atherosclerotic plaque formation are associated with platelet activation and coagulation. Smoking causes to increase platelet agreeability[16] .

Another report suggested the separation of platelets from cigarette users (tobacco), where increased aggregation was observed . the essential pathway of platelet activation is the synthesis of thromboxane[11].

1.3 Comparative studies of narghile and smoking cigarette:

As previously study, for many narghile smokers believe that narghile hold less toxicant and health risks than to smoking of cigar . narghile different from smoking cigar in many sides; the kind of tobacco, effects of different parts of narghile. Therefore, studies showed difficult when compassion toxicity between duration of narghile session and cigarette smoking[17].

Furthermore, the effect of stream smoke of narghile sitting (171 puffs at 17-s intervals, each of 530 ml volume) has around 4 times the carcinogenic PAH and aldehydes compound and 30 times the CO present in that of a single cigar.

Some study shows that a narghile smoker produces, during a one hour sitting, hazards compound like carcinogenic , and toxic in the exhaled mainstream smoke equal to 10 smoking cigar, each smoking at a rate of ten cigarettes per hour[18].

Smoke consists of 2.94 mg of nicotine, 802 mg of tar and 145 mg of carbon dioxide. The number of puffs as well as the volume when using smoking narghile is about 10 times higher than the cigarette, as is the case of metals and heat of combustion where the temperature of the narghile 900℃ while the cigarette 450℃, the concentration of nicotine in cigarettes and narghile is identical but the use of narghile longer than the cigarette nicotine is more effective than cigarettes [19].

1.4 Impact of Smoking on Pulmonary Function in adult:

The utilizes of tobacco by way of young people stays a predominant populace health situation global. There are 1.2 billion of smokers globally, regarding which extra than 50% are young people.

It is evaluate the amount tobacco-related deaths intention keep the almost vital motive on deaths into increasing countries by 2020 cigarette smoke is the principal reason concerning several persistent diseases, such namely attack , heart disease, persistent obstructive pulmonary disease (COPD), periodontal disease, peripheral vascular disease, pneumonia, lung or oral most cancers."

Lung is an immediately affected organ by fag smoking. Various respiratory ailments along with lung cancer, continual obstructive pulmonary disease, interstitial lung diseases, bronchial asthma, are brought on then aggravate by means of fag smoking[20].

1.5 Myeloperoxidase (MPO): E .C (1. 11. 1.7):

The enzyme of myeloperoxidase is consider a hemoprotein subfamily of peroxidase that is produce in the immune system such as polymorph nuclear leukocytes (neutrophils) and excretes through activation, The attendance of peroxidase enzyme of leukocytes exactly in the cytoplasmic granules was suggested by firstly of 20th century but purified it early 1940s [21], which is a potent antimicrobial compound .

There for it was modification oxidative of low-density lipoprotein(LDL) to a high uptake from that is reflected to be a key event in the promotion of modification thermogenesis.

For this reason, MPO is believed to participate in the developed and progress of cardiovascular MPO have potent pro inflammatory properties due to tissue injury. Also MPO implicated in the etiology of "lung cancer, Alzheimer's disease and multiple sclerosis"[22].

As well as MPO suggested the most predictor biomarker in cardiac so that increase the enzyme of MPO in blood of patients , consider as a risk marker for atherosclerosis and coronary artery disease. Complication seem in patients with chest pain in the ensuing 30-day and 6-month periods. Deficiency of myeloperoxidase used by mutations in the MPO gene on chromosome 17. It is the widespread inherited defect of phagocytes. Patients with MPO deficiency have to decrease microbial killing [23].

1.5.1 Structure of Myeloperoxidase:

Myeloperoxidase is a nice dark green heme protein. At 146 k Da in size, it contains two similar heterodimers composed heavy chain 58.5 k Da and light chain 14.5 k Da. Cysteine residues link on heavy chain heterodimers three-dimensional structure of myeloperoxidase has been fixed at high accuracy to provides advantageous insights into how it acts the oxidation potential of

hydrogen peroxide ,Figure (1-2). α-helical is secondary structure predominantly [24].

Each analog half include five helices—four the larger subunit while one from the small therefore a heme was bound covalently in the heavy subunits. So the prosthetic groups occurs in cavities containing the heme are on the same side of the protein and about 40 Å apart.

As well as ,there are five N-glycan's on asparagine residues at location 323, 355, 391, 483, and 729 on heavy subunit [25]. Most of the glycosylated residues located versus the heme cavities mediator between the dimers. When deglycosylation happened, exactly of Asn355 led to lowers enzyme activity. Each heterogeneous bound strongly calcium ion that is connected to the heavy and light subunits. It preserve the stability of the heterogeneous but may also affect redox properties of the enzyme because it is coordinated to Asp96, which is near to His95 the distal histidine that accelerate hydrogen peroxide for reaction with the heme,[26]

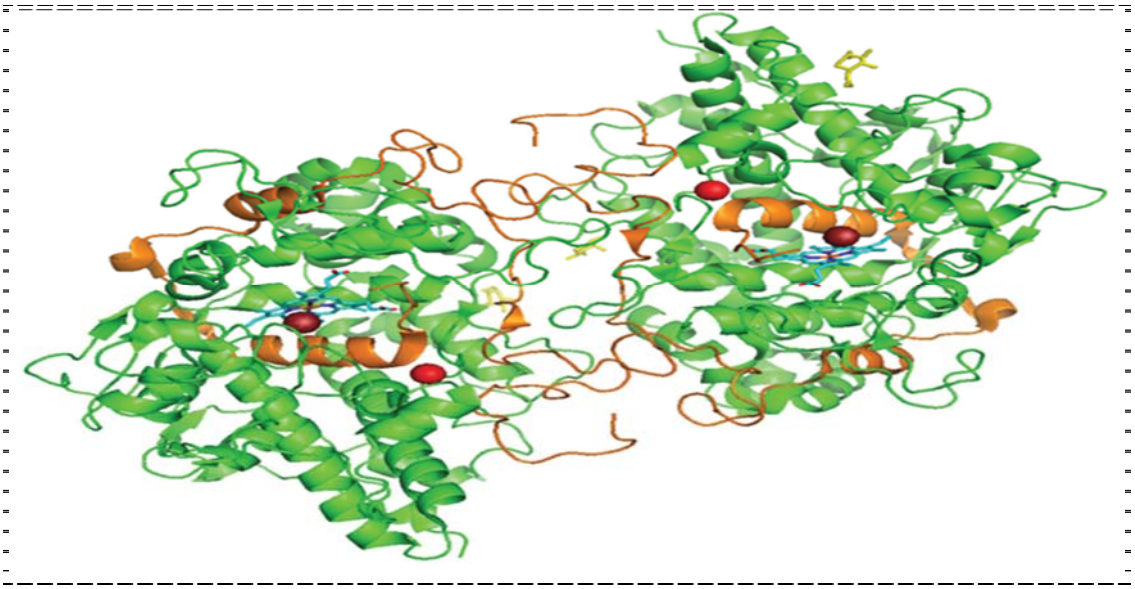


Figure (1-2): Myeloperoxidase structure.

The crystal structure of myeloperoxidase noticed the heavy (green) and light (orange) subunits whilst the heme prosthetic groups (light blue) with a

bromide (brown) restricted in the distal pockets. The calcium ion was shown in red color [27].

1.5.2 Biochemistry of Myeloperoxidase:

The gen for MPO expression was located on chromosome 17 q 12 – 24 and the first transcriptional product of this gene contain 11 introns and 12 exons [28]. Where appeared some alteration this result to signal peptide removal then glycosylation with mannose-rich side chains to synthesis apoproMPO (in active) . This compound enzymatically inactive so bound with accompany to form pro MPO then produce heme after removed. Some amino acid from N-terminal collected to produce of 72–75 k Da protein, split to form heavy chain 58.5 k Da its α subunit includes 467 amino acids and light chain, 14.5kDa, so β subunit contain 112 amino acids Figure (1-3) [29].

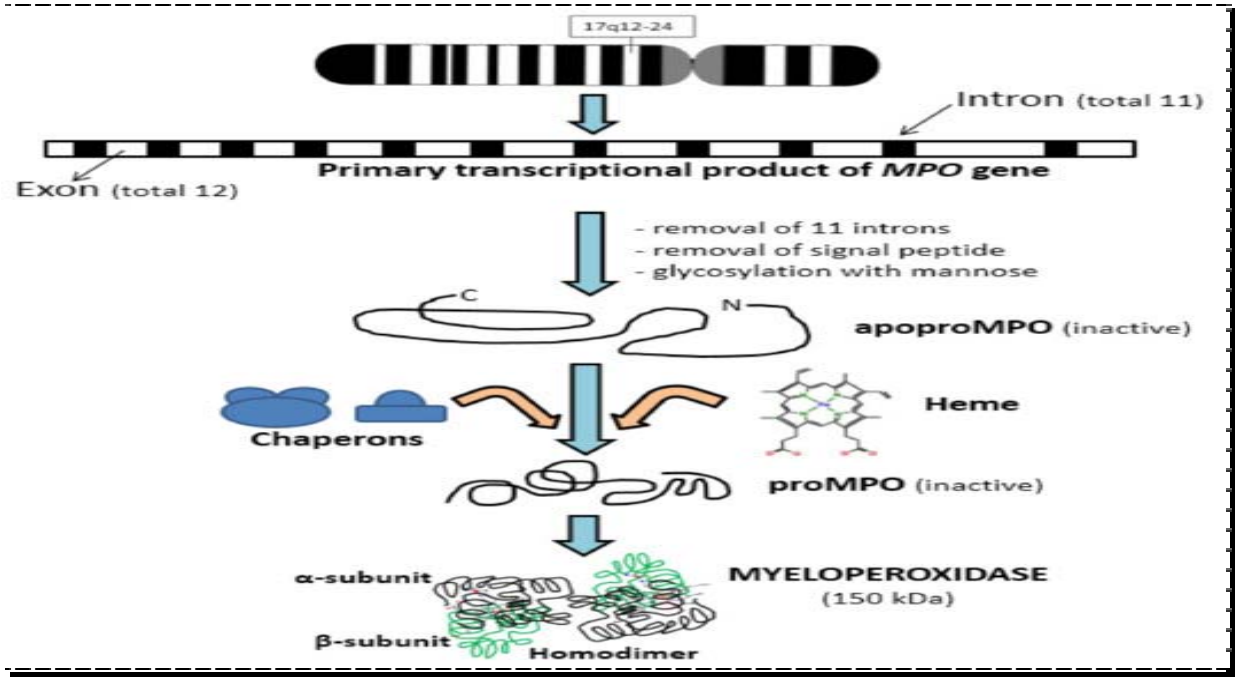


Figure (1-3): Synthesis of myeloperoxidase

1.5.3 Reaction and Mechanism of Myeloperoxidase:

The catalytic cycle was started via the rapid 2-electron oxidation of native Fe(III)-MPO with H_2O_2 to produce water and compound I , a Fe(IV)- Oxo porphyrin radical-cation species (Fe4+=O + porphyrin•+) this was shown in Figure (1 - 4)[30].

Native (MPO) is recycled by reduction of compound I by either the "halogenation cycle", a direct two-electron reduction by pseudo (halides), or via the "peroxidase cycle", a two-step for electron reduction via the formation of the redox intermediate, compound II (which keep the $Fe^{+4} = O$ center) [31].

The mechanism for substrate results depend on PH. throughout the halogenation cycle with halides, Cl^-; Br^-;and SCN^- provides two electrons to compound I to produce hypothalamus acids: hypochlorous acid (HOCl), Hypo bromous acid (HOBr), and Hypothecators acid (HOSCN), respectively.

On physiological PH, the concentrations of HOCL and ^-OCl will be identical, while HOBr will prevalent over ^-OBr while ^-OSCN will predominate over HOSCN[32].

From the other study, the physiological mixtures of these species will be referred to as HOCl, HOBr, and HOSCN.

At neutral PH and physiological concentrations of the (pseudo)halides ions, Cl^- serves as a major substrate for MPO, largely due to the greater abundance of Cl^- relative to Br^- and SCN^- (Cl^-, 100 - 140 mm, Br^- and SCN^-,20- 100 μm), making HOCl the major MPO-derived oxidant.

Risk factors that increase SCN^- concentrations, such as diet and smoking, let SCN^- to be a competitive substrate for MPO Furthermore, HOCl and HOBr can oxidize SCN^-, thus increasing the yields of HOSCN [33]. The peroxidase cycle it means the conversion of compound I to native MPO by a two-step one-electron reduction by compound II through radicals nitric oxide, $NO^•$, and $O_2^{•-}$, organic including tyrosine, ascorbate, steroidal hormones and urate, as well as xenobiotic ,drugs and inorganic substrates nitrite, NO2. In fact, free radical can

be resulting dimers and higher polymers, or react with other biochemical compounds for example lipids and proteins[29]

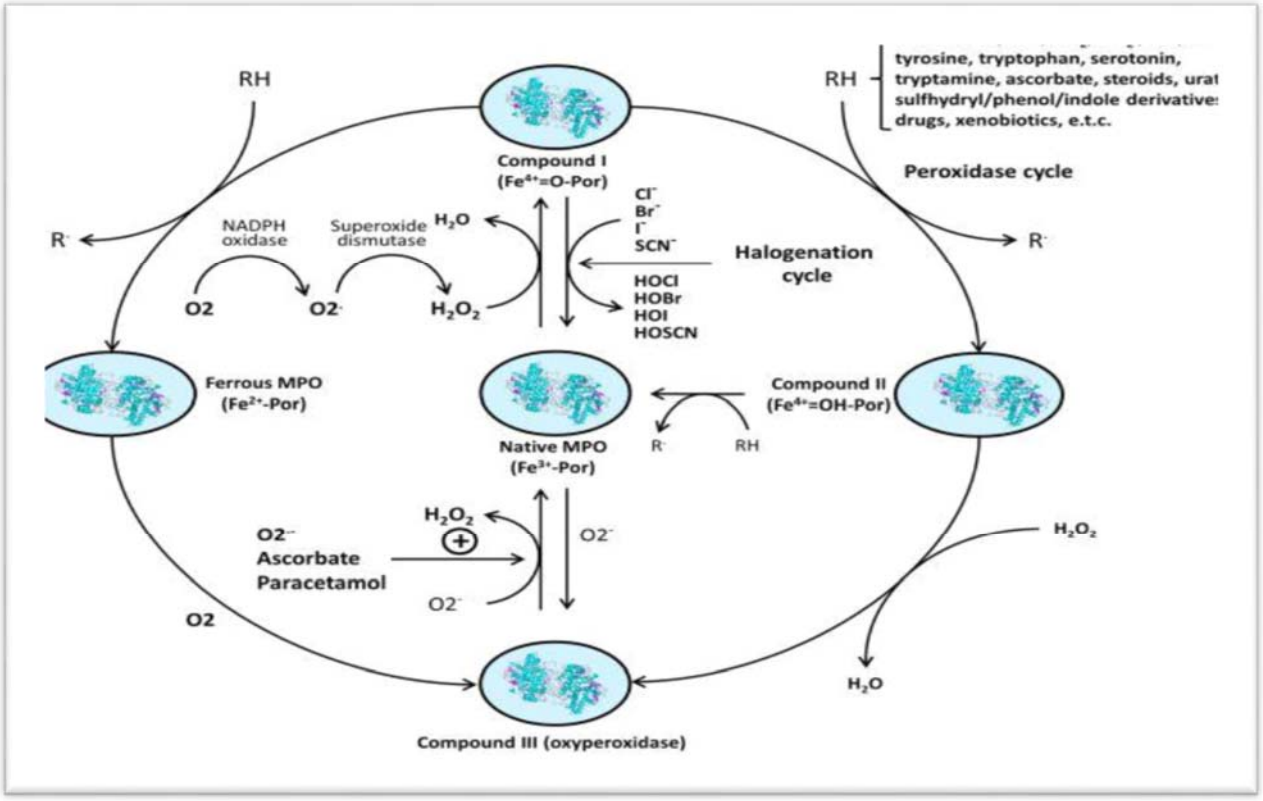


Figure (1-4): Mechanism of Myeloperoxidase.

Homocysteine (HCY):

Homocysteine (Hcy) means a non-protein sulfur containing α-amino acid. It is identical for amino acid cysteine, differing by an additional methylene bridge.

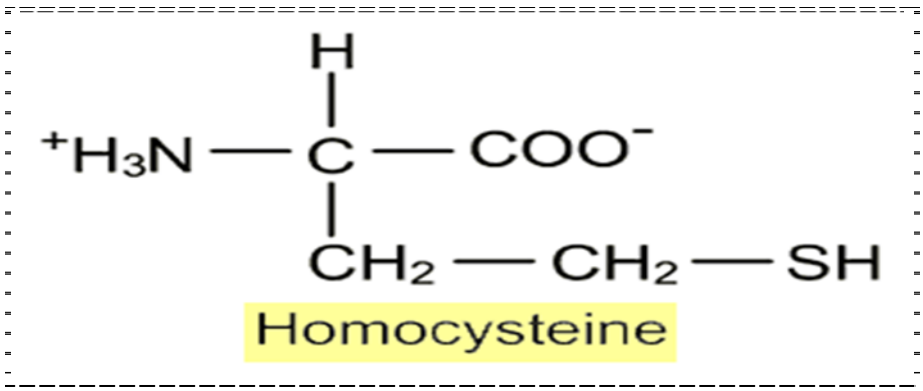


Figure (1-5): Chemical structure of homocysteine

It is synthesized from methionine (an essential amino acid found in meat, seafood, dairy products, and eggs) via removal of its terminal methyl group.

Homocysteine can be converted into methionine or cysteine with the assist of B-vitamins and folate[34].

1.6 homocysteine metabolism

Homocysteine arises during methionine metabolism (Fig(1 - 6). Methionine is activated to S-adenosylmethionine (SAM) by the enzyme methionine adenosyltransferase .SAM is the major biological methyl donor.

The products of these methyltransferase reactions are a methylated product and S-adenosylhomocysteine (SAH), which is subsequently hydrolyzed by SAH hydrolase to homocysteine and adenosine.

Homocysteine has several metabolic fates. homocysteine methy transferase has a rather limited tissue distribution, principally in the liver but, in some species, it occurs in the kidney [35]. Methionine synthase uses a methyl group from the one carbon pool (5-methyltetrahydrofolate, 5-MeTHF) as methyl donor.

Methionine synthase is widely distributed and this remethylation pathway is thought tobe ubiquitously distributed. Together, transmethylation and remethylation comprise themethionine cycle. This cycle does not accomplish methionine catabolism. This involvesthe transsulfuration pathway which consistsof the enzymes cystathionine –synthase and cystathionine –lyase [36].

This series of reactionsconverts homocysteine to cysteine and accounts for the fact that cysteine is not a dietary essential amino acid, provided adequate Supplies of methionine are available. Thetranssulfuration pathway has a limited distribution, being only found in the liver, kidney,small intestine and pancreas. Homocysteinemay also move from cells to the blood. Thetransporter which effects the export ofhomocysteine from cells has not been identified[37].

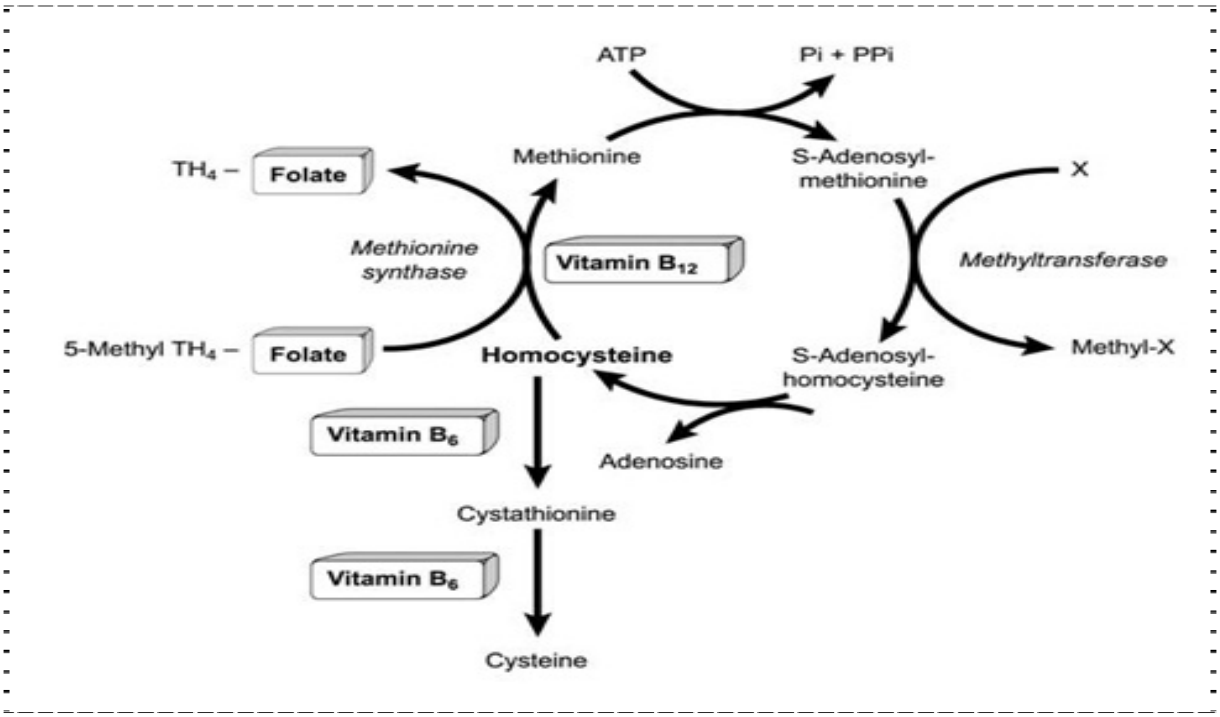


Figure (1- 6): Homocysteine metabolism

1.6.1 Homocysteine related to cardiovascular disease:

Homocysteine has been considered a risk factor since almost 1990, for cases of hypercoagulability, vascular disease and atherosclerosis. In addition, other studies have shown that homocysteine is directly related to the risk of coronary artery disease in people with renal impairment [38].

For a long time, researchers have discussed the fact homocysteine as a risk factor and causes cardiovascular disease, except for only 50% of cardiovascular disease that can be explained by "classic" risk factors.

Researchers have discovered that "new" risk factors can significantly cause cardiovascular disease. At the same time , many authors wonder whether this relationship exists between homocysteine and cardiovascular disease(CVD) [39].

The instrument that utilizes in predicting coronary artery disease known framingham danger score FRS, as important tool in patients with conventional danger factors, such as" dyslipidemia, hypertension, diabetes mellitus DM, and smoking", appear to have decrease the coronary artery disease danger in individuals with high homocysteine plasmatic levels[40].

Homocysteine causes cardiovascular disease by affecting smooth muscle cells and vascular endothelium, thus changes in the structure and function of arteries. In addition , the effects of vascular endothelial damage and proliferation of smooth muscle cells, there are also effects such as oxidation, increased collagen and damage to the arterial wall [41].

However, mechanisms to explain the relationship between excessive homocysteine in the blood and hardening of the aorta is not yet clear. The main assumption is based on that homocysteine plays a vital role in the formation of arterial wall leading to vascular injury, previous studies have shown that high homocysteine levels may have increased oxidation and inflammation of the vascular endothelial cells after due to reduced bioavailability for the production of nitric oxide through the lining.

The other studies proved strong evidence that oxidation is the mechanism result in increased homocysteine and atherosclerosis[42].

Hyper homocysteine has also shown to be linked with higher risk of venous thrombosis, increased homocysteine level promotion of platelet adhesion to endothelial cells then result in higher levels of prothrombotic factors like**, "** β - thrombomodulin, tissue plasminogen activator, and factor VIIC**"**. These leds to augmentation of thrombus formation, and improved arterial stiffness in Hyperhomocysteine can be set in the homocysteine synthesis (LDL) associated with democycin, such as small particle size (LDL) and its oxidative modification[43].

Hyper homocysteine is observed in humans' impairment of endothelium- related vasodilation in temporary or chronic, ultimately, high levels of homocysteine led to modification of LDL and HDL particles, inflammation, coagulation disorders, and fibrinolysis. It may also lead to biochemical effects on endothelial cells and their rupture, dysfunction of the diastolic blood vessels and reduced flexibility in reshaping the vascular wall [44].

1.7 Total cholesterol:

Cholesterol belongs to sterol class of lipid is complex alcohol created of four fused rings and a side chain, physical properties for pure cholesterol is solid at body temperature. Cholesterol is an important structural component of animal cell membranes demand membrane permeability and consider a precursor for the biosynthesis of steroid hormones by the gonads and adrenal cortex, as well as for the biosynthesis of bile acids and vitamins, all animal cells synthesize cholesterol but the liver is the main site, other sites consist of the intestine, adrenal gland, and reproductive organs [44-45].

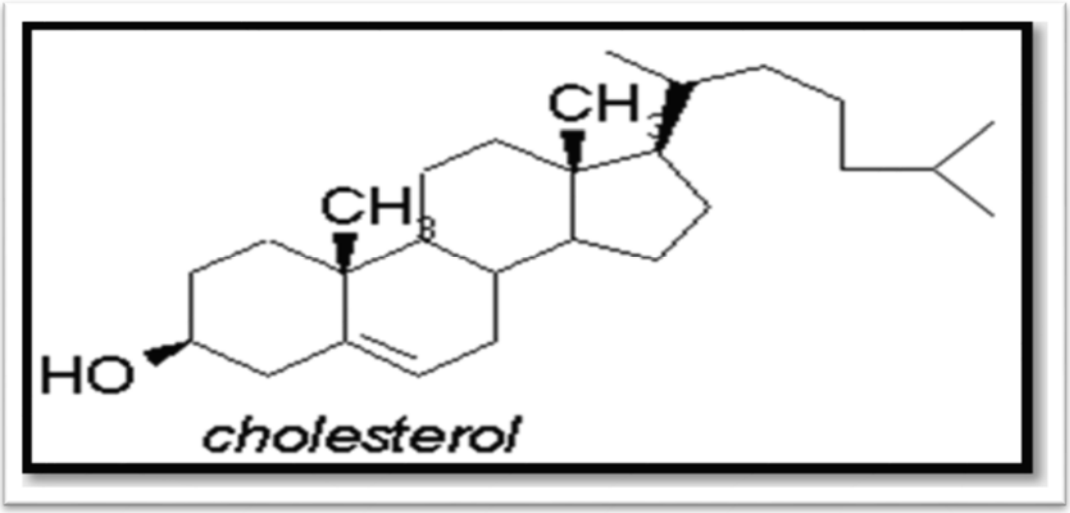


Figure (1- 7): Structure of cholesterol

Cholesterol is transmitted in the body by forming a compound with certain lipoproteins "where the lipoprotein binds to proteins and fats and allows the fat to move through the cells". Protein portions in lipoprotein play an important role in the emulsification of fat molecules, there are examples of lipoproteins including enzymes, carriers, structural proteins, adhesions, toxins and antibiotics. Fatty proteins are distributed as low-density lipoprotein LDL "bad cholesterol" , high-density lipoprotein HDL "good cholesterol" and very low density lipoprotein VLDL which is similar to low-density lipoproteins LDL and triglycerides "relative to the amount of fat to protein content"[45].

1.7.1 High Density Lipoprotein(HDL):

High-density lipoprotein or "good cholesterol" is the one that transports cholesterol from cells and tissues to the liver to reduce cholesterol. These proteins are small and high in density, so they are also called "alpha or heavy lipoproteins". This leads to an increase absorption the cholesterol in the blood during transport in the bloodstream, this absorption called "transfer of cholesterol inverted" as shown in the figure (1- 8) .

It transports cholesterol back from the tissues. The researchers suggested that high-density lipoprotein HDL, "good cholesterol," has genetic factors that can play a role in the protection and prevention of heart disease, as well as inferred for the genetic factors of the individual. In addition, there are other factors such as the volume of high-density lipoprotein HDL molecules and other proteins in the blood[46].

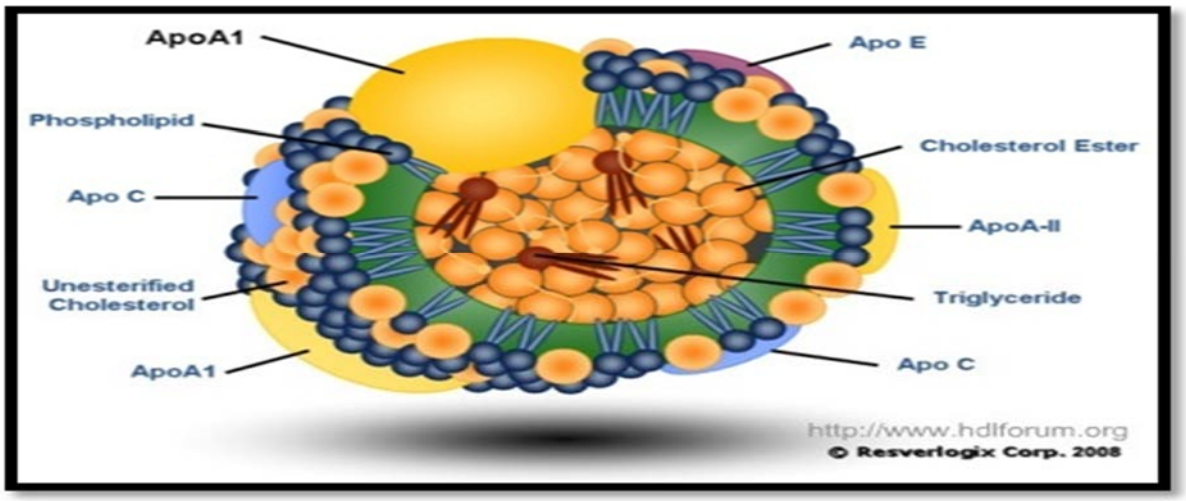


Figure (1- 8): structure of high-density lipoprotein (HDL)

1.7.2 Low-density lipoproteins (LDL):

Low-density lipoproteins "LDL" or called "bad cholesterol" The function of these proteins is the transfer of cholesterol from the intestines and liver to tissues and cells of the body through the bloodstream [47].

It has an essential role in the transfer of cholesterol and metabolism, low-density lipoprotein(LDL) molecules approximately 1.019-1.063 g / ml. A protein well known as Apo lipoprotein B - 100 contains (4536) amino acid residues in each particle of low-density lipoprotein (LDL), so it is considered to diameter each particle LDL [48].

The hydrophobic nucleus contains enhanced cholesterol molecules. In addition, a version of the lipoprotein Apo B-100 and phosphor lipid is found in the surface layer [49].

1.7.3 Very Low-Density Lipoprotein (VLDL):

Very low-density lipoprotein or(VLDL) is a kind of lipoprotein have the highest quantity of triglycerides. In addition, it's considered as a kind of "bad cholesterol" because at last gets converted into LDL then due to buildup of cholesterol in the walls of arteries, VLDL plays a vital role in carrier of triglycerides from the liver to the peripheral tissues for storage. VLDL transport approximately most of the plasma triglyceride so that the levels of triglycerides in VLDL and plasma triglycerides at the same amount. VLDL consist of Apo lipoprotein B-100, Apo lipoprotein C1, Apo lipoprotein E, cholesterol, cholesteryl esters, and triglycerides. The normal range for VLDL cholesterol about (2-30) mg/dl and higher levels are related with stroke and heart diseases [45].

1.8 Triglyceride:

The chemical composition of triglyceride comes from triglyceride consist of three molecules of fatty acids and one molecule of glycerol this fat found in food, in blood plasma and with cholesterol they form plasma lipids. Triglyceride is the main compound that storage fat in the body and the essential source of energy for cardiac muscle, all the time it is utilized for energy for all organ of the body except the brain. Ultimately, triglyceride and phospholipids are present in

the plasma membrane of the cell to make each cell different from one to another[50].

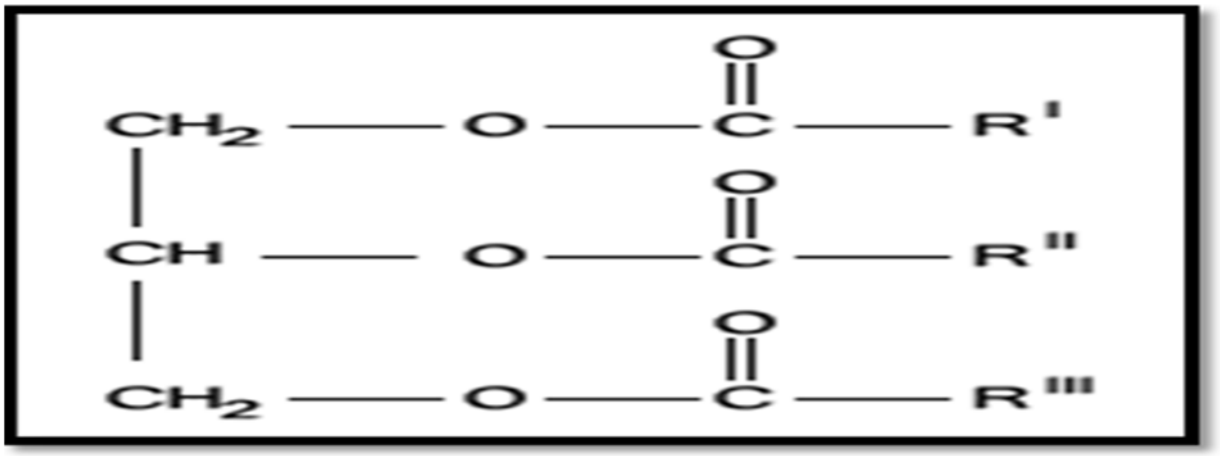


Figure (1- 9): Triglyceride structure

1.9 Atherogenic Index of Plasma (AIP):

The atherogenic index of plasma (AIP) can be calculated by the equation as log (TG / HDL) has been proposed as a biomarker of plasma atherogenicity. AIP may be a remarkable measurement for analyzing the results of clinical trials. This marker gives the related between TG and HDL in this simple ratio indicator reflects the balance between risk and protective lipoprotein forces. Previous studies demonstrated that the ratio of triglycerides to HDL was a strong indicator of myocardial infarction as well as findings the relationship between HDL and TG [51].

The Framingham study decided cholesterol as an important risk factor for CAD. In addition, other studies found the relationship between serum lipids, atherosclerosis and ischemic heart disease (IHD). AIP gives to consider the accurate metabolic interactions within the whole lipoprotein complex. From other studies It has been suggested that an AIP value of under (0.11) is related to low risk of CVD, the values between (0.11 to 0.21) highest than (0.21) are correlating with intermediate and increased risks, respectively [52].

1.10 The Packed Cell Volume (PCV):

The hematocrit (HCT) or packed cell volume (PCV) is known as the volume percentage (%) of red blood cells in the blood. It is nearly 45% for men and 40% for women. It is measured as an integral part of a person's complete blood count (CBC) results, hemoglobin concentration, white blood cell count, and platelet count. High level of hematocrit reflects hemoglobin concentration, reduced plasma volume, or absolute (increased red blood cell mass). Other complication increased red cell mass -the primary (polycythemia) or chronic lung disease, smoking, (hematoma, fibroids, hypernephroma). In mammals, finally, hematocrit marker is independent of body size[53].

Aim of the study:

This study was designed to:

1. Determination of mpo and hcy levels in smoking cigarettes and narghile subject and compare results with control group
2. Find the correlation coefficient of Mpo, Hcy with Hb , pcv and lipid profile in smoking cigarette and narghile daily, and study the probability of exposure these subjects to cardiovascular and atherosclerosis diseases in future.

CHAPTER

TWO

MATERIALS

&

METHODS

2.1 Materials

2.1.1 Chemicals

Kits that used in this study and its companies are listed in table (2 – 1).

Table (2-1): Chemicals and their sources.

Chemicals	*Sources*
Human Myeloperoxidase (MPO) ELISA- Kit	My biosource / USA
Human Homocysteine (Hcy) ELISA- Kit	My biosource / USA
Total cholesterol - kit	Randox, Germany
Triglyceride - kit	Randox, Germany
HDL-c kit	Randox, Germany

2.1.2 Materials and Manufacturers:

Types of equipment and manufacturers that used in this study were shown in table (2-2).

Table (2-2): Materials and Manufacturers.

Equipments	Manufacturers
Centrifuge	Hermle-(Germany)
Incubator	Gallenkamp, (U.K.)
ELISA	Biotech, (USA)
Spector photometer	APEL,(Japan)
Automatic pipettes	Slammed, (Germany)
Shaker	Khan Shaker, (Italy)
CBC	BC3000-midary,(China)

2.2 Subject and Methods :

This project conducted between January and April (2019) in Baghdad, Iraq. All samples were randomly selected. Subjects were divided into three groups,

cigarette smokers (G2= 30), narghile smokers (G3= 30) and non-smoking groups (G1= 30). The age ranges between (16-20) years. Five ml of venous blood samples was divided into two tubes one of them contained anticoagulant such as EDTA utilized to evaluate Hb and Pcv % .Another part of blood put in plain tube .

After centrifugation, the serum was frozen at -8 ° C until analysis. Total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), The measurement was done in all subjects after fasting (12) hours Myeloperoxidase and homocysteine were also measured. Comprehensive screening, and routine investigations to exclude any underlying diseases and exclude any diseases that affect lipid levels.

2.3 Methods :

2.3.1 Calculation of Body Mass Index

BMI was measured to evaluate by weight in kilograms (kg) divided by height in meter, squared(m^2) .Results indicited if the number of this indicater is high this mean person have more fatty acid [54].

BMI were divided in to :

- Underweight: this mean BMI less than eighteen
- Normal value: - BMI from 18.5 to 24.9.
- Overweight: - BMI from 25 to 29.9
- obese approximately 30. As shown in figure (2 – 1)

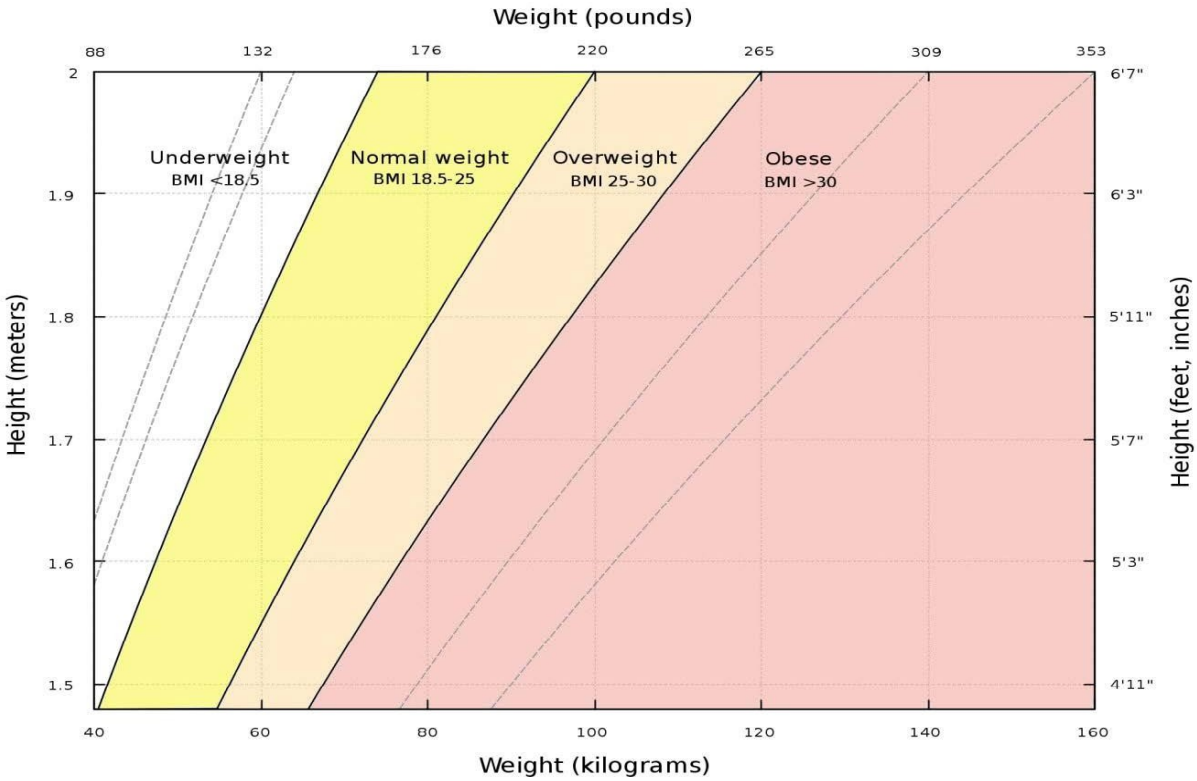


Figure (2-1): categorization of (BMI) [55]

2.3.2 Evaluate of myeloperoxidase:

Principle:

The kit was used to test the level of MPO, based on the principle of biotin double antibody sandwich technology enzyme-linked immunosorbent assay (ELISA) . Standard and sample were added to the wells that pre-coated with objective antibody, then add streptavidin HRP to form an immune complex.

So incubation and washing to removal of unbound enzyme, then substrate a and b were add .Solution will turn blue and finally change into yellow via the effect of acid media . The color depth or light was positively correlated with the concentration of myeloperoxidase .

Washed plate method:

1. Hand-washed plate method: the liquid within the ELISA plate was get rid; by utilized paved a few layers of absorbent paper, the plate was pat hard several times

downward; the diluted washing solution at least(0.35)ml was injected into the well, then soaking was done (1-2) minutes.

Assay procedure:

1. All reagents before starting assay procedure were prepared and then recommended that all standards and samples were added in duplicate to the micro Elisa strip plate.
2. Fifty µl was added from standard to standard well.
3. Ten µl from sera of G1 , G2 and G3 were added to a testing sample well, then(40)µl from diluent reagent was added to the testing sample well; while blank well does not add anything. So that one hundred (100)µl of{ HRP-conjugate reagent} was added to each well (standard wells and testing sample wells). After that it was covered with seal plate membrane, then wells were gently shaken and mixed for (60) minutes at (37) ° C incubation.
4. Preparation of washing solution: (20X) from dilute washing was diluted with distilled or deionized water for later use.
5. Washing by hand: the sealing film was removed carefully then was drain the liquid, was dried up, Each well was filled with washing solution, and was putted it aside for (1) min then was drain the liquid this way was repeated (5) times.
6. Colour was developed firstly that (50) µl chromogen solution A was added to each well, then (50)µl chromogen solution B was added to each well was shaked gently to mixed then was incubated for (15) minutes at (37)°C, the tubes must be away from light for color developing.
7. Fifty µl of stop solution was added to each well to stop the reaction the (blue) color changes into(yellow) immediately at that moment). Besides that, If the color in the wells was (green) or the color change does not appear uniform we must be, gently tap the plate to ensure thorough mixing.

8. Assay: the blank well was made at zero, so the absorbance was measured (OD) of each well one by one under(450)nm wavelength, which should be carried out within the (15) minutes after having added the stop solution
9. The results of concentration for myeloperoxidase levels were calculated by using the standards curve

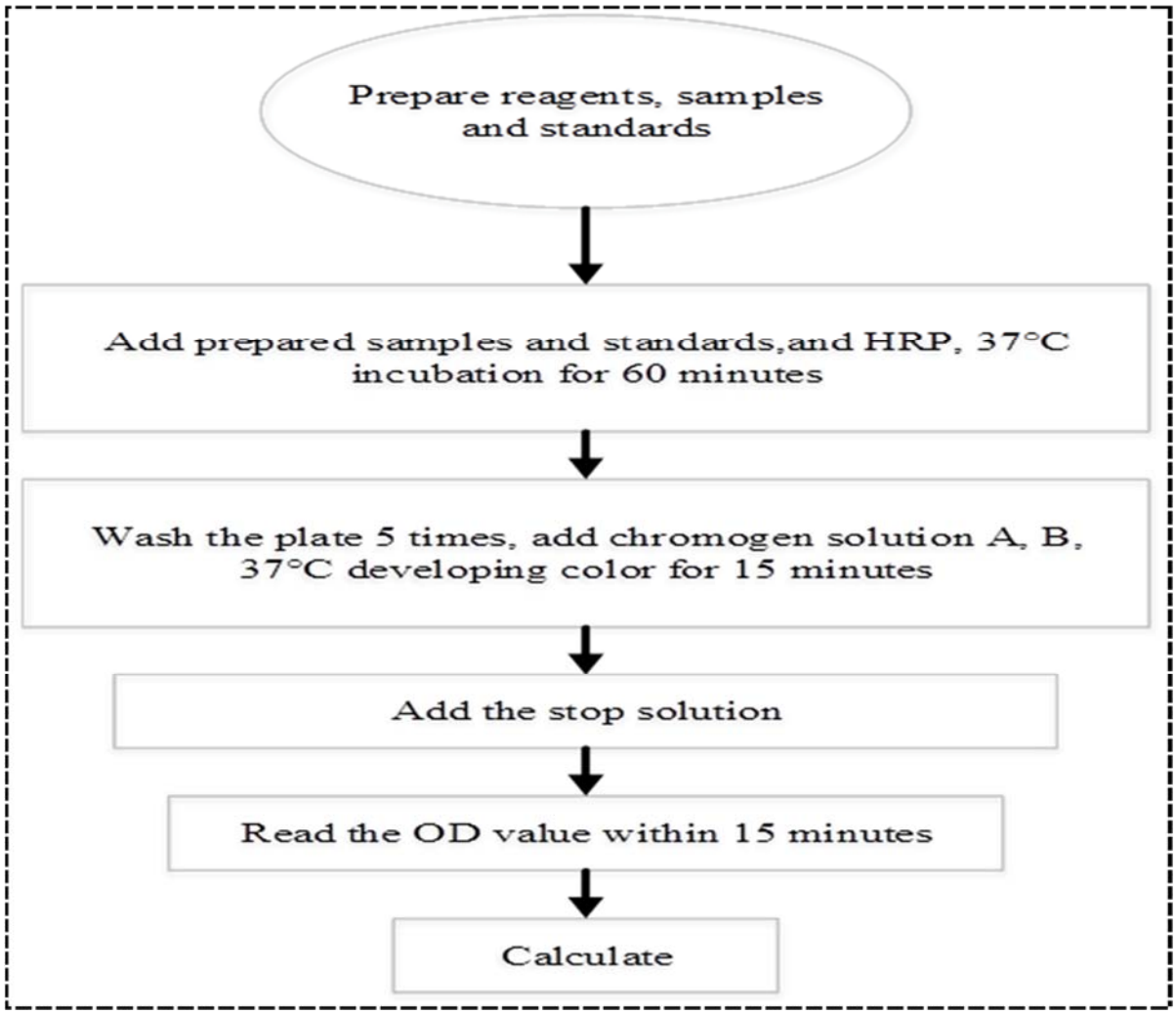


Figure (2-2): Summary of operating procedures of myeloperoxidase

The standard curve was desaiged by plotting the mean O.D. (450) nm for all six standard concentrations on the vertical (Y) axis Vs the parllear concentration on the horizontal (X) axis.

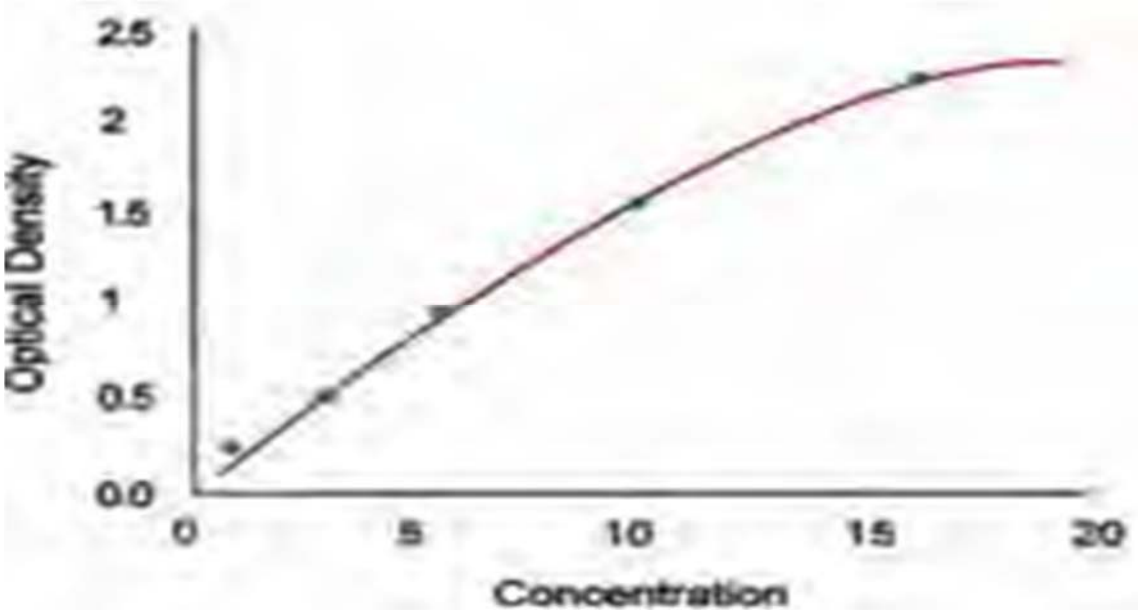


Figure (2-3): Standard curve of myeloperoxidase

2.3.3 Determination of homocysteine :

Principle:

kit was used to test the level of HCY, based on the principle of biotin double antibody sandwich technology enzyme linked immunosorbent assay (ELISA). Add Standard and Sample to the wells that pre-coated with objective antibody, then add streptavidin HRP to form an immune complex, incubation, by incubation and washing, removal of unbound enzyme, and then add the substrate A and B, then the solution will turn blue and finally change into yellow at the effect of acid. The color depth or light was positively correlated with the concentration of Homocysteine (HCY).

Washed plate method :

1.Hand-washed plate method: get rid of the liquid within the ELISA plate; in the experimental bench paved a few layers of absorbent paper, pat hard the plate several times downward; the diluted washing solution at least 0.35ml inject into the well, soaking 1-2 minutes.

Assay procedure:

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the Micro elisa Strip plate.
2. Add standard: Set Standard wells, testing sample wells. Add standard 50µl to standard well.
3. Add Sample: ℃Add Sample 10µl to testing sample well, then add sample diluent 40µl to testing sample well; Blank well doesn't add anything. ℃ Add 100µl of HRP-conjugate reagent to each well(Standard wells and testing sample wells), then cover it with seal plate membrane, gently shake and mix for 60 minutes at 37 ° C incubation.
4. Preparation of washing solution: Dilute the washing concentration (20X) with distilled .
5. Washing by hand: carefully remove the sealing film, drain the liquid, dried up, each well filled with washing solution, put it aside for one min then drain the liquid, so repeat 5 times.
6. Color developing: firstly add 50µl chromogen solution A to each wells, then add 50µl chromogen solution B to each well as well. Shake gently to mix up. Incubate for 15 minutes at 37℃,away from light for color developing.
7. Add 50µl Stop Solution to each well to stop the reaction (the blue color changes into yellow immediately at that moment). If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
8. Assay: Take blank well as zero, measure the absorbance (OD) of each well one by one under 450nm wavelength, which should be carried out within the 15 minutes after having added the stop solution.
9. According to standards' concentrations and the corresponding OD values, to calculate the linear regression equation of the standard curve.

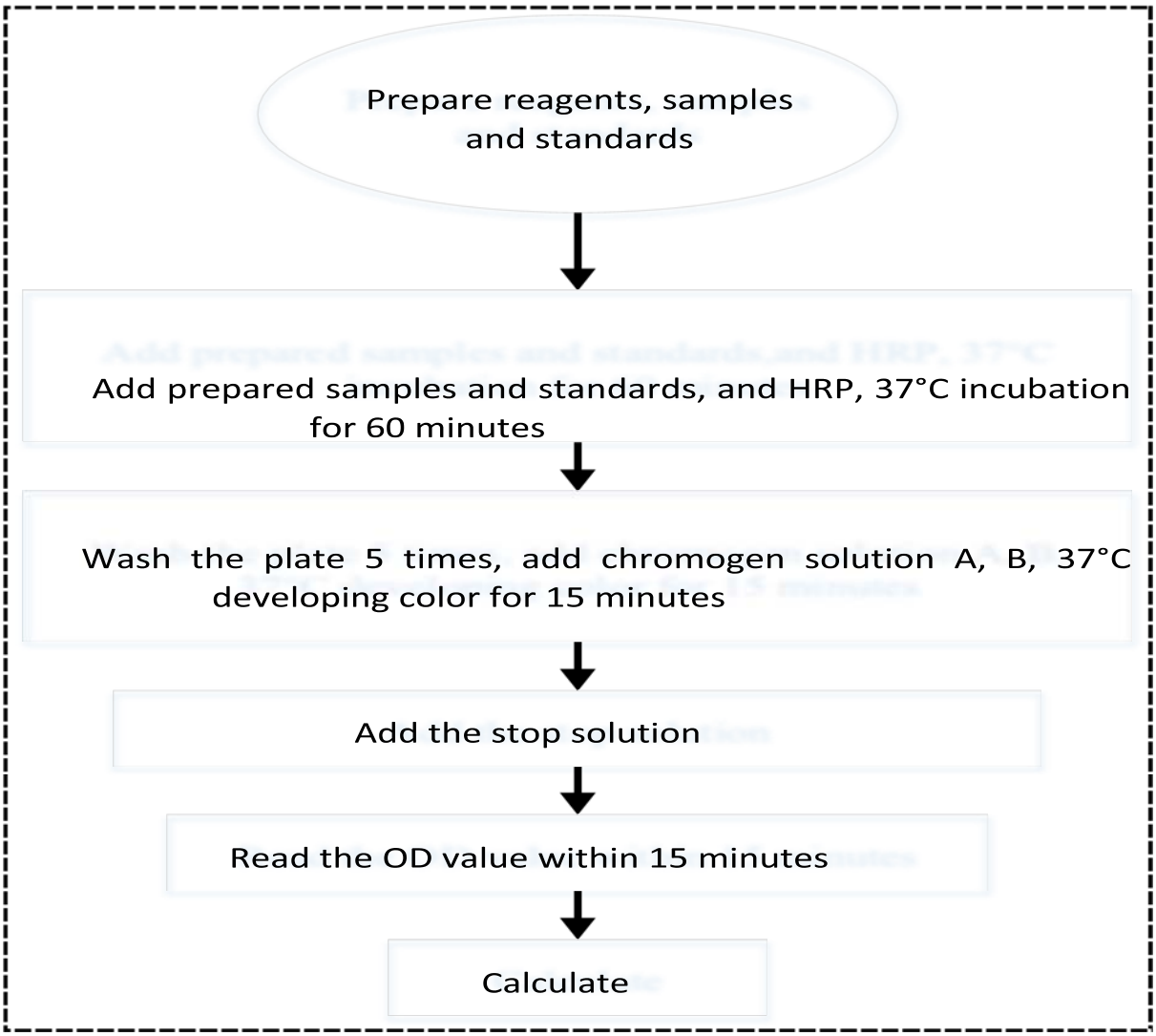


Figure (2-4): Summary of operating procedures of homocysteine.

The standard curve was desaiged by plotting the mean O.D. (450) nm for all six standard concentrations on the vertical (Y) axis Vs the parllear concentration on the horizontal (X) axis.

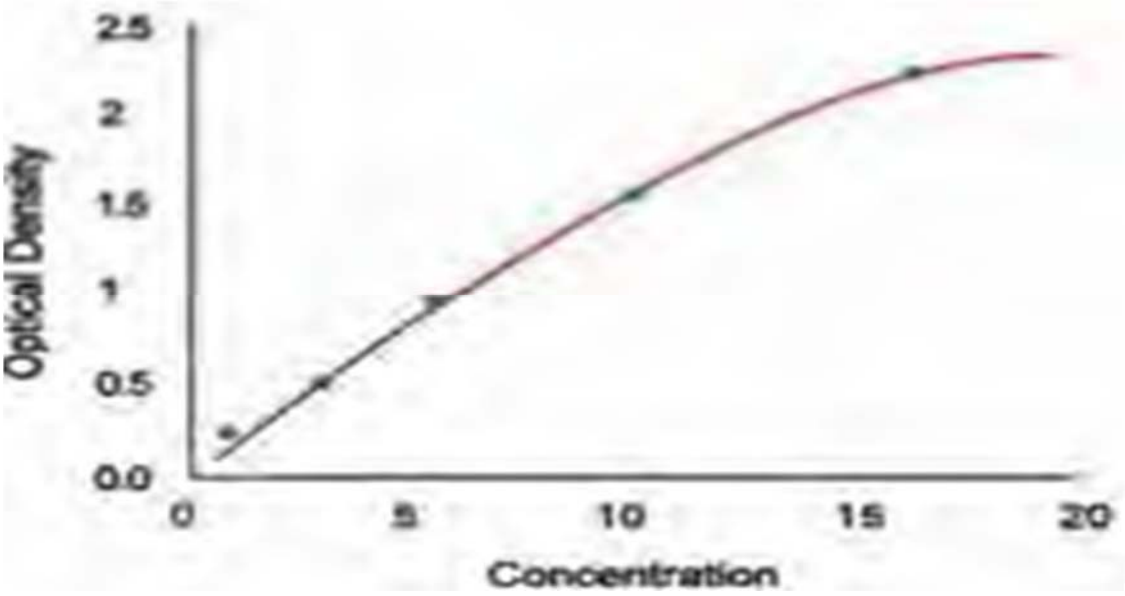


Figure (2-5): Standard curve of homocysteine

2.3.4 Determination of Lipid Profile:

2.3.4.1 Determination of serum (TG):

Principle:

enzymatic method was used to determination of TG as following the reaction[56].

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipases}} \text{Glycerol} + \text{Fatty acids} \quad (1)$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol Kinase (GK)}} \text{Glycerol} - 3 - \text{phosphate} + \text{ADP} \quad (2)$$

$$\text{Gelycerol} - 3 - \text{phosphate} + O_2 \xrightarrow{\text{GPD}} \text{Dihydroxyacetone} - \text{phosphate} + H_2O_2 \quad (3)$$

$$2H_2O_2 + 4 - \text{aminophenazone} + 4 - \text{chlorophenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + \text{HCL} + 4H_2o \quad (4)$$

Reagent:

Reagent type	Material	Concentration
Reagent (1) a Buffer	Insulating tubes 4-chloro-phenol Magnesium-ions	(40)mmol/L, ph(7.6) (5.5)mmol/L (17.5)mmol/L
Reagent(1)b Enzymes	Glycerol-3-phosphate oxidase 4-aminophenazon ATP Lipases Glycerol – kinase Peroxidase	≥ (1.5) U/ml (0.5)mmol/L (1.0)mmol/L ≥ (150) U/ml ≥ (0.4) U/ml ≥ (0.5) U/ml
Reagent (2) Standard	Triglycerides	(2.15)mmol/L (200)mg/dl

Procedure:

Working Solution: (15) ml of R1a (Buffer Solution) was taken blended with R1b (Enzyme Reagent) and was left for (5) min.

	Reagent Blank µl	Standard µl	Sample µl
Sample	---	---	10
Standard {CAL}	---	10	---
Reagent {R1}	---	100	1000
Distilled Water	1000	---	---

Combine incubate for(5) minutes at (37) Ċ. the absorbance of the sample (A sample) and standard (A standard) were measured against the reagent blank, at(500)nm.

Calculation:

$$\text{Concentration of TG} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Standard}} \times \text{Standard Conc. } 200 \left(\frac{\text{mg}}{\text{dll}}\right).$$

Expected values of TG

The ANCEP (American National Cholesterol Education Program) has set up the accompanying characterization for triglyceride levels as per the danger of creating coronary heart infections[57].

- ❖ Normal < 150 mg/dl
- ❖ Borderline – high 150-199 mg/dl High 200-499 mg/dl
- ❖ Very High ≥ 500 mg/dl

2.3.4.2 Determination of serum (TC):[55]

Principle:

After enzymatic decomposition and oxidation, cholesterol is the only guarantee. From hydrogen peroxide and amineptine are formed the quinonimine marker at the sight of phenol and peroxid.

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Lipcholestrol esterase}} \text{cholesterol} + \text{Fatty} \quad (5)$$

$$\text{Fatty acids cholesterol} + O_2 \xrightarrow{\text{Glycerol Kincholestrol oxidase}} \text{Cholesterol} - 3 - \text{one} + H_2O_2 \quad (6)$$

$$2H_2O_2 + \text{phenol} + 4 - \text{aminophenazone} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O \quad (7)$$

Reagent:

Reagent type	Material	Concentration
Reagent(1) Buffer	Pipes Buffer 4-Aminoantipyrine Phenol Peroxidase Cholesterol esterase Cholesterol oxidase	80mmol/L,ph6.8 0.25mmol/L 6 mmol/L ≥ 0.5 U/ml ≥ 0.15 U/ml ≥ 0.10 U/ml
Reagent (2) Standard	Cholesterol	5.27mmol/L 200mg/dl

Procedure:

	Reagent Blank µl	Standard µl	Sample µl
Sample	---	---	10
Standard{CAL}	---	10	---
Reagent {R1}	---	100	1000
Distilled Water	1000	---	---

Tube were mixed and incubated for(5)minutes at (37) Ċ. Absorbance of the sample (A Sample) and standard (A standard) were measured against the reagent blank, at(500) nm.

Calculation:

Concentration of cholesterol

$$= \frac{\Delta A \text{ Sample}}{\Delta A \text{ Standard}} \times \text{Standard Conc.} 200 \left(\frac{\text{mg}}{\text{dl}}\right).$$

Normal Values in Serum/Plasma (Risk levels)[57]

Value	Interpretation
< 5.17mmol/l (200 mg/dl)	Desirable blood cholesterol
5.17-6.18 mmol/l (200-239) mg/dl	Borderline-high blood cholesterol
≥6.20 mmol (240 mg/dl)	High blood cholesterol

2.3.4.3 Determination of serum (HDL): [56]

Principle:

Low-density lipoproteins (LDL) and Very low-density lipoproteins (VLDL) and chylomicron portions are encouraged quantitatively by the expansion of Phosphotungstic corrosive within the sight of magnesium particles. After centrifugation, the cholesterol in the HDL division, which stays in the supernatant, was determined.

Reagent:

Reagent type	Material	Concentration
Reagent{1} Solution	Phosphotungstic acids Magnesium Chloride	L/0.55 mmol L/25mmol

Procedure:

	Reagent Blank µl	Standard µl	Sample µl
Distilled Water	100	---	---
Supernatant	---	---	100
Standard	---	100	---
Reagent	1000	1000	1000

The samples were mixed and allowd (10) minutes at room temperature, then centrifugation for(10) minutes at 4000rpm.Absorbance of the example (A sample) and standard (A standard)were determind blank at(500) nm.

Calculation:

$$\text{T concentration of HDL} - \text{TC} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Standard}} \times \text{Standard Conc.} 55 \left(\frac{\text{mg}}{\text{dl}}\right).$$

Expected Values [57]

Mg/dl	**Mmol/L**	
<40	<1.04	Low
≥60	≥1.55	High

As HDL cholesterol is affected by various factors, like, hormones, practice, smoking, age, and sex, every research center must be built ought to put to own reference ranges.

2.3.4.4 Calculation Of LDL

LDL-c was calculated by using the equation :

LDL -C = (TC - TG/5 – HDL -C).

Optimum value of LDL must be less than one hundred mg/dL[56].

2.3.4.5 Calculation VLDL-C in sera

VLDL was calculated by this equation:

$$(\boldsymbol{VLDL - C = \frac{TG}{5}})$$

The VLDL level must be less than (30) mg/dL [56].

2.4 Calculation of AIP:

The equation was utilized for AIP estimation is ; log (TG/HDL) if AIP equal 0.11 its normal range but more than 0.21 it's dangerous due to CVD [57].

2.5 Haemoglobin and packed cell volume (Hb and pcv):

Hemoglobin concentration and hematocrit percent were measured by using automatically device (BC3000-midary).

2.6 Statistical analysis:

Data were expressed by utilizing Excel 2016 as mean ± SD. The comparison between patients and control groups was expressed by T.test P-value of ≤ 0.001 < 0.05, P> 0.05 were considered highly significant , significant and non- significant, respectively . The correlation coefficient (r) test is used for describing the association between the different studies parameters .

CHAPTER

THREE

Results

&

Discussions

3.1 Subject characteristic:

In this study, Blood samples were taken from participants which were classified into three cohort groups, cohort group one as control G1, cohort G2 who taken cigarette while cohort G3 taken narghile.

The results explained that the mean ± SD, P-value was extracted, the difference of between groups variation was consider highly significant when P-values are (P≤ 0.001)were shown in table (3 – 1) .

Table (3-1): Mean ± SD of characteristics parameters (Age, BMI, Pcv and Hb) in Control(G1), Cigarette smokers (G2) and narghile smokers (G3):

Parameters	G1 No.(30)	G2 No.(30)	G3 No.(30)	G1vs. G2	G1vs. G3	G2 vs. G3
PCV%	42.31±2.85	43.46±2.90	44.37±72.71	S	S	NS
Hb mg/dl	14±0.91	14.49±096	14.74±2.84	S	S	NS
Age (year)	17.9±3.50	17.7±1.49	18.2±1.21	NS	NS	NS
BMI kg/m^2	19.30±3.76	20.5±1.57	20.63±4.18	S	NS	NS

HS: p≤ 0.001 S: p≤0.05 NS: P>0.05

3.2 Hemoglobin (Hb) and the packed cell volume(PCV) :

Figure (3 – 1) showed a significant increase in PCV levels (p<0.05) in G2(43.46±2.90) % when compared G1(42.31±2.85) %. Study showed also significant increase (p<0.05) in Pcv levels in (G3) (44.37±72.71) % when compared with G1, but there was non-significant increases (p>0.05) between G3 and G2.

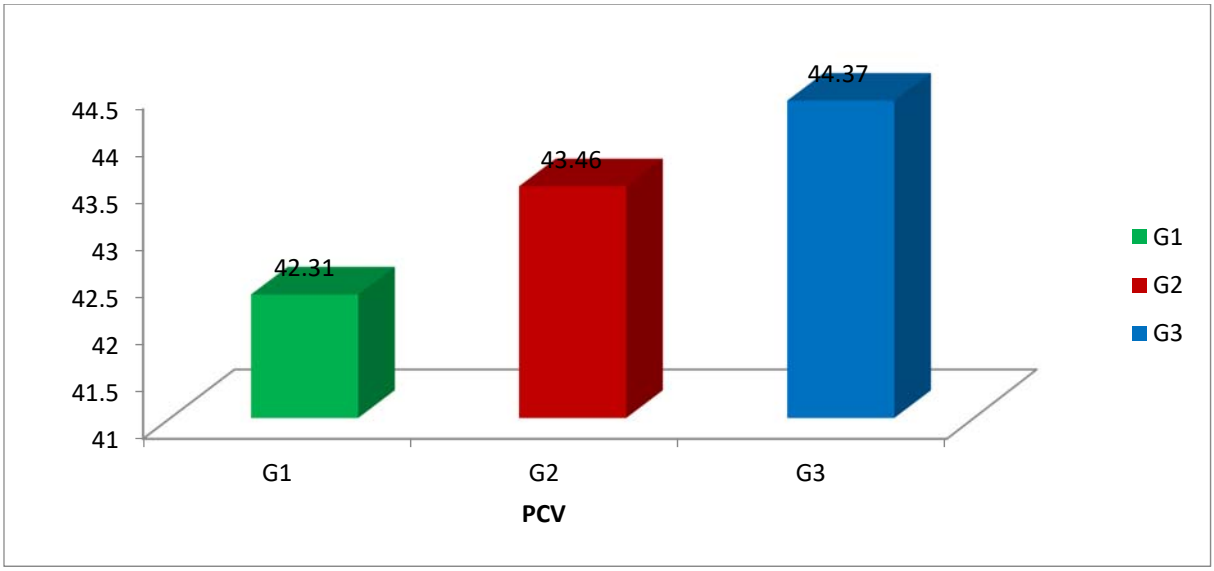


Figure (3 –1): Levels of Pcv % in G1 , G2 and G3

Figure (3- 2) showed significant increase in Hb ($p<0.05$) level in G2(14.49±0.96) mg/dl when compared with G1(14±0.91) mg/dl , also there was significant increase ($p<0.05$) in Hb in G3 (14.74±2.84) mg/dl when compared with G1, but there was non-significant($p>0.05$) between G3 and G2.

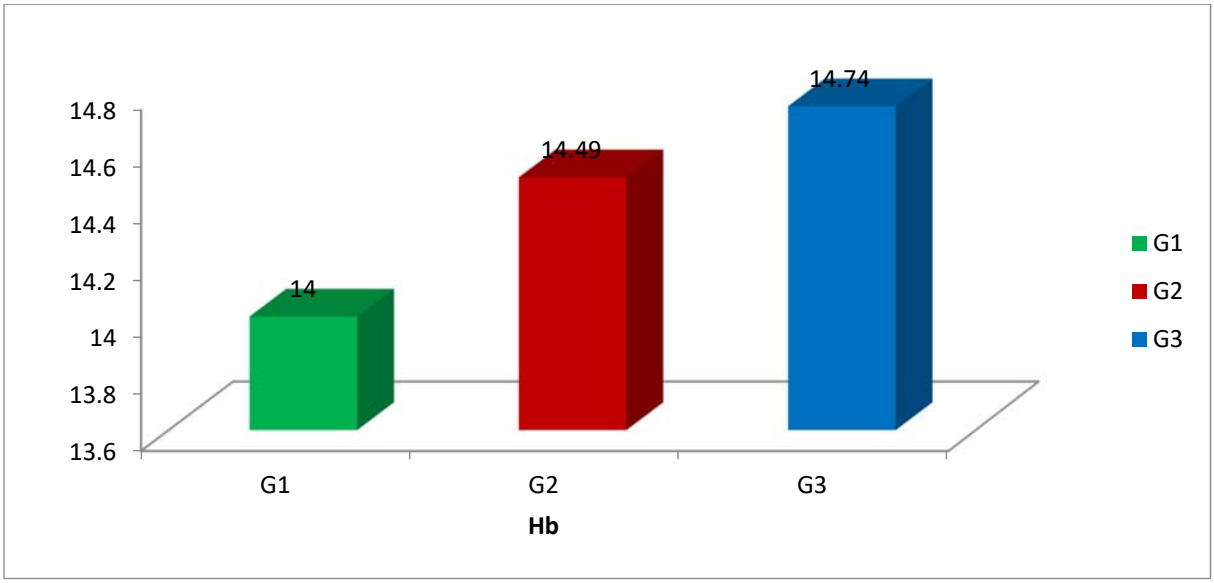


Figure (3 –2):Levels of Hb mg/dl in G1, G2 and G3

From previous study showed that cigarette smoking and narghile cause many health problems in people, this agreement with resent study that also observed cigarette smoking and narghile have severe adverse effects on blood parameters such as Hb and PCV among the study population. In this study it was found that, hemoglobin concentration and PCV levels increased significantly in cigarette and narghile smokers when compared with non-smokers. This indicates that cigarette and narghile smoking can develop polycythemia in the future due to high levels of carboxyhemoglobin, which causes dramatically hypoxia and causes increased RBCs[58].

The increase in hemoglobin concentration is thought to be mediated by exposure to carbon monoxide, and some scientists have proposed that increasing the hemoglobin level in smokers' blood can be a compensatory mechanism. Carbon monoxide binds to Hb to form a carboxy hemoglobin, an sluggish form of Hb that has no oxygen carrying capacity. Carboxyhemoglobin converts the Hb dissociation curve on the left side, resulting in a decrease in Hb's ability to deliver oxygen to the tissues. To compensate for the low oxygen delivery capacity, smokers retain a higher level of hemoglobin than non-smokers[59].

Increased erythrocytes and PCV values in male smokers can be explained by the fact that hypoxia in tissues caused by increasing the creation of carboxy hemoglobin leads to increase the excretion of erythrocytes, there, by increasing erythrocytes. The carbon monoxide (CO)from tobacco smoke also leads to an increase in capillary permeability which reduces the volume of plasma, which ultimately mimics polycythemia, which is characterized by increasing the share of red blood cells in the volume of blood. [60].

In this study we note a difference when comparing the age of cigarette smokers and narghile smokers this difference is due to the selected group be small size and the number of smoking be in adults less by virtue of their young age .

In this study, a difference in body mass index in smokers compared with non-smokers was due to the difference in metabolic rates, dietary patterns and percentage of body fat between the selected groups as well as the age of the selected group and the duration of smoking have an effect on that.

3.3 Myeloperoxidase (MPO) :

Table (3-2): Mean ± SD of (MPO , Hcy , lipid profile and AIP) levels in control (G1), cigarette smokers (G2) and narghile smokers (G3) .

HS:p≤0.001 S:p<0.05 NS: p>0.05

Parameters	G1 No. (30)	G2 No. (30)	G3 No. (30)	G1 vs G2	G1 vs. G3	G2 vs. G3
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	5.54 ± 1.36	HS	HS	NS
TC(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 ± 13.91	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
AIP	0.15 ± 0.12	0.22 ± 0.09	0.28 ± 0.10	S	HS	S

Result in table (3 – 2) and figure (3- 1) show the(mean ± SD) of myeloperoxidase levels in studied groups a highly significant increase (P≤ 0.001) in sera of G3 (32.10± 7.29) U/L and G2 (26.30 ± 5.48) U/L when compared with G1 (21.60 ±5.07) U/L respectively, was observed the results show highly significant increases (p ≤0.001) when compared MPO levels between G2 and G3 that noticed level of MPO higher in G3 than in G2. Another study agreement

with this study that demonstrated MPO levels expressed into the azurophilic grains of neutrophils and a low amount into monocytes that known as oxidative enzyme .

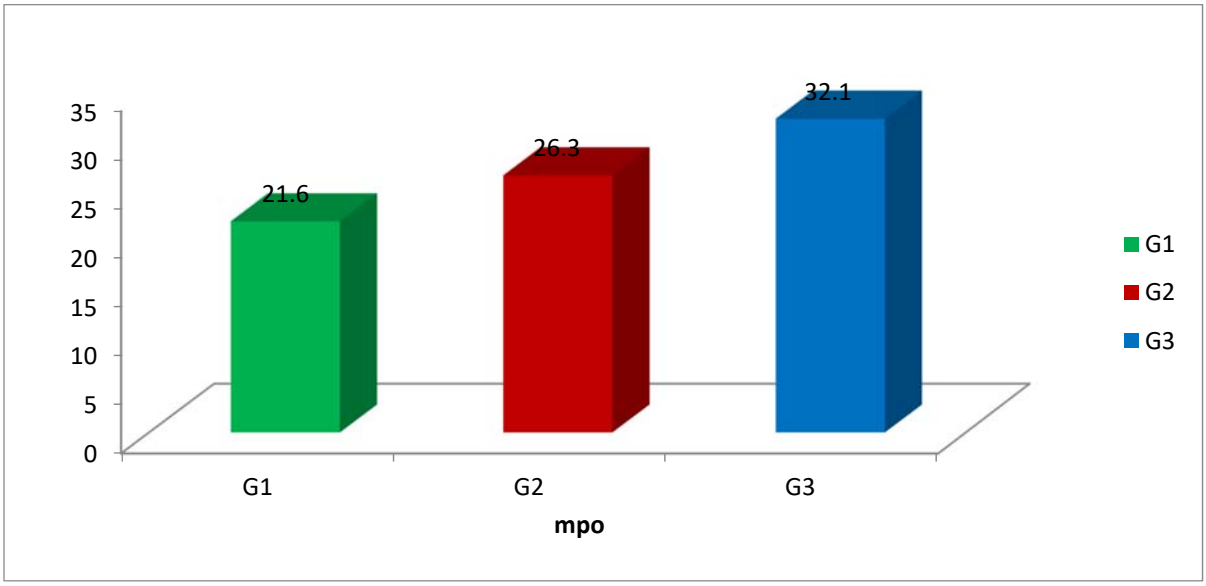


Figure (3- 3): Levels of MPO (U/L) in G1, G2 and G3.

As well as, the mechanism that explain the biological role for MPO action was hypothesis that when MPO react with H_2O_2 to form MPO-H_2O_2 compound then produce hypochlorous (HOCl) ,Oxygen chloride (OCl), chloride ion (Cl^-).

However, hypochlorous is the physically powerful oxidant in the active neutrophils ,the noxiousness of H_2O_2 increases when it reacts with MPO enzyme. In additional important mechanism effect of MPO led to depletion of NO ; and led to of endothelial dysfunction [61]. myeloperoxidase consider as catalytic sink for NO, weaken nitric oxide related vasodilation, and decreases NO bioavailability in the cultured cells [62].

MPO Levels in the blood serve as a strong indicator of endothelial dysfunction in human cells. They suggested that MPO-mediated of endothelial dysfunction may be a major mechanical link between oxidative inflammation which has a key role in cardiovascular disease[21].

Recently study demonstrated that smoking is an important risk factor for the onset and development of coronary atherosclerosis and this agreement with other study that emerge the effect of smokers are at risk of complications of coronary artery disease (CAD), vasodilation, peripheral artery disease (PAD) or stroke[63].

Smoking increases blood pressure, increases the levels of low-density lipoprotein (LDL) and cholesterol (TC)levels, facilitates oxidation and promotes plaque build-up 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. 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 to increase the vascular diseases and heart diseases[64].

Other study showed that nicotine stimulate nucleotide neutrophils, as well as nicotine activation adenine phosphate in the nucleotide, which leads to reduced the oxidase production[65].

Other study also agreement with present study by noticed the levels of MPO derived from neutrophils are higher in smokers compared to nonsmokers when study suggested PMN is stimulated within the circulatory system, due to released MPO in the blood stream with its substrates. By other hand clinical trials over the past decade have also discovered of MPO is not only essential role in the pathophysiology of hemorrhagic vascular disease, but it is also a valuable diagnostic biomarker in patients with acute coronary syndrome[66]. The Mpo has been widely linked to many aspects of human cardiovascular disease and it is believed that this enzyme acts on both the initiation and propagation of cardiac pathologies [67].

The information of MPO plasma levels are already high when plaque is ruptured and thus happened before myocardial necrosis - support the hypothesis

that MPO would be suitable as an early sign of acute coronary syndrome. In fact ,increasing body of evidence now indicates that MPO produce from activated PMNs significantly due to initiation and development of smoking-dependent blood vessels in atherosclerosis. So atherosclerosis caused by smoking via decrease the bioavailability of NO ventricular due to increasing LDL, HDL oxidation, arterial plaque development and instability .However, MPO is a major biomarker contributor to the progress of coronary artery disease in smokers and may consider as a new biomarker in patients with cardiovascular disease[29].

While other study demonstrated the relationship between asbestos exposure and cigarette smoking and highly increasing in MPO consecration due to lung cancer [68].

Other study findings that relationship provided insight into the interaction between MPO-related chlorinating activity with other biomarkers, expressing different domains like neurohormonal, inflammatory, metabolic-nutritional, and oxidative stress, all potentially interested in the prognosis of heart failure[69].

Finally, production of reactive oxidant species by MPO-catalyzed .Pathways may have a substantial effect on the promotion of inflammatory events that due not only to immune defenses but also to the tissue damage that outcome from a range of inflammatory conditions, consist of atherosclerosis.

The MPO appears to join in a range of actions involved in the initiation, propagation and subsequent complications of atherosclerotic plaque. In this results the components of the MPO pathway represent agreeable targets for the enhancement of prognostic biomarkers and therapeutic interventions to stop atherosclerotic cardiovascular disease [70].

The present study regard as the first study deal with the sampling directly Community samples were taken from the Coffey (Bekhal Coffey) in Palestine

street and like those study , thought highly significant for appear the side bad for smoking by cigarette and narghile that more spreader at present so to occurrence the relationship between the newly biomarker MPO in sera of those cohort groups to predict their atherosclerosis so that consider one the studies designed to serve society

3.4 Homocysteine (Hcy) levels :

Results in Table (3-2) and Figure (3- 2) show (Mean ± SD) levels of Hcy in sera of G1, G2 and G3. The results show a highly significant ($p \leq 0.001$) increase in levels of both G2(5.97 ± 1.59) µ mol/L and G3 (5.54± 1.36) µ mol/L when compared with G1 (4.01 ± 1.11) µ mol/L and there was non-significant ($p > 0.05$) different in Hcy level between and G3 when compared between them there fore, the finding for this study agreement with another study that noticed highly significant increase between patients group with coronary artery diseases in indian patients and control group.

So, There is found no significant correlation between plasma homocysteine level and other conventional risk factors of cardiovascular diseases hypertension, diabetes mellitus, smoking, and alcohol consumers[71].

Other study found that hemorrhagic, and intracranial hemorrhage incidence, independent of long-recognized factors like hyperlipidemia, hypertension, diabetes mellitus, and smoking[43]. Also, elevated levels of HCY are implicated in the development and progression of vascular disease. Homocysteine has a role in cardiovascular morbidity and mortality with its atherogenic and prothrombotic properties.

The metabolite of homocysteine can accompany with LDL-cholesterol to synthesis foam cells and atherosclerotic plaques. Produce free radicals during the oxidation of homocysteine may be directly injured endothelial cells [72].

Led to platelet aggregation after that produce pro-aggregator effects of homocysteine. A result of this process impairs shows that the production of nitric oxide through prolonged exposure of endothelial cells to homocysteine more studies demonstrated the linked between hyperhomocysteinemia and myocardial infarction and frequency coronary events. Homocysteine leukocyte induction by up-regulating monocyte chemoattractant protein-1 and interleukin-8 expression and excretion, so that homocysteine raise smooth muscle cell proliferation and enhance collagen production[73].

It is known that HCY led to endothelial dysfunction and has been refer to impaired bioavailability of NO , first mechanism for reduced bioavailability of NO is mediated via asymmetric dimethyl 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. Elevated plasma levels of ADMA have been related with HCY and endothelial dysfunction in both animals and humans. Apart from inhibiting the production of NO. The 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[72].

It is worthwhile this study consider the first study in Iraqi exactly in Baghdad city for determination of homocysteine level in cohort groups and compare this parameters in G2 and G3. The result show homocysteine levels was in border line with normal range so it thought this fact depend the nature of the group, for example, the number of times taken cigarette or narghile daily, as well as depend on small size group was selected and period of smoking because the sampling was younger.

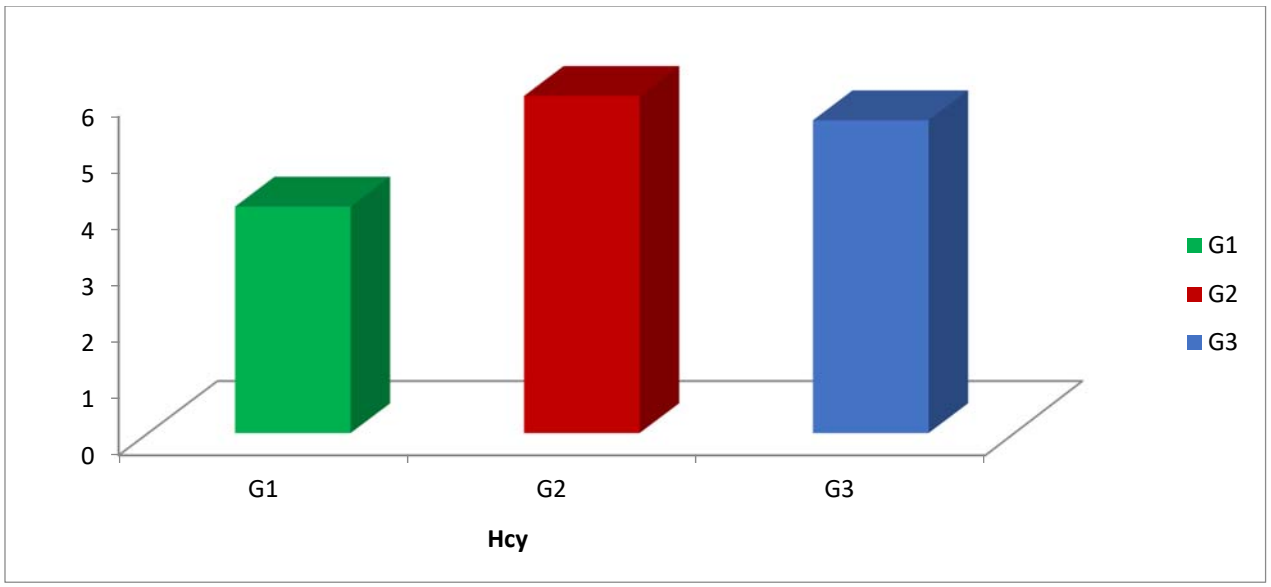


Figure (3 –4): Levels of HCY µ mol/L in G1 , G2 and G3.

3.5 Lipid profile:

The result in Table (3 – 2) and Figure (3 – 3) represent the levels of TC in G1,G2 and G3. The present study showed non-significant increase(p> 0.05) was found in sera of G1 (156.77± 15.61) mg/dl when compared with G2 (159.13± 17.75) mg/dl , while there was significant increase (p< 0.05) when compared between G1 and G3 (164 ± 12.18) mg/dl, the study showed also non-significant increase (p> 0.05) in G3 when compared with G2.

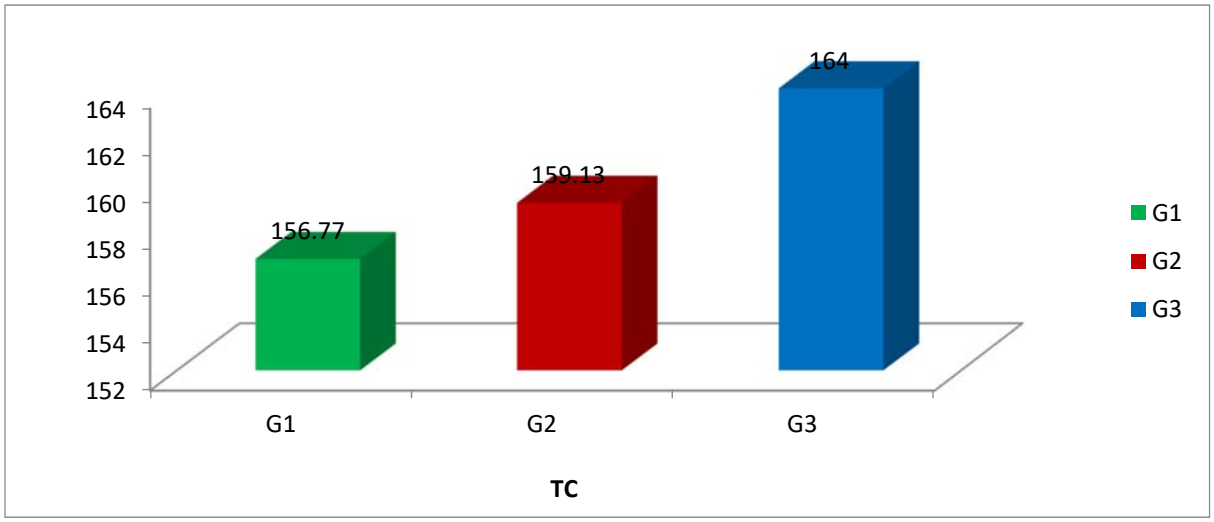


Figure (3 –5) : Levels of TC mg/dl in G1 , G2 and G3.

The present study showed significant increase ($p<0.05$) when compared G2 (73.8 ± 13.77)mg/dl with G1(65 ± 15.61) mg/dl ,the study showed also highly significant increase ($P\leq 0.001$) for TG levels in sera of G3 (79.91 ± 13.91) mg/dl when compared with G1, but there was non-significant ($p>0.05$) between G3 and G2

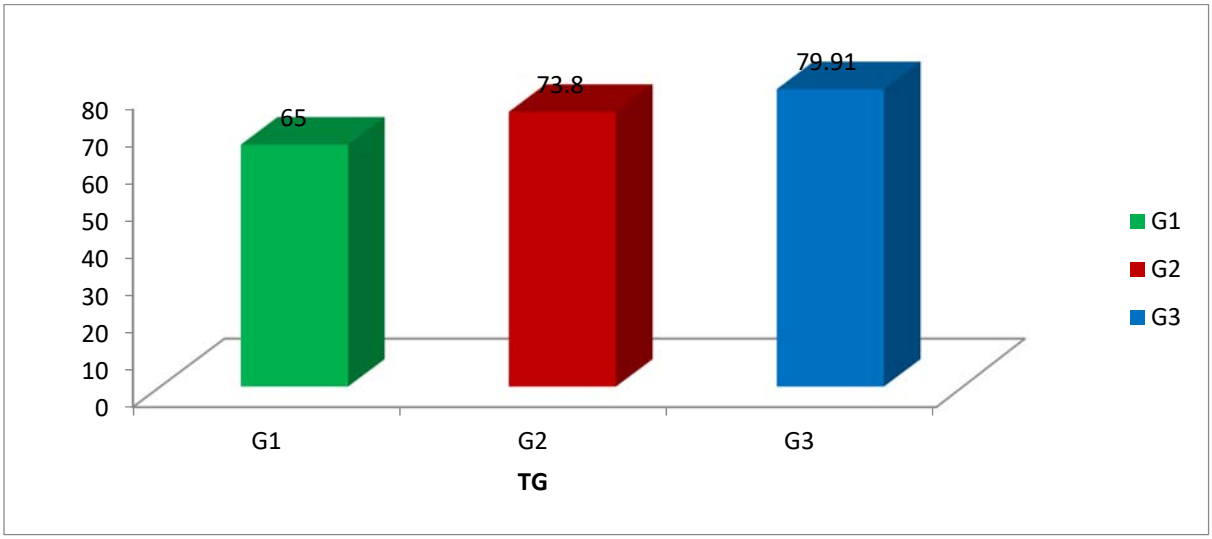


Figure (3 –6): Levels **of TG mg /dl in G1 ,G2 and G3**

The present study showed non-significant decrease ($p>0.05$) in HDL for In sera of G2 (43.33± 3.30) mg/dl when compared with G1 (44.53±3.10) mg/dl but there was a highly significant decrease ($P\leq 0.001$) for G3(41.47± 2.92) mg/dl when compared withG1.as well as there was significant decrease ($p<0.05$) when compared G2 and G3

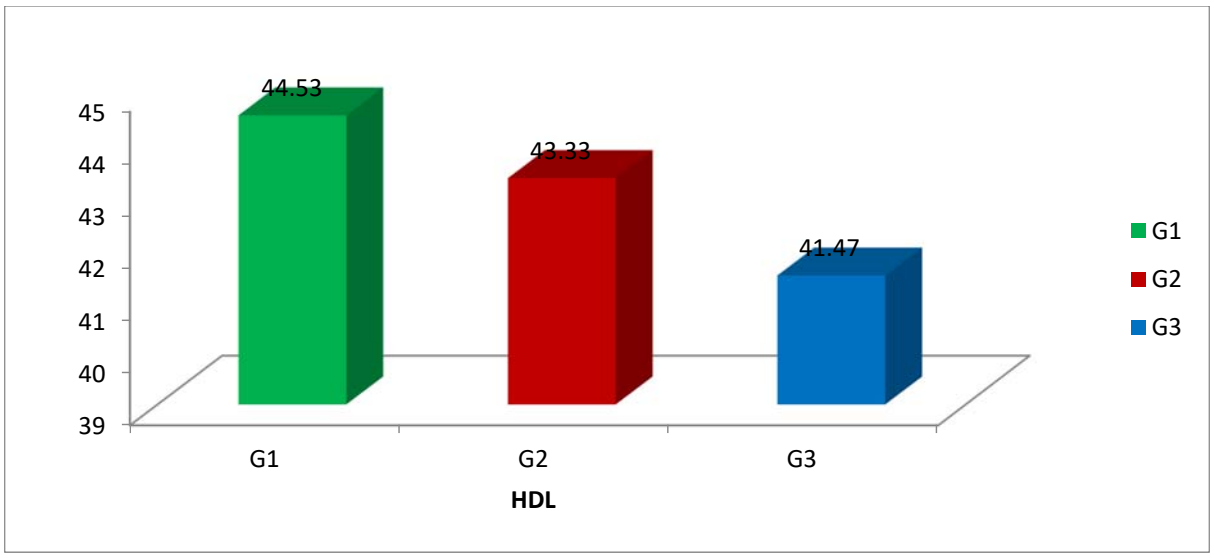
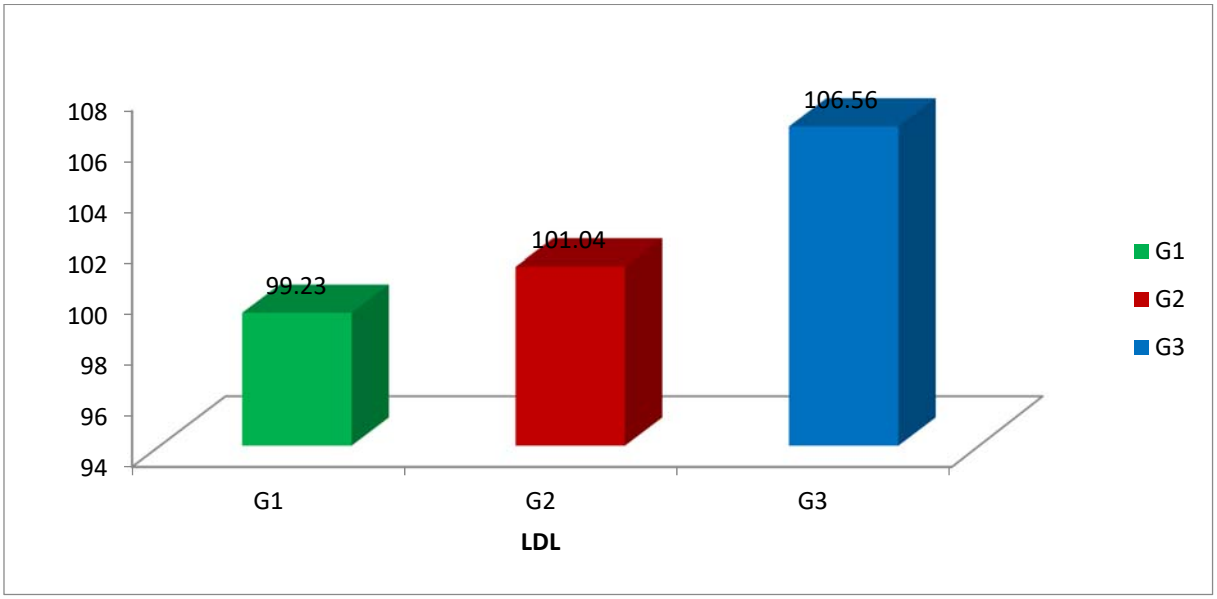


Figure (3 –7) :Levels of the HDL mg /dl in G1 , G2 and G3

The study showed non-significant increases($p > 0.05$) in LDL levels for G2 (101.04 ±19.11) mg/dl when compared with G1 (99.23 ± 16.29) mg/dl as well as there was significant increase ($p < 0.05$) in G3 (106.56± 12.24) mg/dl when compared with G1 .the study also showed non- significant ($p > 0.05$) differences when compared between G3 and G2.



Figure(3 –8): levels of LDL mg/dl in G1, G2 and G3

The present study showed a significant increase ($p<0.05$) in VLDL level when compared G2 (14.76 ± 2.75)mg/dl with G1(13 ± 3.12) mg/dl .Study

showed also highly significant increase (P≤ 0.001) in VLDL levels in sera of G3 (15.98 ± 2.80) mg/dl when compared with G1, but there was non-significant differences between G3and G2.

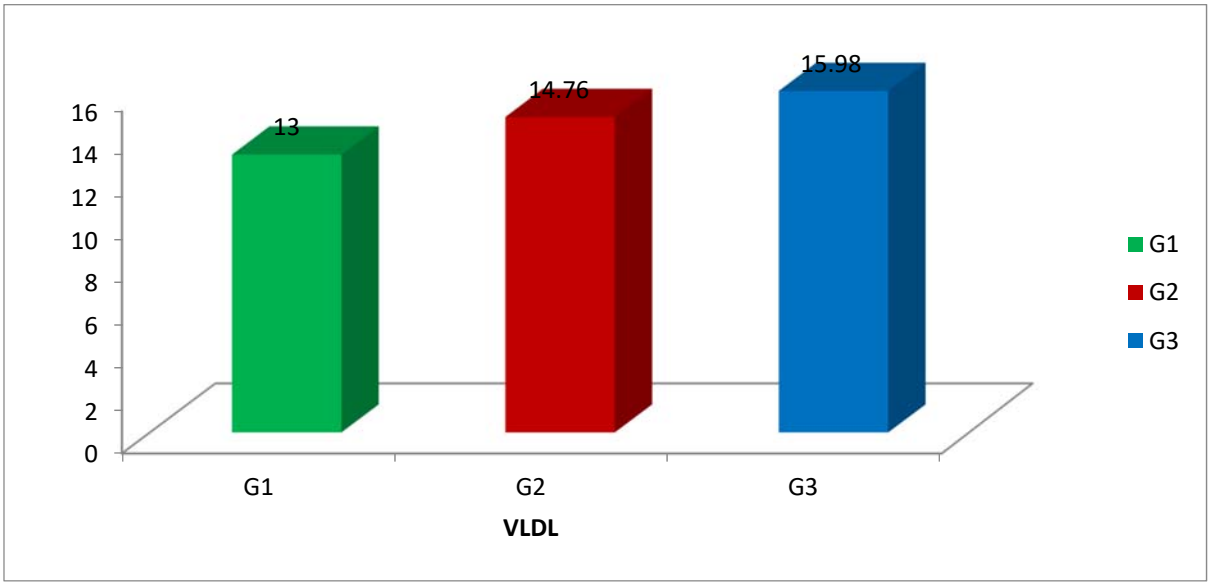


Figure (3–9): levels of VLDL in G1 ,G2 and G3

The study presented a high concentration for triglyceride, VLDL in G2and G3 compared with G1 as shown in table (3-2) . This study agreement with other study which 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 catecholamines[74]. These excess free fatty acids in the liver due to increase the formation of triglyceride and sulfur, and this increasing the concentration of triglyceride and VLDL in the blood .This result for current study disagreement with other study and this difference due to the lack of smoking period compared to other studies that took the group of smokers for long periods duration [75].

Several studies have reported a high levels of homocysteine in plasma in chronic smokers. Homocysteine plasma is negatively associated with HDL and Apo A-I[76]. In addition, another study reported that MPO enzyme has also been shown to be implicated in lipid peroxidation and nitrate by generating a

product of oxidation,NO_2. MPO also have ability to oxidize low density lipoprotein and high density lipoprotein , 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[77].

Other study illustrated the relationship between monocyte to high density lipoprotein cholesterol ratio (MHR) in smoker and non-smoker group the suggested elevated MHR is relationship with cigarette smoking and may be a useful biomarker of systemic inflammatory response in smokers. Smoker who has high MHR levels can easily be recognized during routine complete blood count (CBC) analysis and could possibly assistance from preventive treatment[78].

Other study demonstrated that cigarette smoking group produce substantial amount of oxidative stress noticed smoke inhaled by cigarette or shisha lead to increase effect of nicotine compound due 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 [79].

3.6 Atherogenic index of plasma (AIP) :

This study showed that the AIP levels that significant increase ($p< 0.05$) in G2 (0.22± 0.09) when compared with G1 (0.15± 0.12) but there was significant increasing ($p\leq0.001$) for G3 (0.28 ± 0.10) with G1 , and G2 were found significant increase ($p< 0.05$) between G3 and G2.

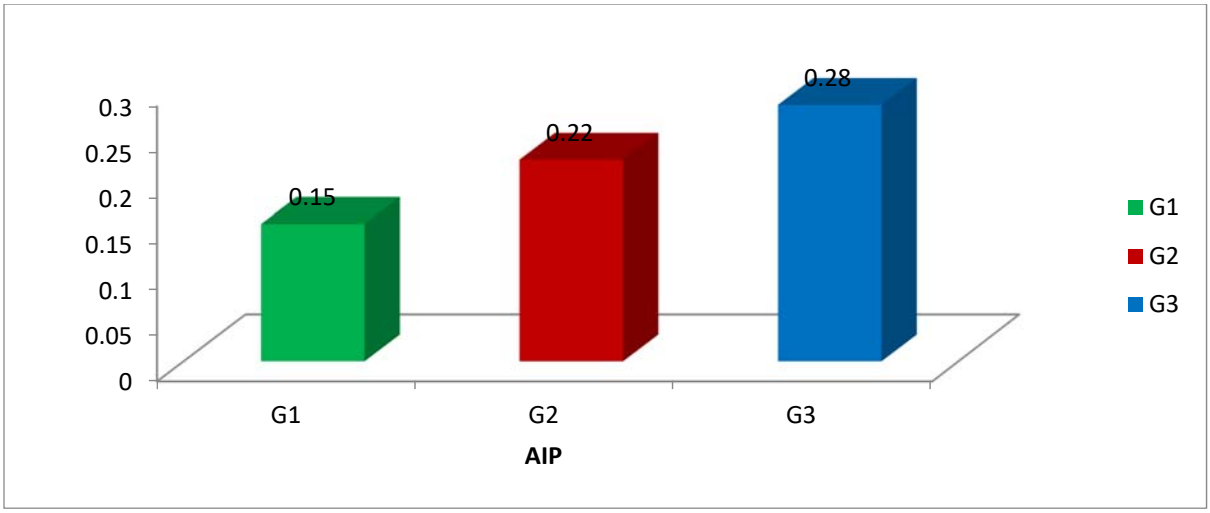


Figure (3–10) :Levels of Atheros Index of plasma G1 , G2 and G3

This study showed, that the significant increase in AIP between smoking group via cigarette or narghile as well as we show smoker with narghile group highly significant than cigarette group and this agreement with other study which illustrated a strong correlation between AIP and lipoprotein particle size, therefore AIP could be considered as a biomarker of atherogenic lipoprotein status in cardiovascular disease [80].

3.7 Correlation Study:

Table (3 – 3): Correlation between MPO with HCY, TC, TG, HDL, LDL , VLDL , AIP , PCV and Hb in cigarette and narghile smokers groups :

parameter	MPO / G2		MPO /G3	
	r- value	**p-value**	**r- value**	**p-Value**
HCY	0.18	HS	0.04	HS
TC	0.31	HS	-0.03	HS
TG	-0.07	HS	-0.12	HS
HDL	-0.25	HS	0.12	HS
LDL	0.34	HS	-0.02	HS
VLDL	-0.07	HS	-0.12	HS
AIP	-0.04	HS	-0.15	HS
PCV	-0.26	HS	0.29	HS
HB	-0.23	HS	0.23	HS

In this study , Table (3-3) and Figure (3-11a,b) showed highly significant ($p \leq 0.001$) positive association between MPO ,HCY in G2(r =0.18) and G3 narghile (r = 0.04) respectively.

Table (3-3) and Figure (3-12a,b) show highly significant ($p \leq 0.001$) positive association between MPO and TC in G2(r =0.31) ,highly significant ($p \leq 0.001$) negative correlation with TC in G3 (r =-0.03).

Figure (3-13a,b) and Table (3-3) explained highly significant ($p \leq 0.001$) negative association between MPO and TG in,G2 (r =-0.07), and G3 (r =-0.12), respectively.

The findings in which are listed in Table (3-3) and Figure (3-14 a,b) explained highly significant ($p \leq 0.001$) negative correlation between MPO and HDL levels in G2 (r= -0.25), as well as highly significant positive association in G3(r = 0.12).

The data in Table (3-3) and Figure (3-15a,b) explained a highly significant ($p \leq 0.001$) positive association between MPO and LDL in G2 (r= 0.34), highly significant($p \leq 0.001$) negative association with LDL inG3 (r =-0.02).

The result that shown in Table (3-3) and Figure (3-16a,b) explained highly significant ($p \leq 0.001$) negative association between MPO and VLDL in G2 (r =-0.07),G3 (r =-0.12) respectively.

It can be seen from the data in Table (3-3) and Figure (3-17a,b) showed highly significant ($p \leq 0.001$) negative correlation between MPO and AIP in to cigarette (r =-0.04), narghile (r = -0.15) respectively.

In the current study, there was a highly significant ($p \leq 0.001$) negative correlation between MPO and PCV in cigarette (r =-0.26) ,highly significant($p \leq 0.001$) positive correlation in PCV in to narghile (r = 0.29), figure (3 -18a,b).

The findings in which are listed in Table (3-3) and figure (3-19a,b) showed highly significant ($p \leq 0.001$) negative correlation between MPO and Hb in cigarette (r =-0.23) ,highly significant ($p \leq 0.001$) positive correlation with Hb in to narghile (r= 0.23).

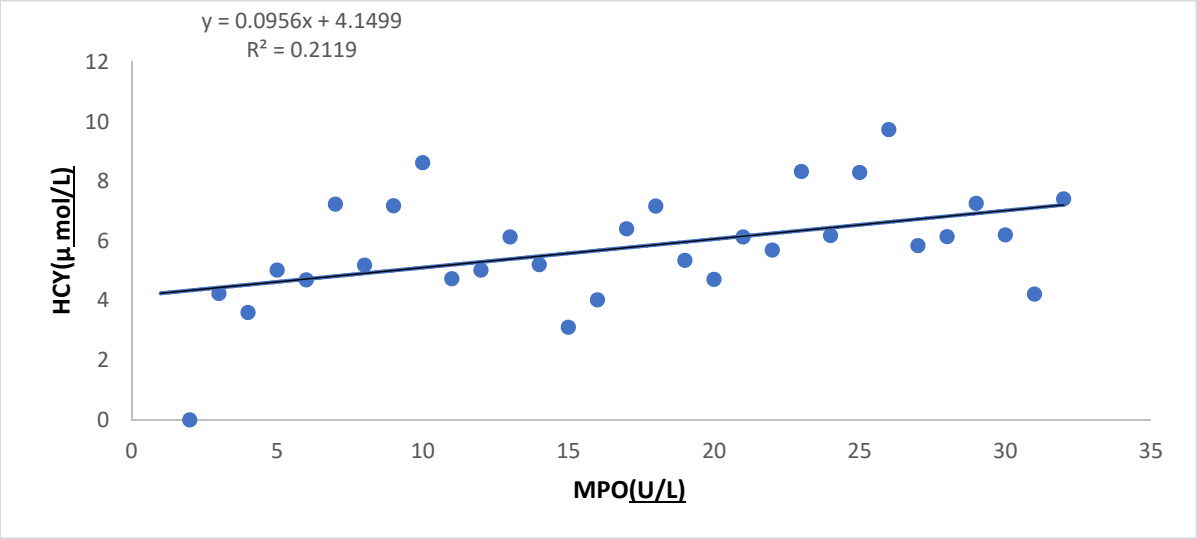


Figure (3- 11a): correlation between MPO and Hcy in G2.

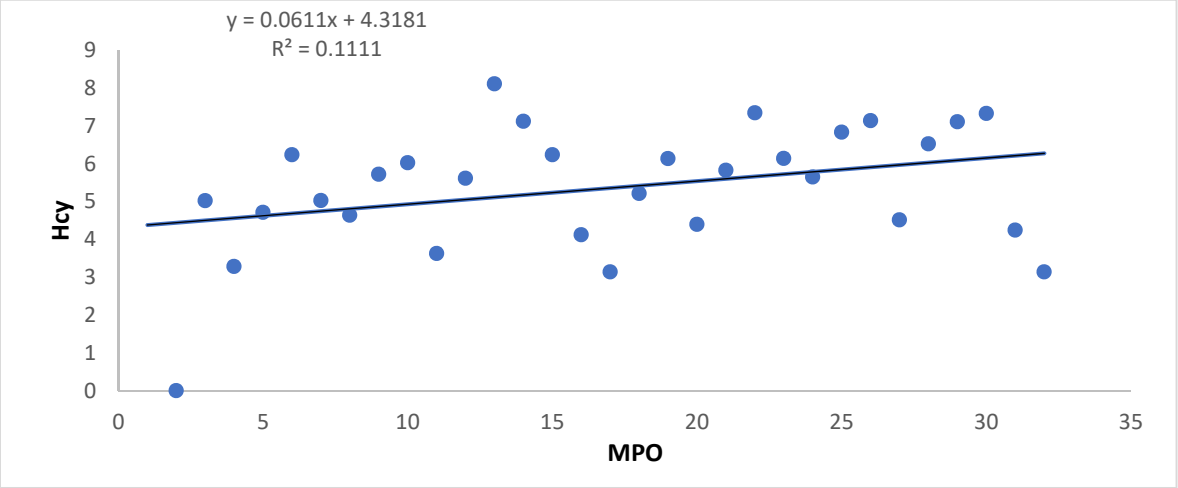


Figure (3-11 b): correlation between MPO and Hcy in G3.

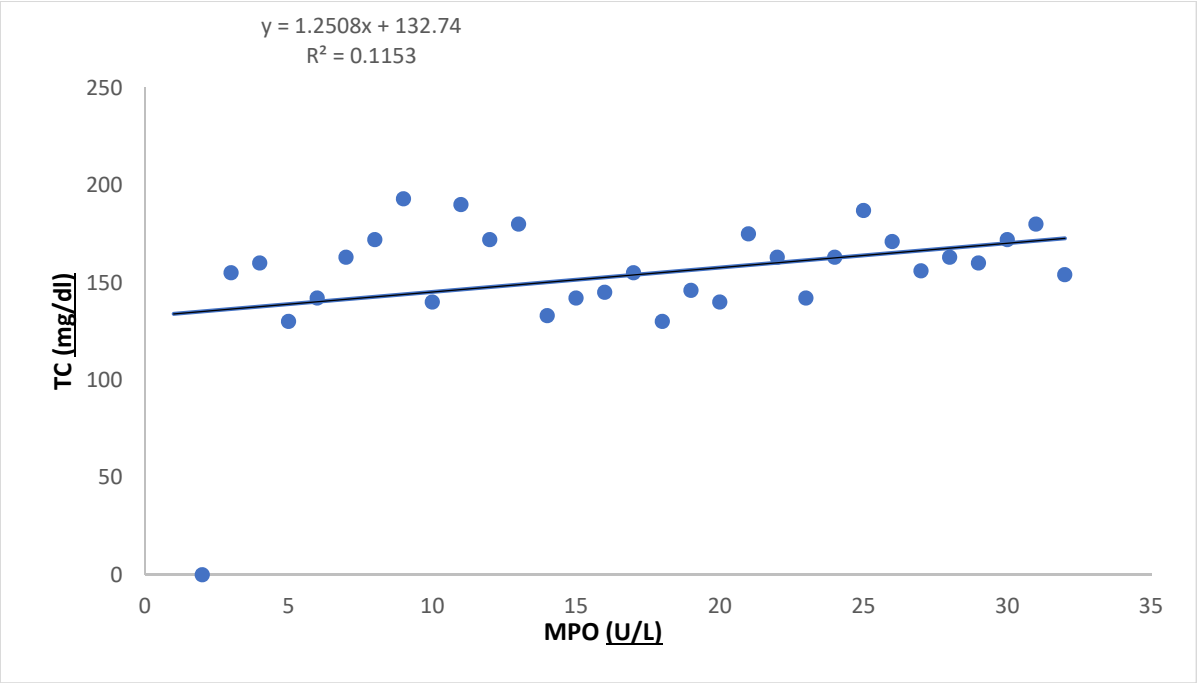


Figure (3- 12a): correlation between MPO and TC in G2.

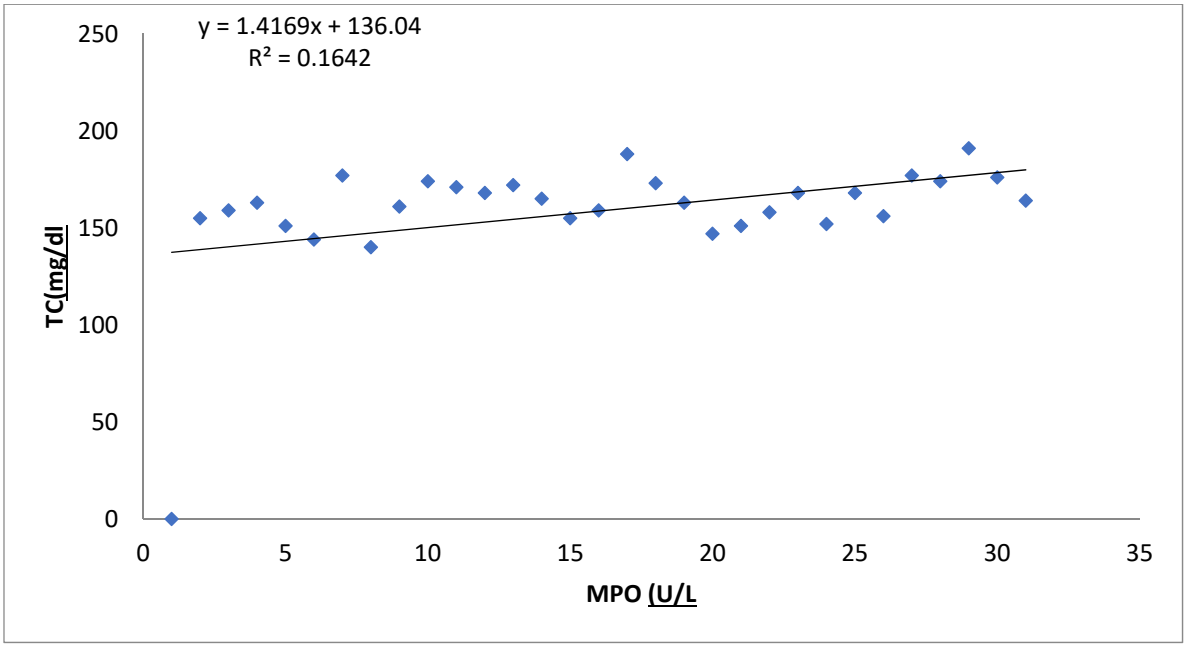
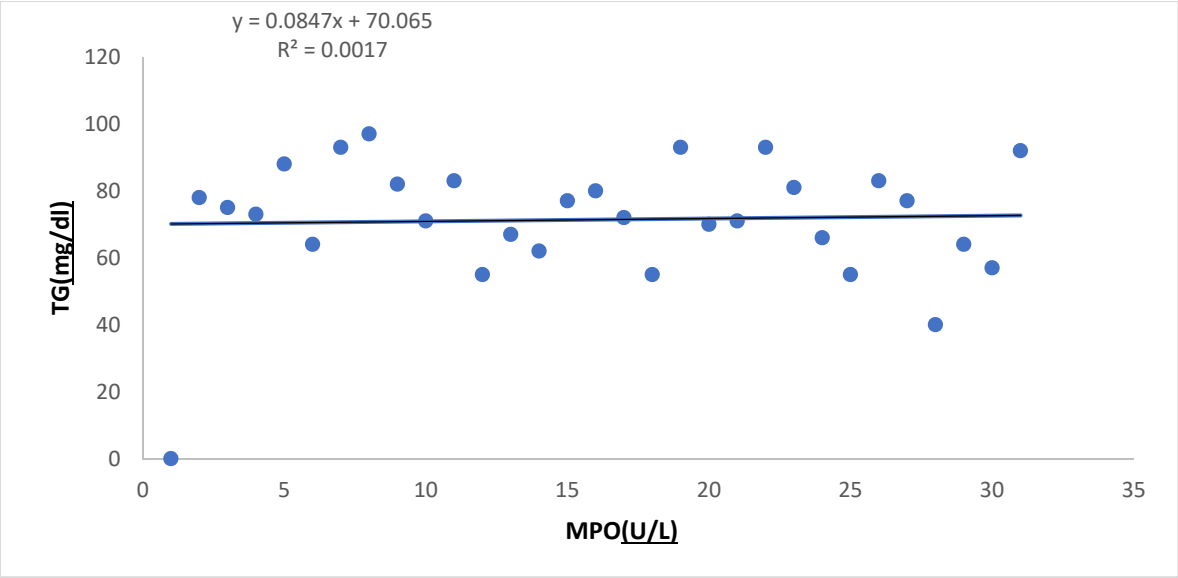


Figure (3-12 b): correlation between MPO and TC in G3.



Figure(3-13a): correlation between MPO and TG in G2 .

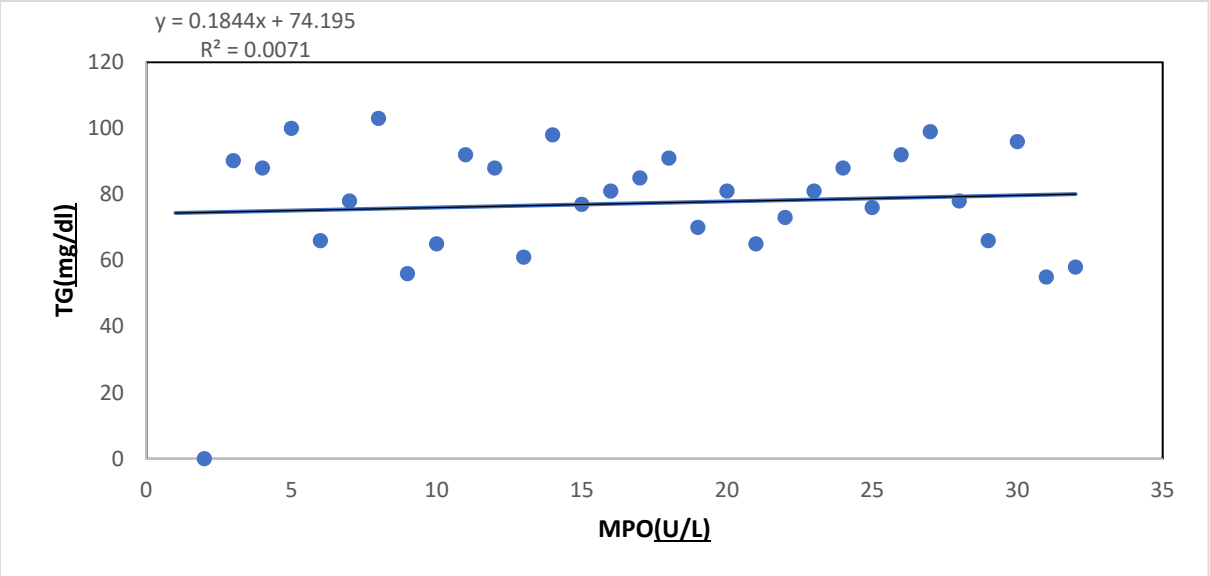


Figure (3-13 b): Correlation between MPO and TG in G3

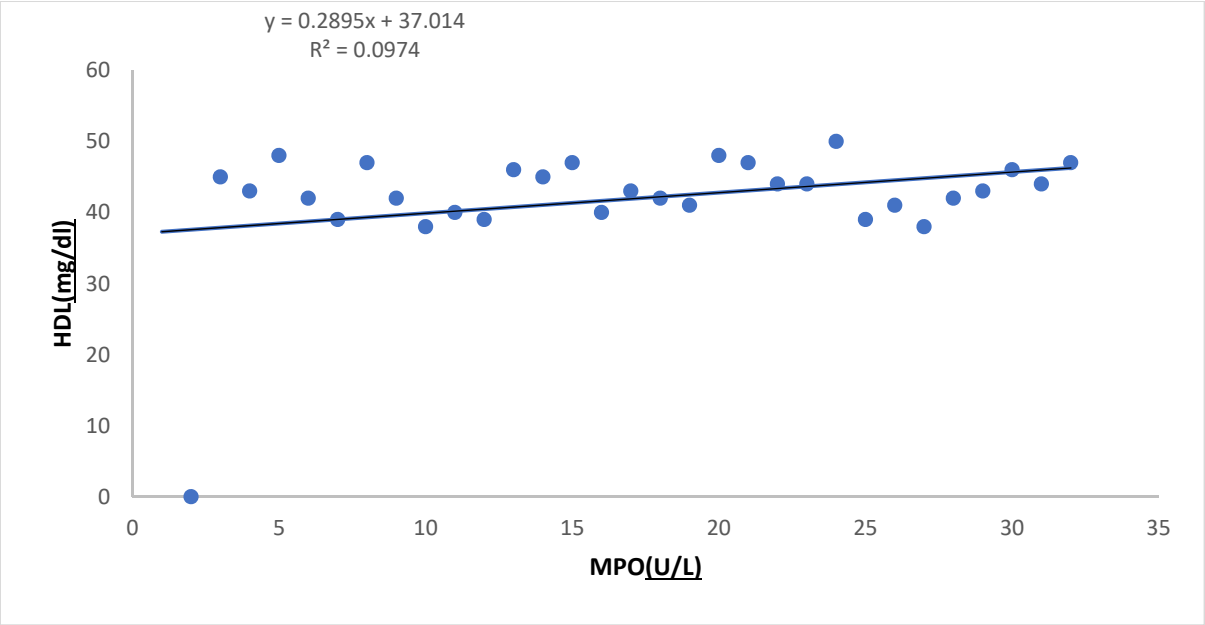


Figure (3- 14a): correlation between MPO and HDL in G2.

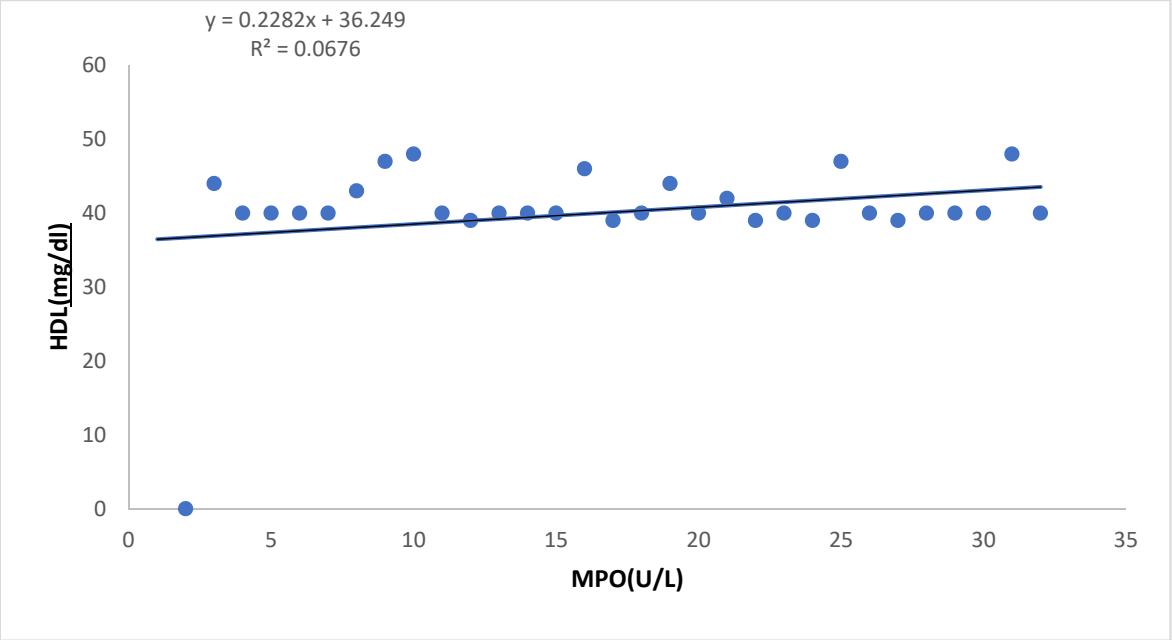


Figure (3-14 b): correlation between MPO and HDL in G3.

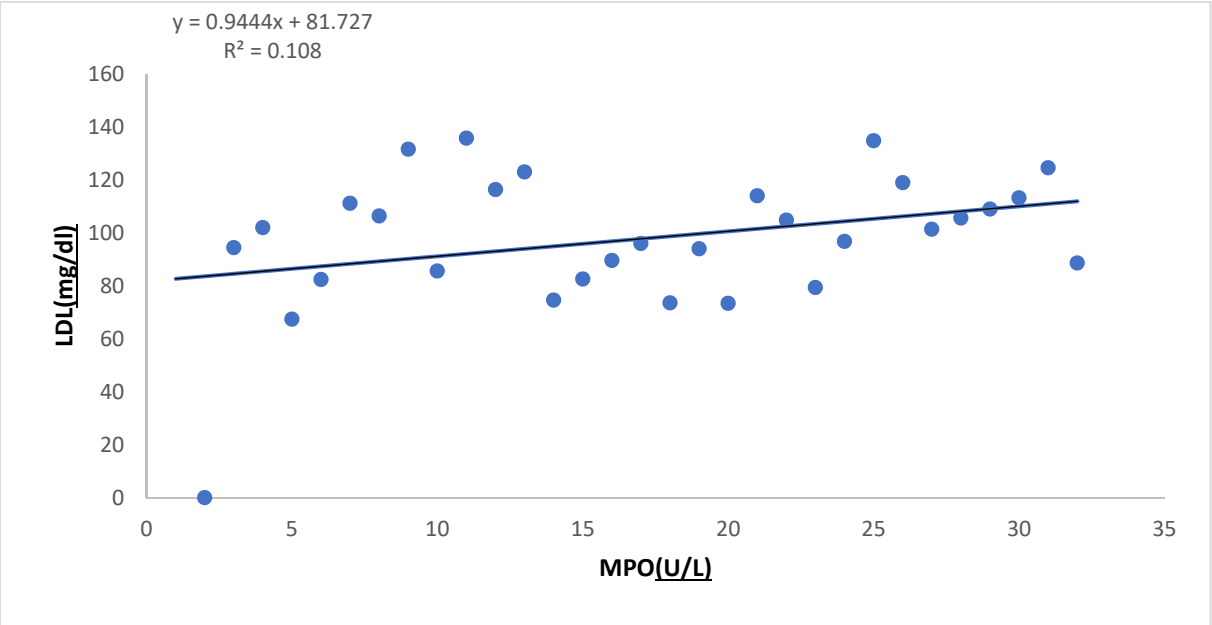


Figure (3-15a): correlation between MPO and LDL in G2.

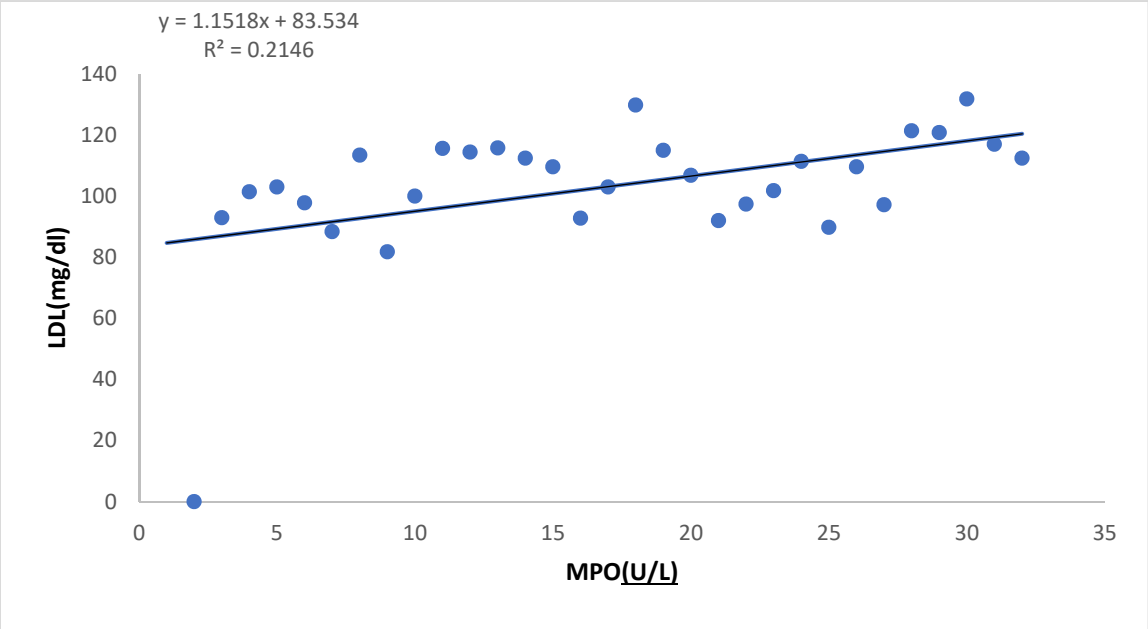


Figure (3-15 b): correlation between MPO and LDL in G3.

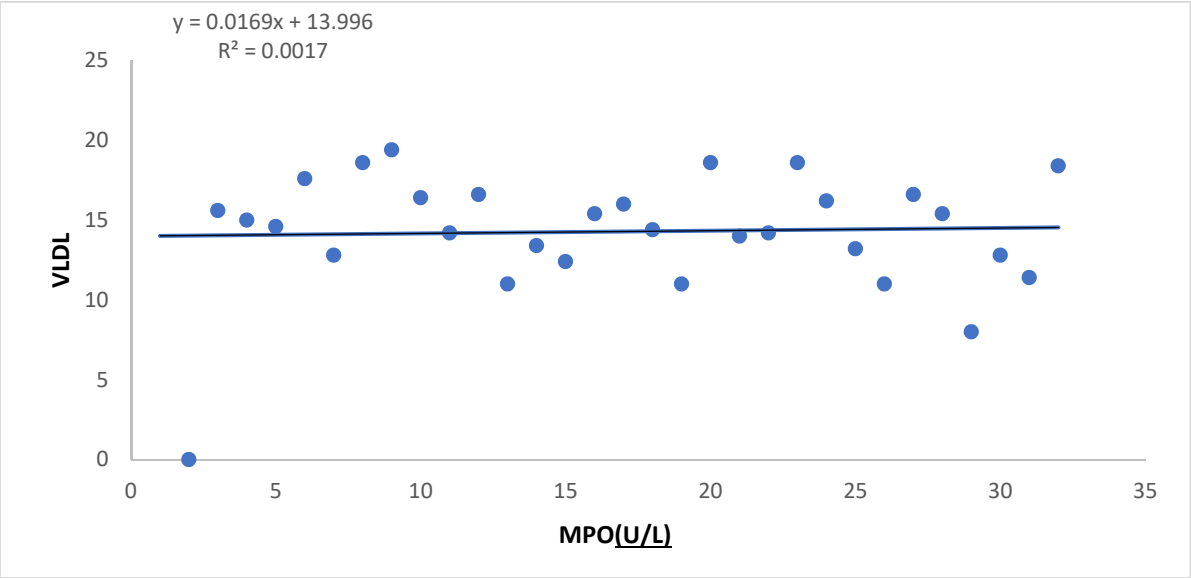


Figure (3- 16a): correlation between MPO and VLDL in G2.

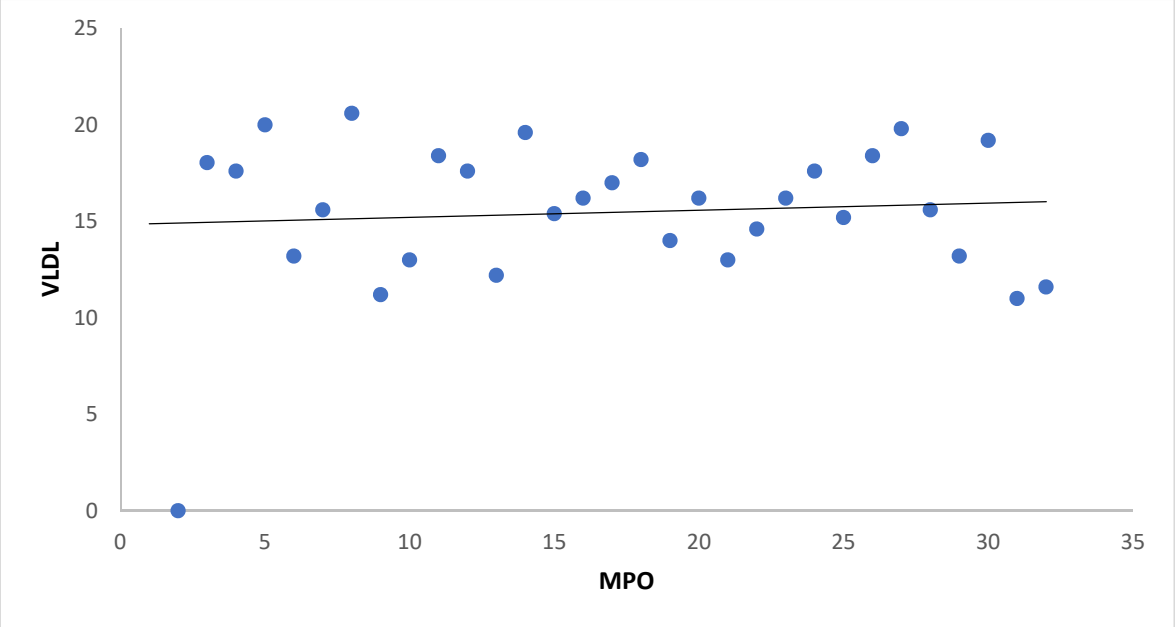


Figure (3-16 b): correlation between MPO and VLDL in G3.

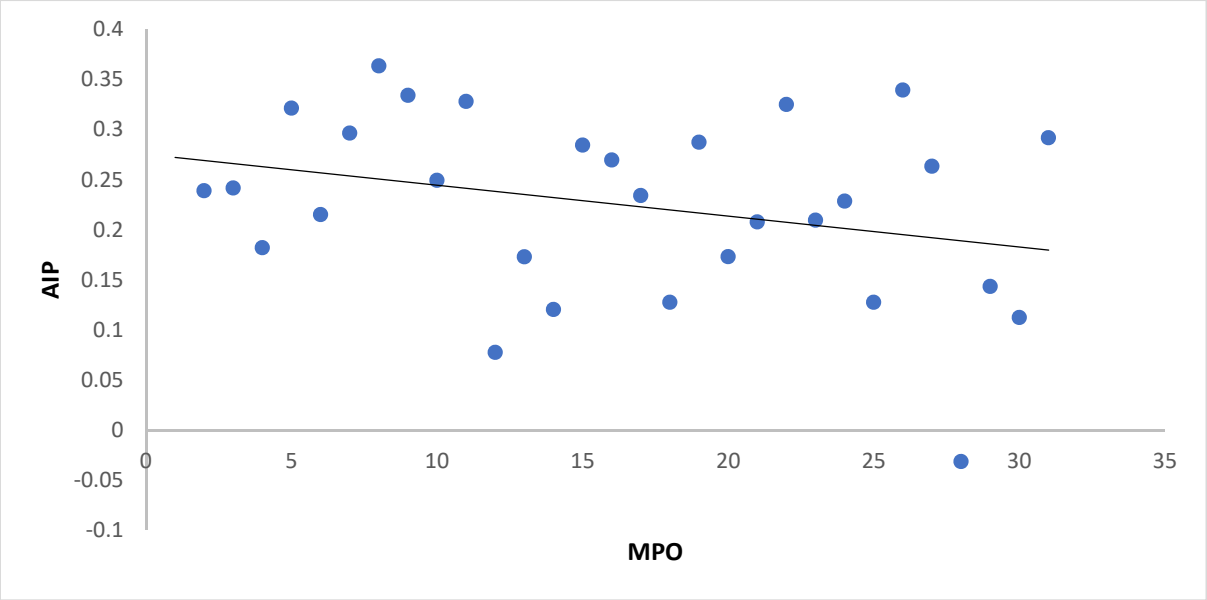


Figure (3-17a) : correlation between MPO and AIP in G2.

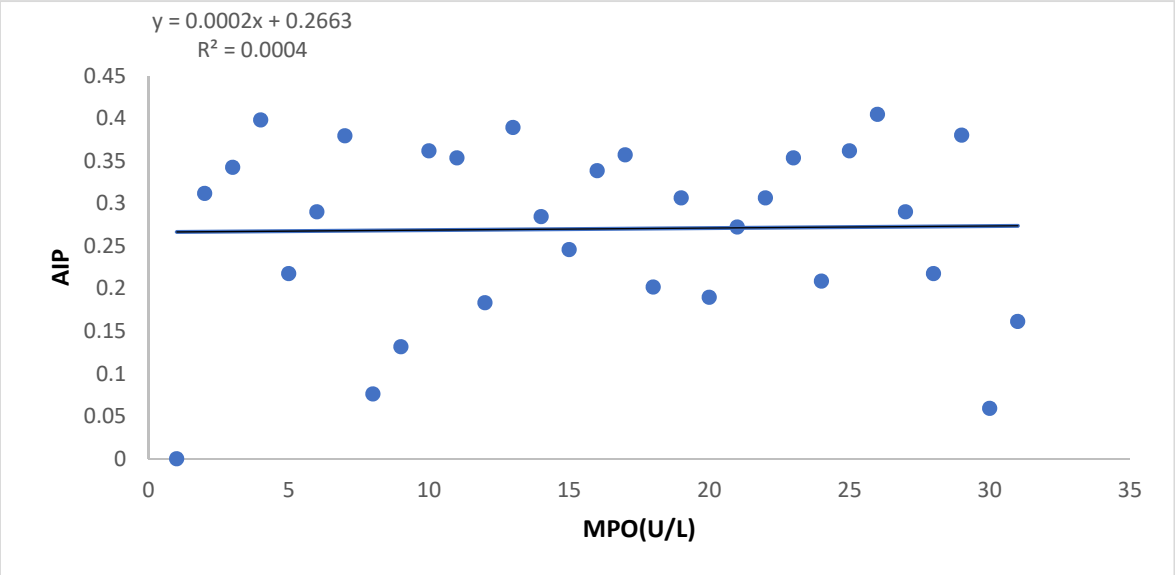


Figure (3-17b): correlation between MPO and AIP in G3 .

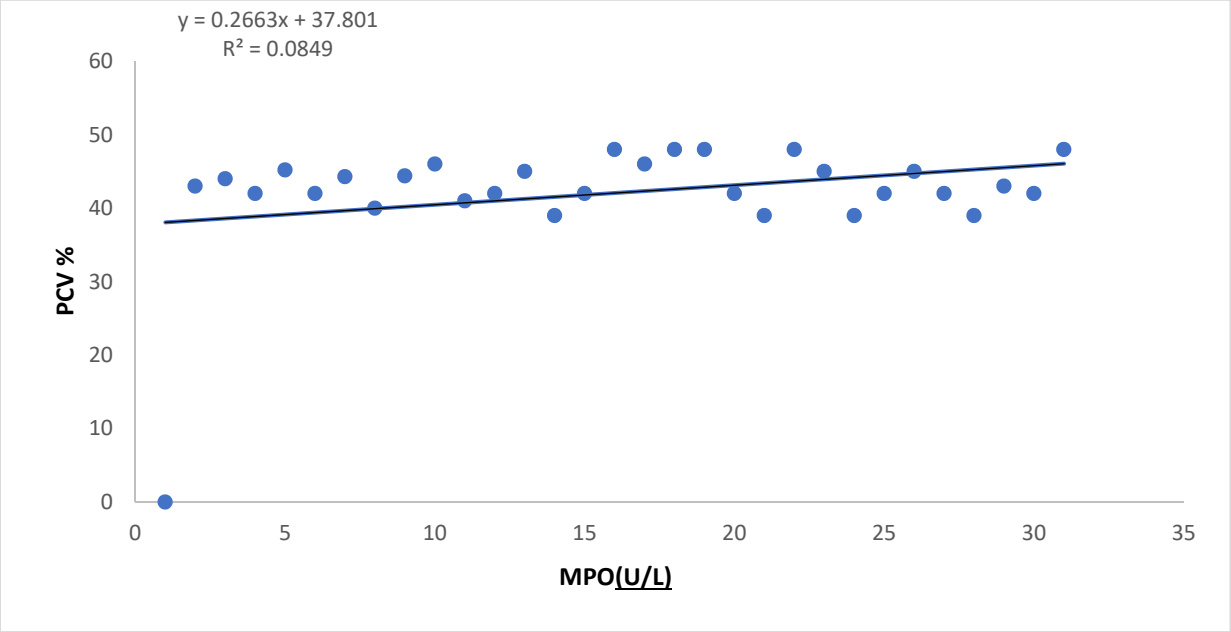


Figure (3- 18 a) : correlation between MPO and PCV in G2.

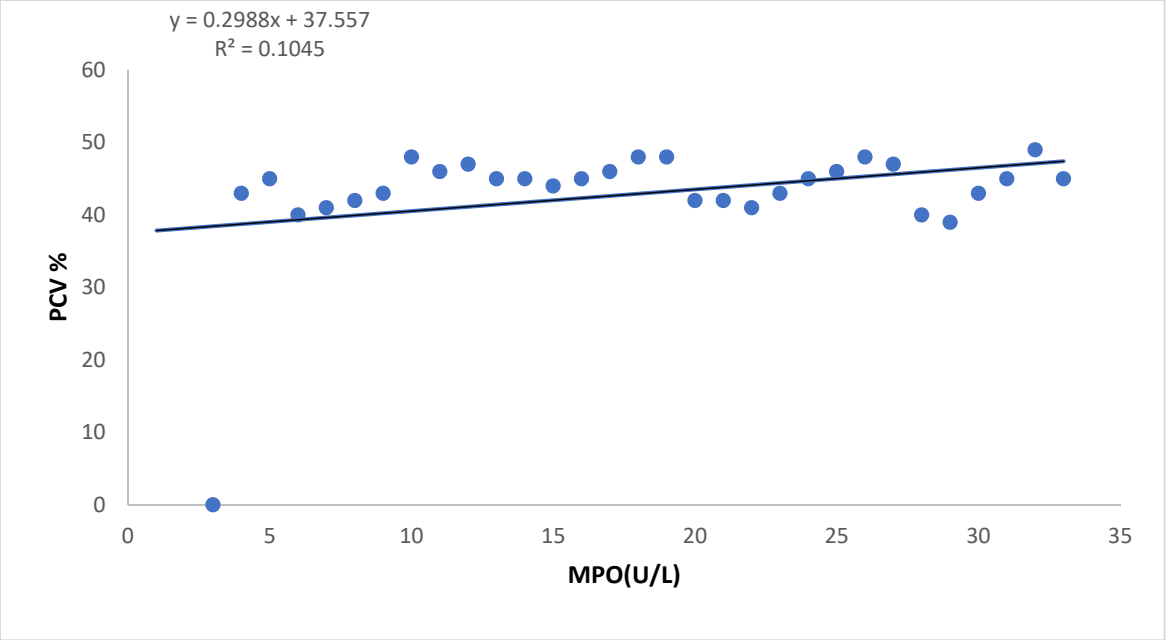


Figure (3-18 b): correlation between MPO and PCV in G3.

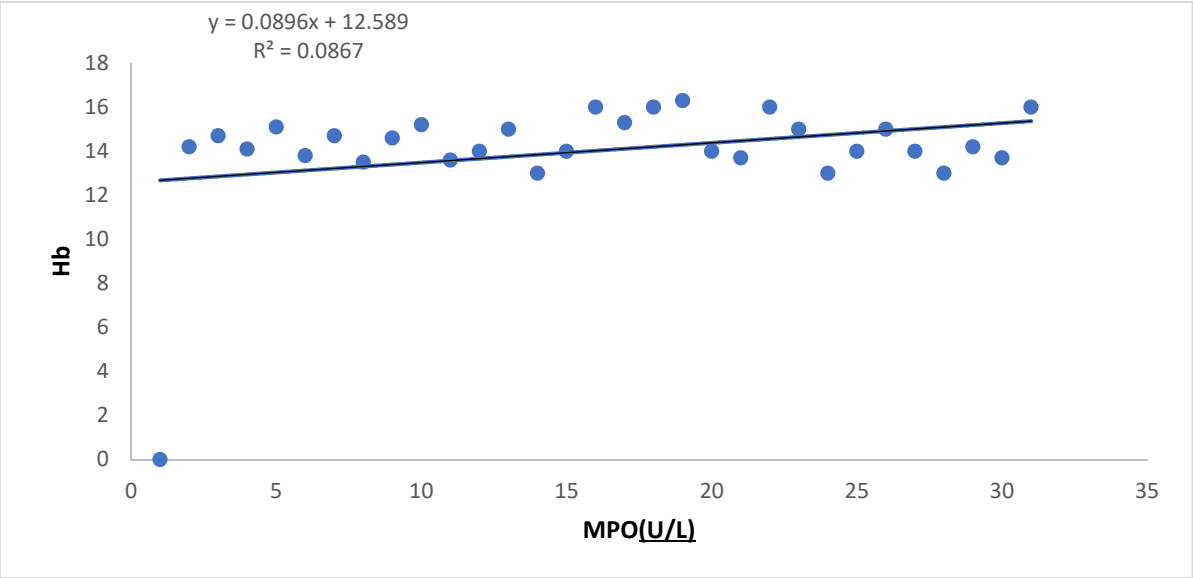


Figure (3- 19 a) : correlation between MPO and Hb in G2.

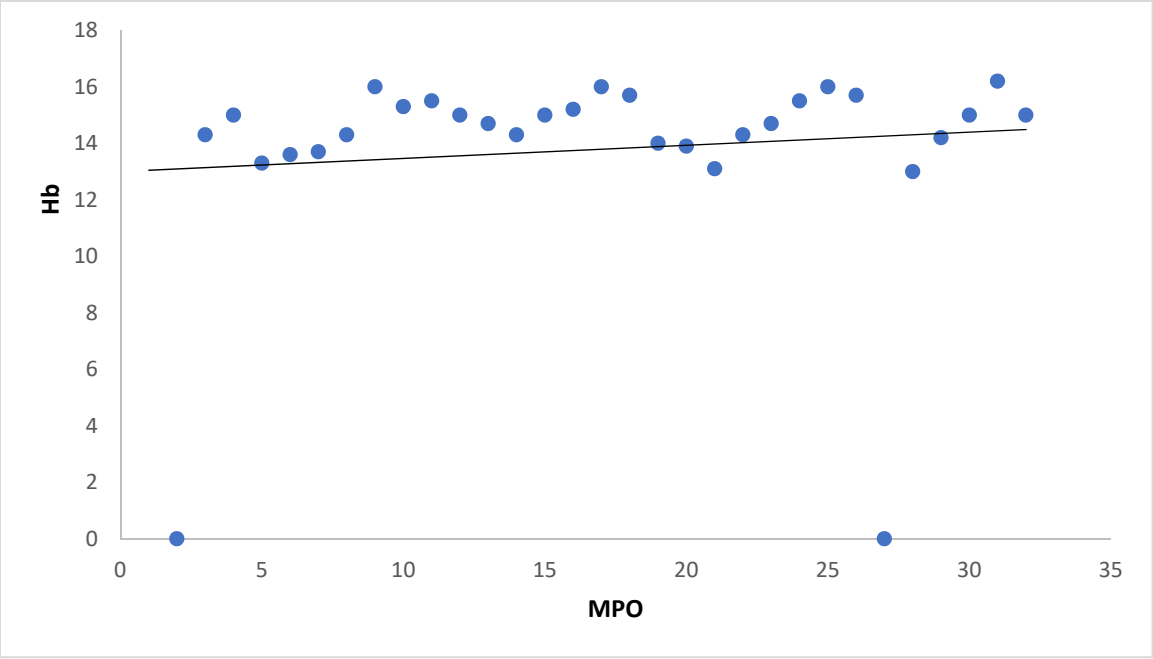


Figure (3- 19 b) : correlation between MPO and Hb in G3.

Table(3- 4) : The correlation between HCY with MPO, TC, TG, HDL ,LDL ,VLDL , AIP , Pcv and Hb in cigarette smokers and narghile smokers groups.

parameter	HCY /G2		HCY /G3	
	r-value	**p-value**	**r-value**	**p-Value**
MPO	0.18	HS	0.04	HS
CH	0.14	HS	0.11	HS
TG	-0.04	HS	-0.15	HS
HDL	-0.27	HS	.0.04	HS
LDL	0.19	HS	0.16	HS
VLDL	-.0.04	HS	-0.15	HS
AIP	0.03	HS	-.0.10	HS
PCV	0.03	HS	-0.10	HS
HB	0.02	HS	-0.19	HS

The findings in which are Listed in Table (3-4) and Figure (3-20 a,b) explained highly significant ($p \leq 0.001$) positive association between HCY and TC in G2($r = 0.14$) and G3($r = 0.11$) respectively.

The results in table (3- 4) and Figure (3-21 a,b), explained highly significant ($p \leq 0.001$) negative association between HCY and TG inG2 ($r = -0.04$) and G3 ($r = -0.15$) respectively.

The data in Figure (3- 22 a,b) and Table (3-4) explained highly significant ($p \leq 0.001$)negative association between HCY and HDL inG2 ($r = -0.27$) and G3 ($r = 0.04$) respectively.

It can be seen from the data in Table (3- 4) and Figure (3-23 a,b) explained highly significant ($p \leq 0.001$) positive association correlation between HCY and LDL in G2 ($r = 0.19$)and G3 ($r = 0.16$) respectively .

The results in Table (3- 4) and Figure (3-24 a,b) explained highly significant ($p \leq 0.001$)negative association between HCY and VLDL inG2 (r= -0.04)and G3 (r =-0.15) respectively.

The results that shown in table (3-4) and Figure (3- 25 a,b), explained highly significant ($p \leq 0.001$) positive association between HCY and AIP in G2(r =0.03), and a highly significant ($p \leq 0.001$) negative association with AIP inG3 (r =-0.10).

The results in figure (3- 26 a,b), explain highly significant ($p \leq 0.001$) positive association between HCY and PCV in G2 (r =0.03), and highly significant ($p \leq 0.001$) negative association with PCV in G3 (r =-0.10).

The findings in Table (3 – 4) and Figure (3- 27 a,b) also explain highly significant ($p \leq 0.001$) positive association between HCY and HB in G2 (r =0.02), and highly significant ($p \leq 0.001$) negative association with Hb in G3 (r = -0.19).

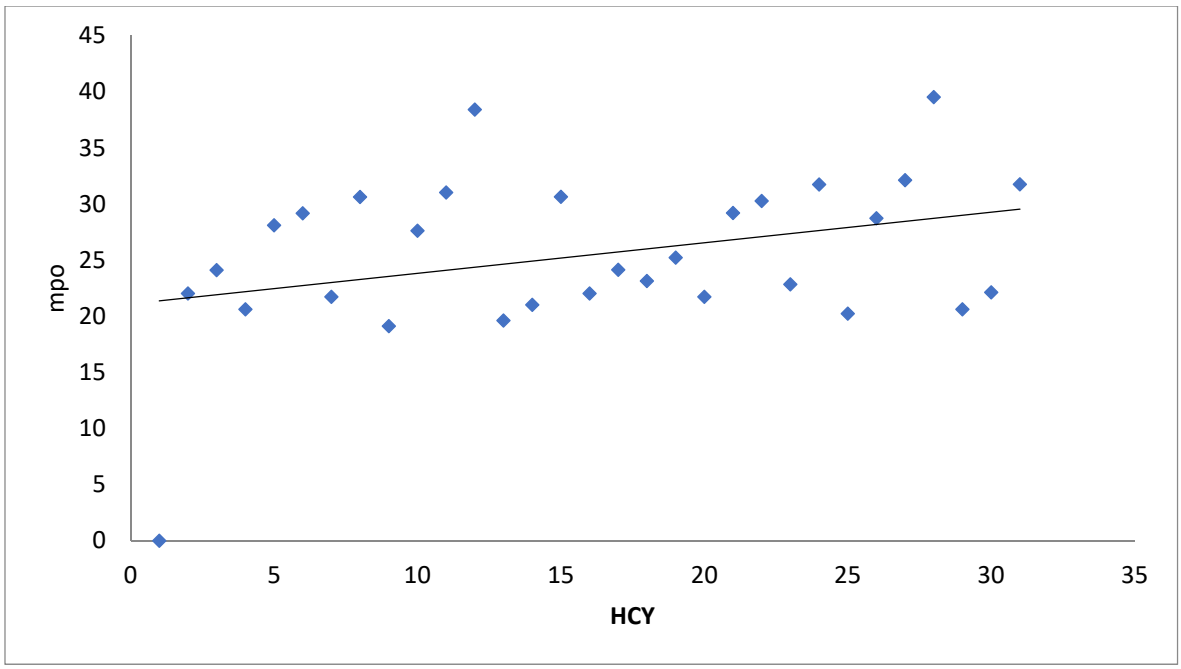


Figure (3-20 a): Correlation between Hcy and MPO in G2 .

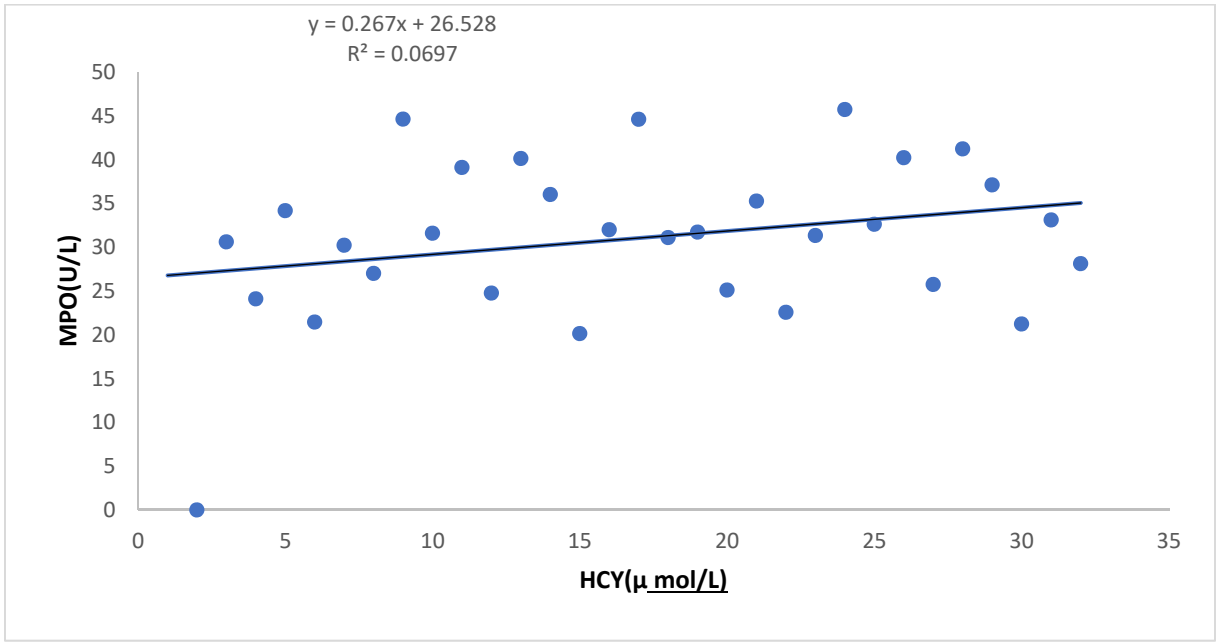


Figure (3-20 b): Correlation between Hcy and MPO in G3.

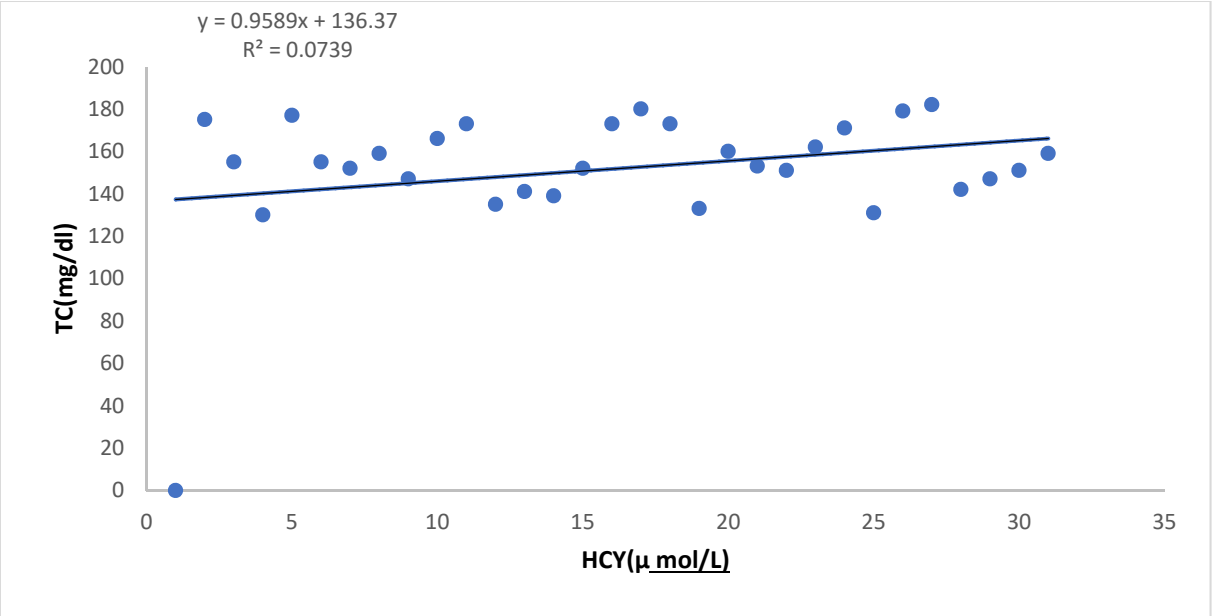


Figure (3-21 a): Correlation between HCY and TC in G2.

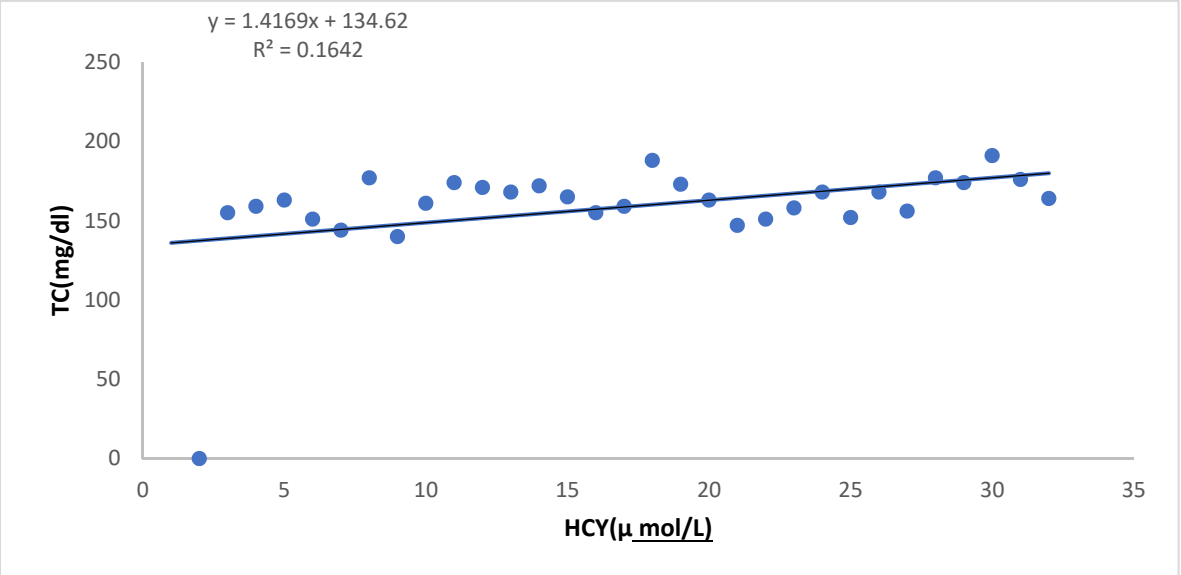


Figure (3-21 b) : Correlation between HCY and TC inG3.

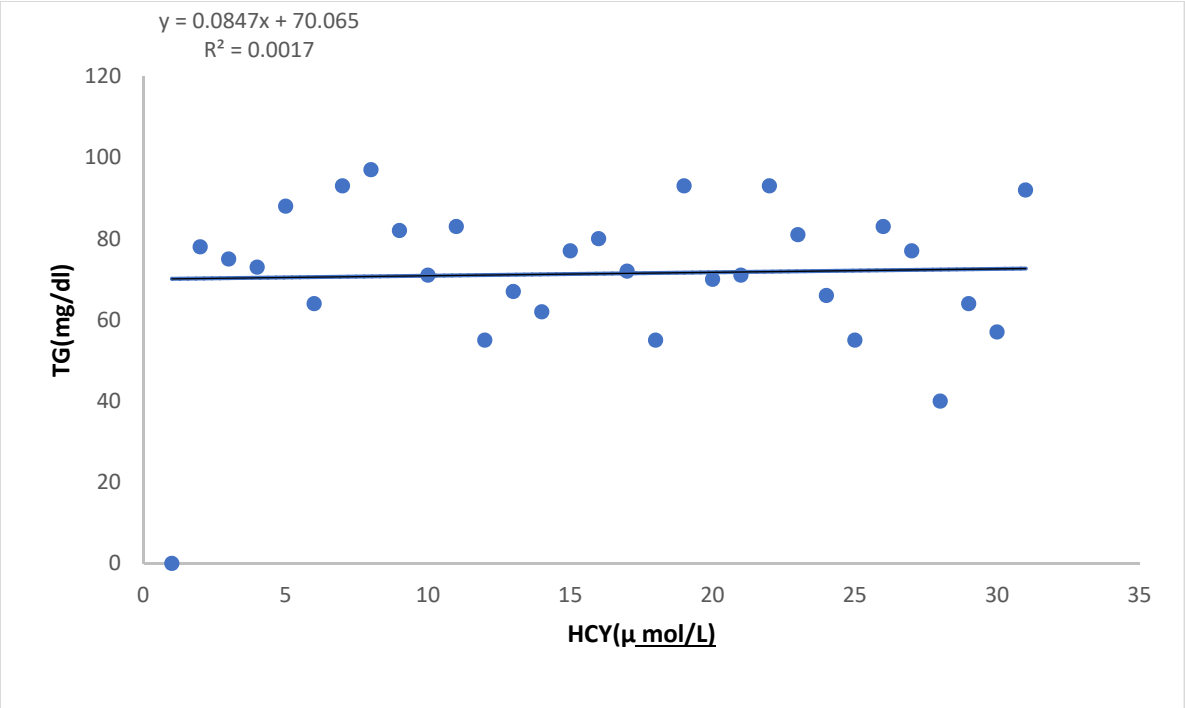


Figure (3-22 a): Correlation between HCY and TG in G2.

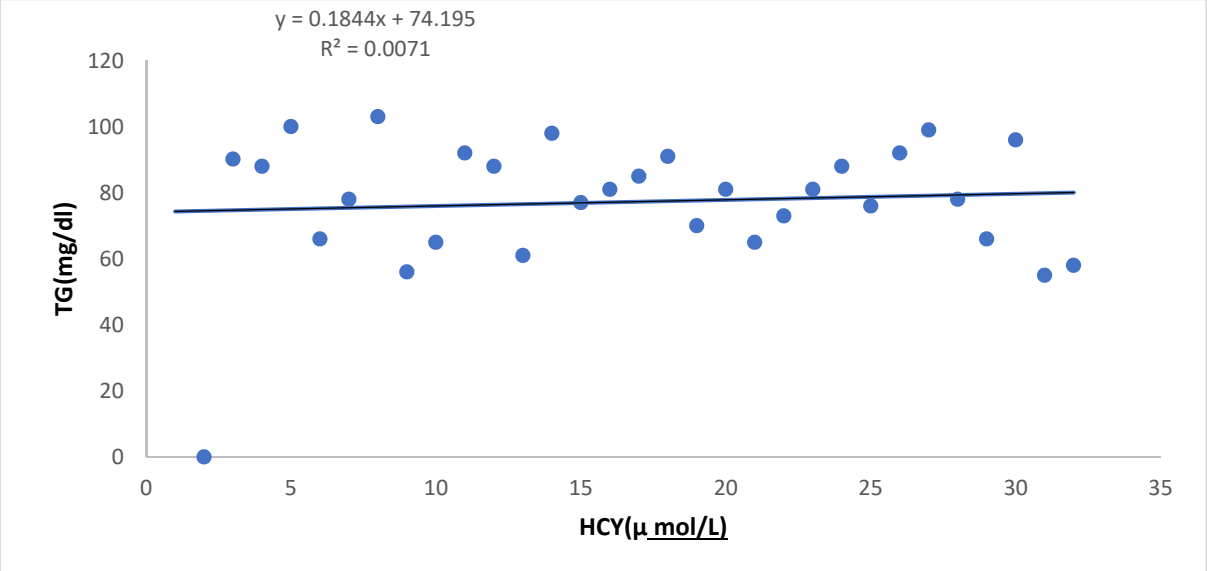


Figure (3-22 b): Correlation between HCY and TG in G3.

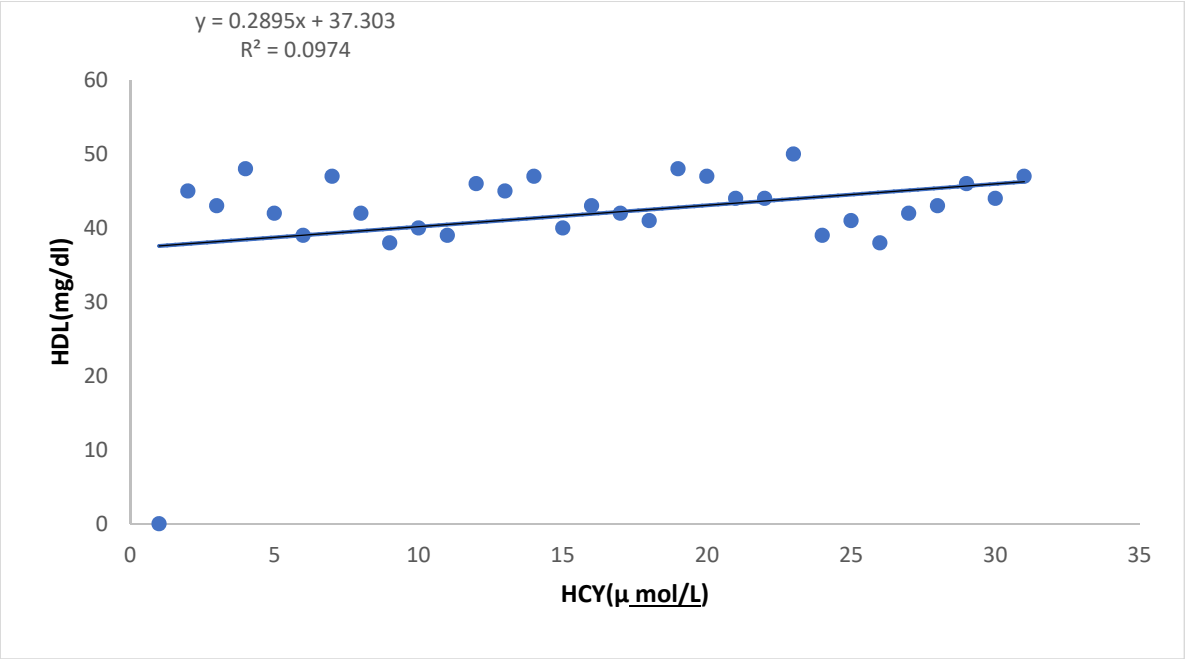


Figure (3-23a): Correlation between HCY and HDL in G2 .

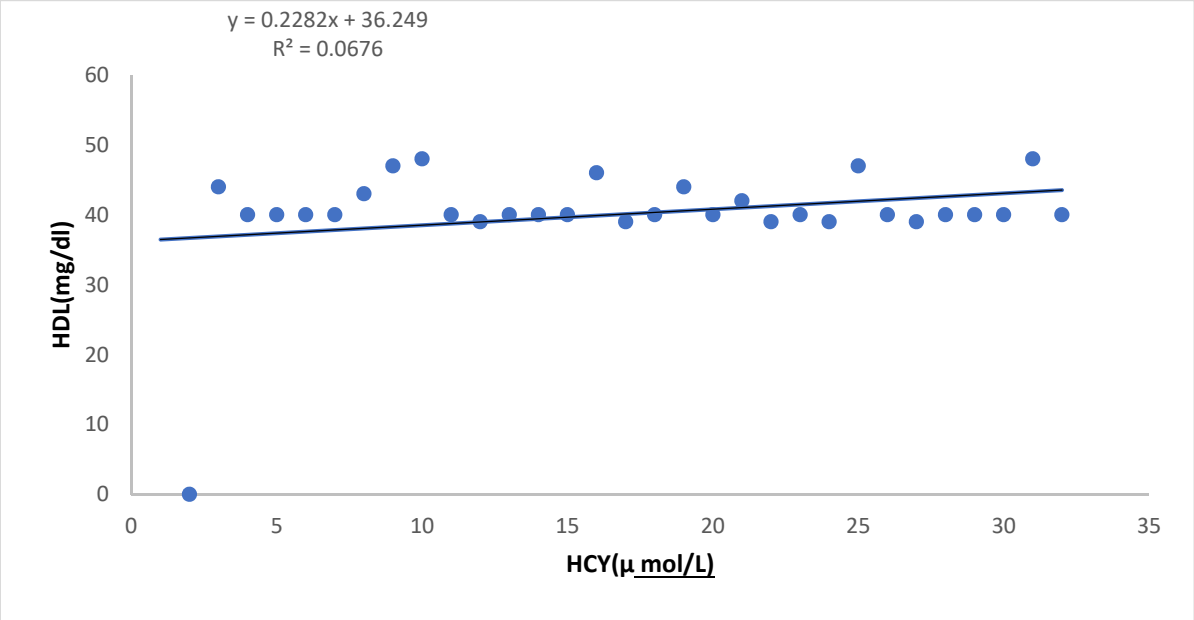


Figure (3-23 b): Correlation between HCY and HDL in G3.

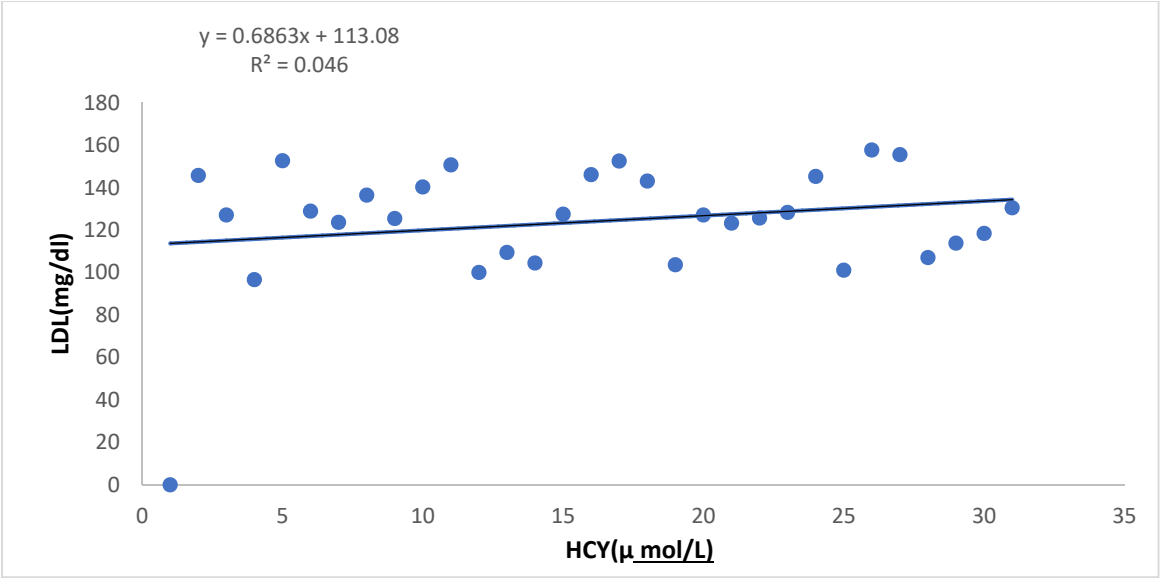


Figure (3-24a): Correlation between HCY and LDL in G2.

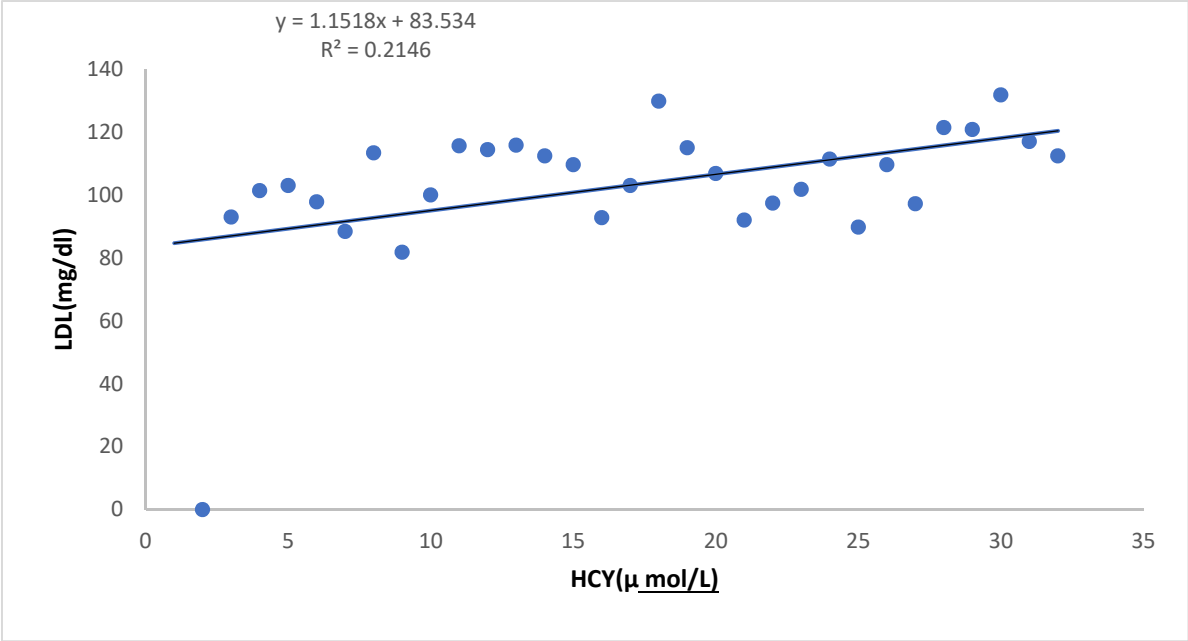


Figure (3-24 b): Correlation between HCY and LDL in G3.

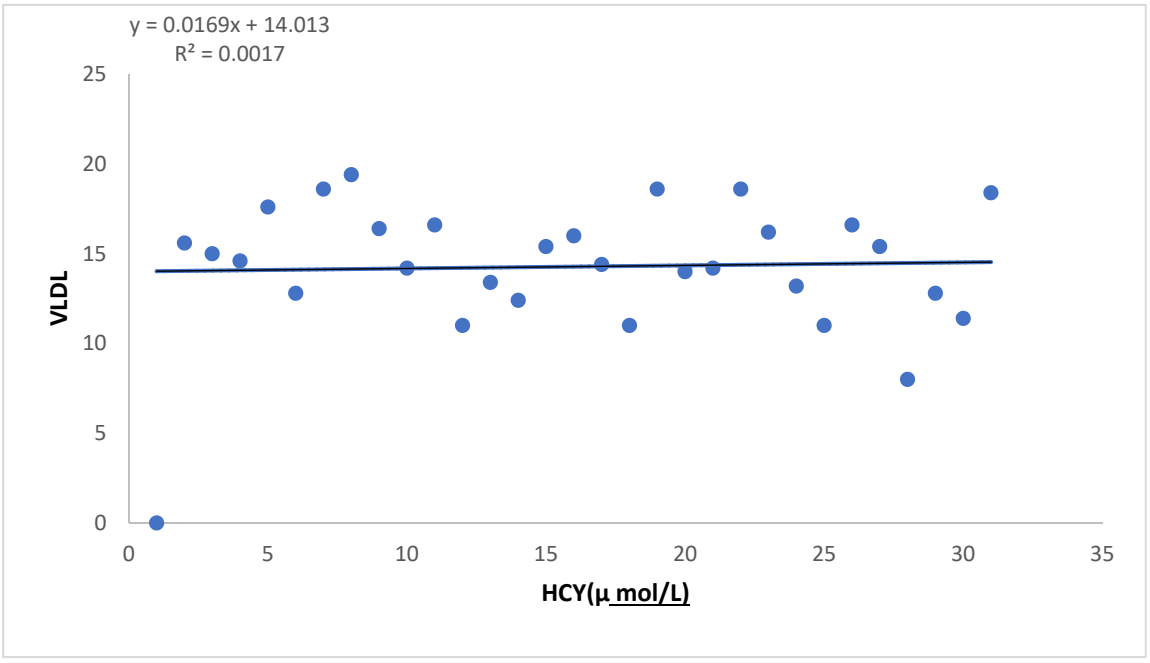


Figure (3-25 a): Correlation between HCY and VLDL inG2.

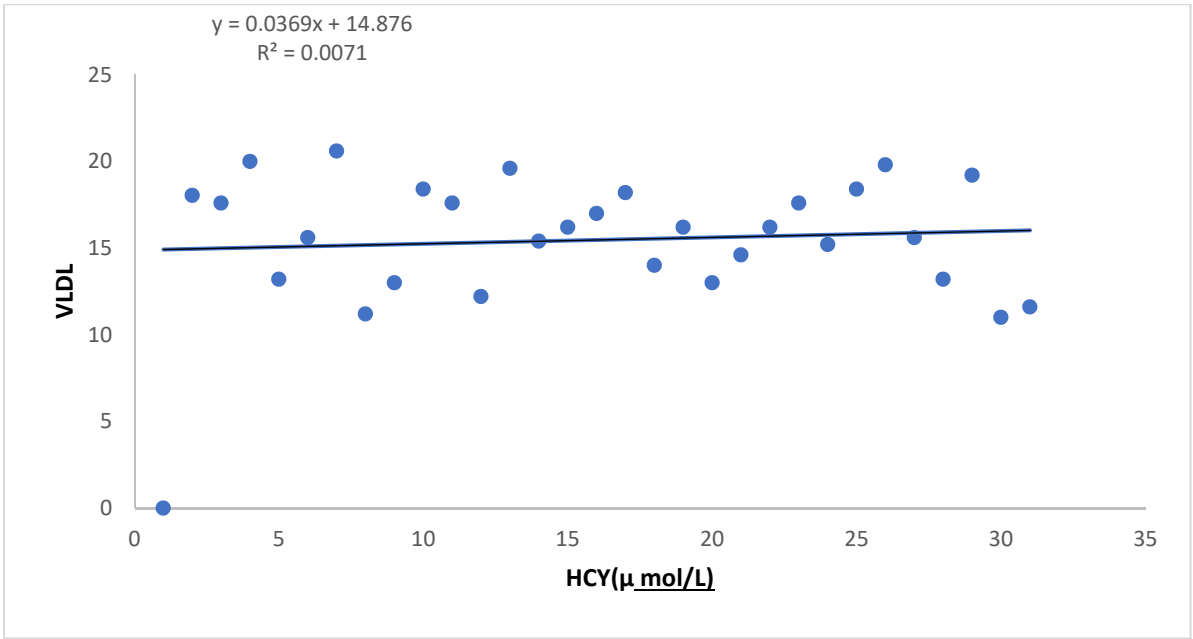


Figure (3-25 b): Correlation between HCY and VLDL in G3.

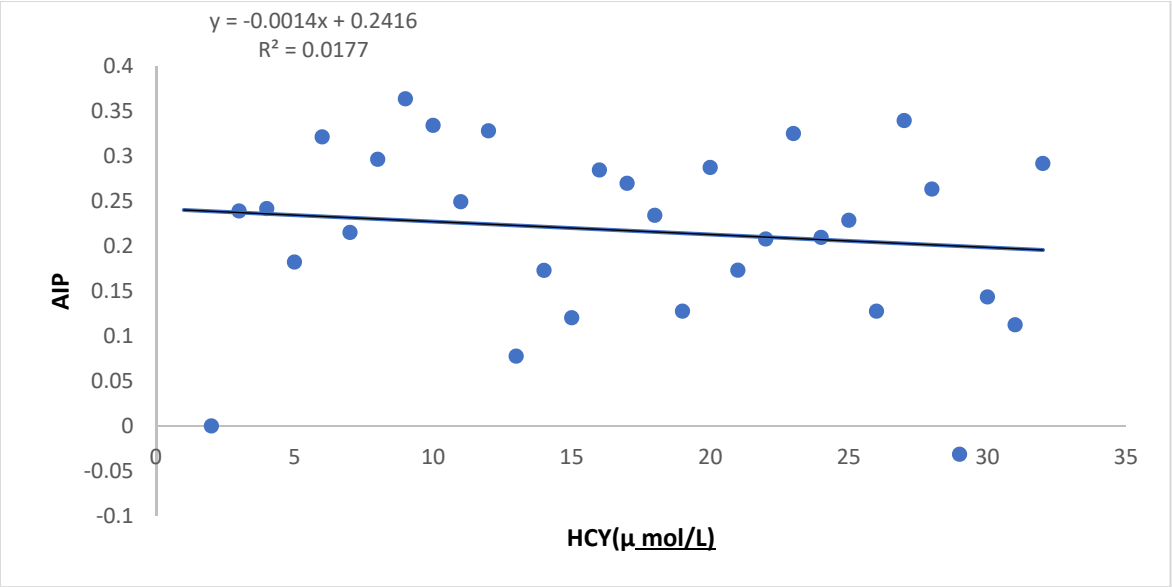


Figure (3-26 a): Correlation between HCY and AIP in G2.

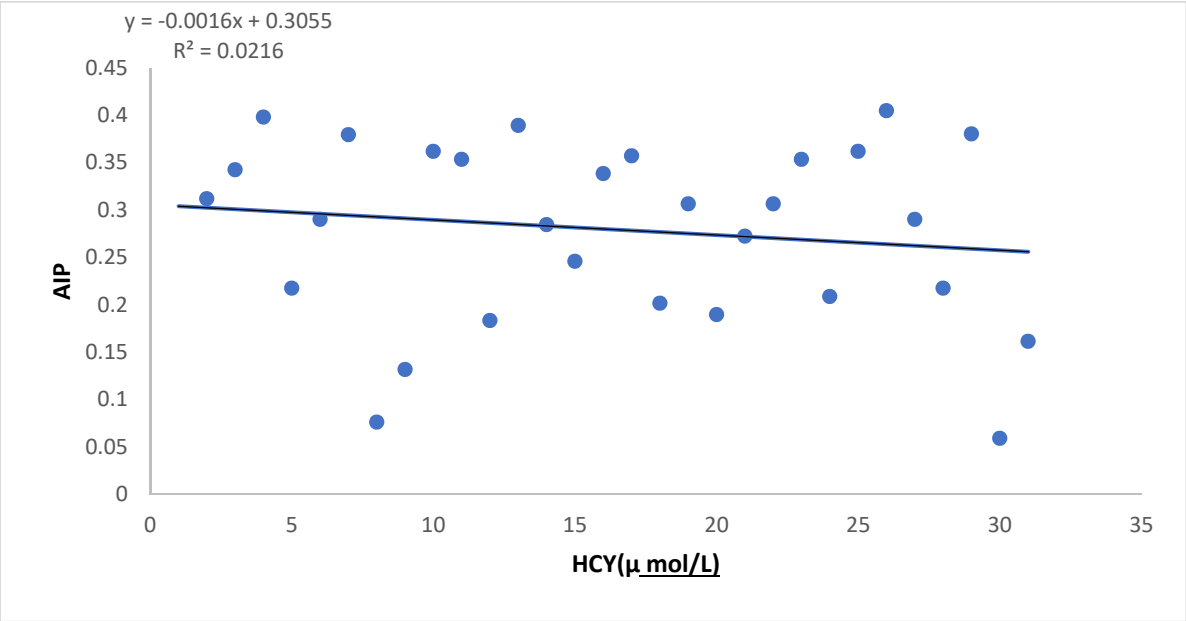


Figure (3-26 b): Correlation between HCY and AIP in G3.

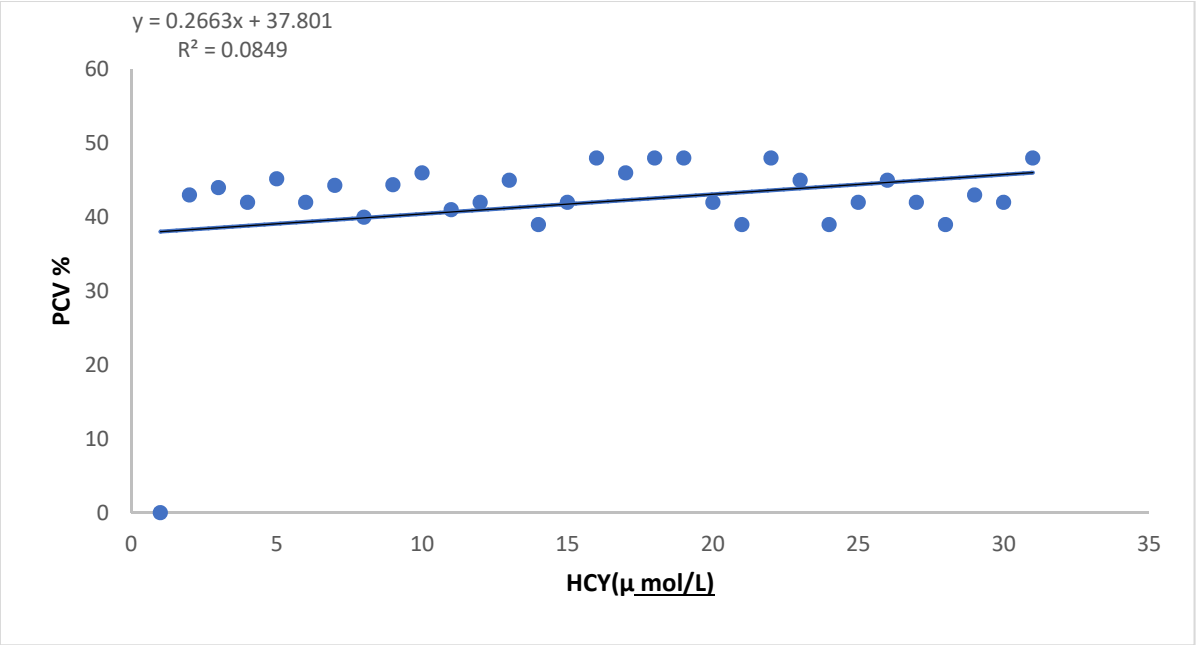


Figure (3-27 a): Correlation between HCY and PCV in G2.

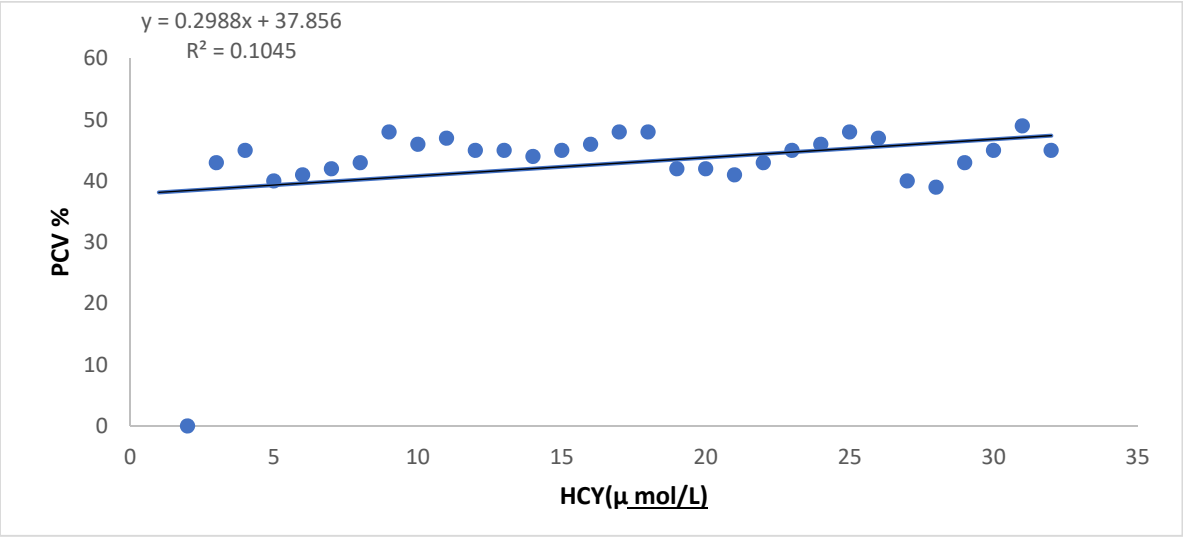


Figure (3-27 b): Correlation between HCY and PCV in G3.

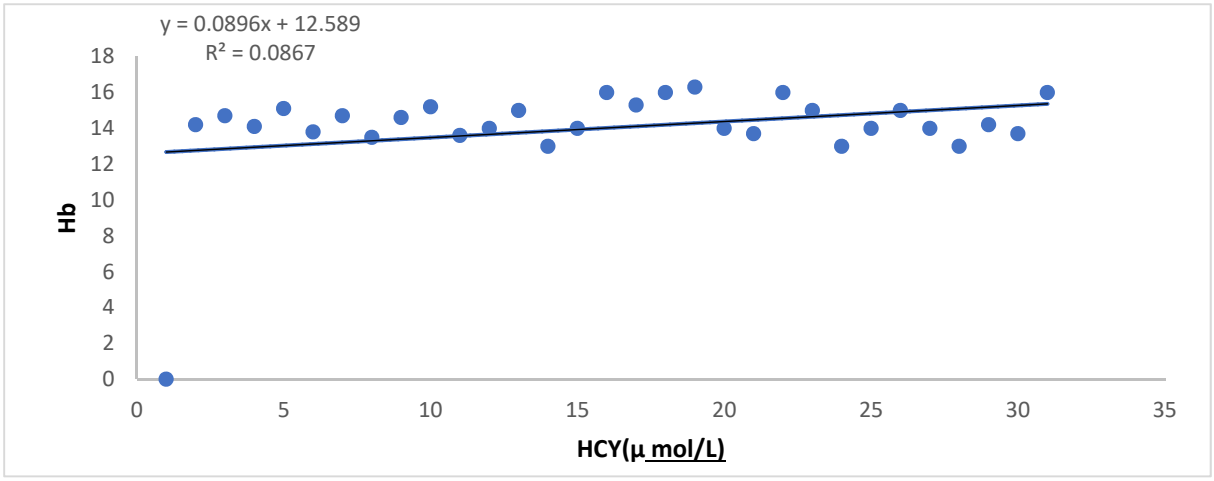


Figure (3-28 a): Correlation between HCY and Hb in G2.

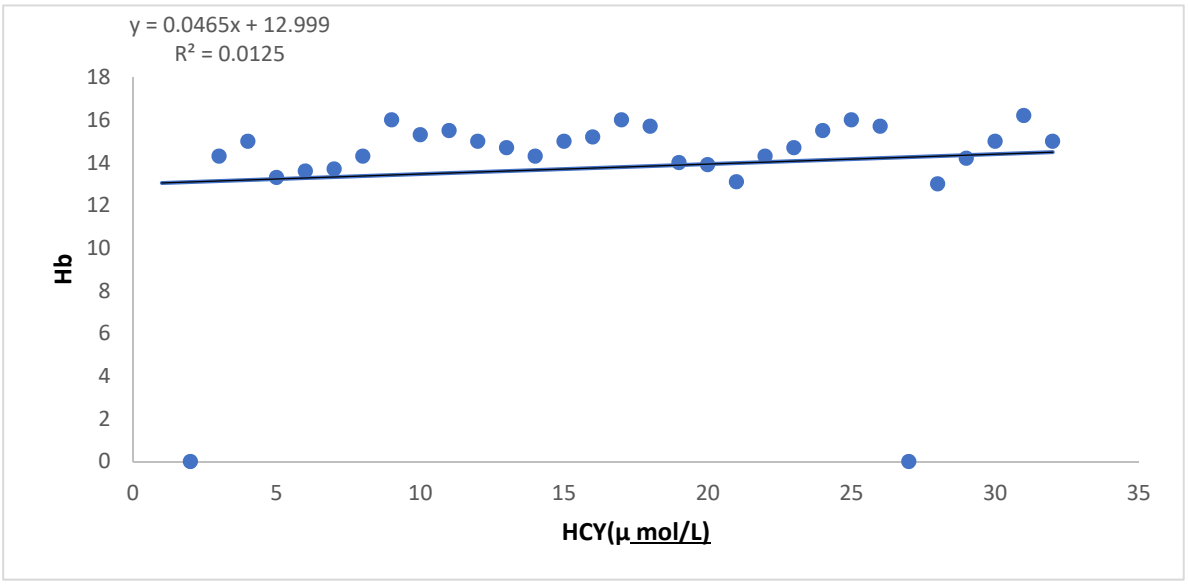


Figure (3-28 b): Correlation between HCY and Hb in G3.

Conclusions:

The Conclusions of the current study were :

1. The levels of MPO in narghile smokers group is higher than that in cigarette smokers group .
2. The study proved a highly significant elevation of Hcy levels in cigarette smokers group compared to narghile smokers group .
3. From the present data showthat the levels of TC , TG , LDL . VLDL, AIP , Pcv and Hb in narghile group are higher than that of cigarette group while the levels of Hcy ,HDL in cigarette group are higher than that narghile group.
4. Because of the increasing in MPO and Hcy levels in sera of cigarette and narghile smokers when compared with control group , so these individuals are may be more prone to cardiovascular disease and atherosclerosis in the future .

Future Studies:

Depend On the findings obtained from the study, proposed future work:

1. Dynamic study for myeloperoxidase enzyme.
2. Study impact homocysteine in the field of macrovascular disease according to sex.

   Study impact of narghile and cigarette on cytokine like interleukin.

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PATIENT NAME		Date Of Sample	
Age		Time Of Sample	
Length		Other Disease	
Weight			

APPendix I: **NO.of sample**

1-MPO

2-HCY

3-Lipid profile includes

- cho
- TG
- HDL-C
- LDL-C
- VLDL-C
- AIP

4- PCV

5- Hb

الخلاصة

صُممت هذه الدراسة لتحديد آثار تدخين السجائر والنركيلة على المستويات الميلوبيروكسيديز، الهوموسستين والملف الذهني من خلال تحديد بعض البارامترات في المجموعة الأصغر سناً لمدينة بغداد، وذلك لإيجاد معامل ارتباط الميلوبيروكسيديز والهوموسستين وتلك البارامترات في المجموعات التي تدخن السيجارة والنركيلة بشكل يومي، وكذلك، اعتبار الميلوبيروكسيديز كمؤشر حيوي للتنبؤ بأمراض القلب التاجية.

أجريت الدراسة في بغداد في عام 2019 في الفترة ما بين يناير وأبريل، العراق. تم اختيار جميع العينات بشكل عشوائي , ثم قُسمت العينات إلى ثلاث مجموعات ، المجموعة الأولى :(الغير مدخنين وعددها (30) عينة) ,المجموعة الثانية : (مدخني السيجارة مدخني السجائر وعددها (30) عينة) والمجموعة الثالثة: (مدخني النركيلة وعددها (30) عينة) , جميع الأشخاص (العينات) تتراوح أعمارهم بين (20-16) عامًا ، وقد تم أخذ(5 مل) من عينات الدم الوريدي من المجموعات ثم قسمَ إلى جزأين أحدهما يستخدم لقياس الهيموغلوبين وحجم الخلية المعبأة , والجزء الآخر وضعت في أنبوب عادي وتم استخدام جهاز الطرد المركزي بسرعة 3000 دورة في الدقيقة لمدة 10 دقيقة للحصول على المصل ، والذي تم تقسيمه في أنبوب إيبندورف وتجميدها في (8-) درجة مئوية لتحديد كل من (الميلوبيروكسيديز, الهوموسستين , الدهون الثلاثية , الكوليسترول الكلي ، البروتين الدهني عالي الكثافة ، البروتين الدهني منخفض الكثافة و البروتين الدهني منخفض الكثافة جدا).

استخدمت الطريقة الأنزيمية لتحديد الدهون الثلاثية والكوليسترول الكلي، في حين أن البروتين الدهني عالي الكثافة تستخدم طريقة المشاركة، ومع ذلك فإن (البروتين الدهني منخفض الكثافة والبروتين الدهني منخفض الكثافة جدا) تقوم بالتقييم وفقًا لحساب المعادلة بينما تم استخدام طريقة تقييم المناعة المناعية الخاصة بشطيرة الإنزيم (ساندويتش) لتقييم الميلوبيروكسيديز، الهوموسستين.

أظهرت النتائج زيادة كبيرة للغاية في مستويات الميلوبيروكسيديز ($P \leq 0.001$) في الأمصال من المجموعة الثانية والثالثة مقارنة مع المجموعة الأولى، وفي المجموعة الثانية والثالثة لمستويات الميلوبيروكسيديز كما لوحظ من النتائج أن مستويات الميلوبيروكسيديز تكون في المجموعة الثالثة أعلى من المجموعة الثانية. أظهرت النتائج زيادة كبيرة ($p \leq 00101$) في مستويات كل من المجموعة الثانية والثالثة مقارنة مع المجموعة الأولى وكان هناك اختلاف كبير ($p> 0.05$) في مستوى الهوموسستين بين المجموعة الثانية والمجموعة الثالثة.

ومع ذلك، لوحظ انخفاض في مستويات الكوليسترول الكلي في الأمصال من المجموعة الاولى، بالمقارنة مع المجموعة الثانية. فضلا عن وجود زيادة كبيرة (P <0.05)، في المجموعة الثالثة عند مقارنتها مع المجموعة الاولى.

لوحظ أيضا زيادة الدهون الثلاثية (P <0.05)في المجموعة الثانية عند مقارنتها بـالمجموعة الاولى وكان هناك اختلاف كبير (p≤0.001) في المجموعة الثالثة عند المقارنة مع المجموعة الأولى.

كذلك، لاحظ زيادة كبيرة (P <0.05) في المجموعة الثالثة والمجموعة الثانية عند مقارنتها بـ المجموعة الاولى لمستويات الدهون الثلاثية، بينما أظهرت فرقًا غير مهم في مستويات البروتين الدهني عالي الكثافة بين المجموعة الثانية والمجموعة الثالثة، ولوحظت زيادة كبيرة (P <0.05) في البروتين الدهني منخفض الكثافة في المجموعة الثالثة عند مقارنة مع المجموعة الاولى وكان هناك اختلاف غير مهم (p> 0.05) في المجموعة الثانية عند مقارنته بـ المجموعة الاولى.

لوحظ ايضا زيادة كبيرة (P <0.05) في البروتين الدهني منخفض الكثافة جدا في المجموعة الثانية عند مقارنتها بـ المجموعة الاولى وكان هناك اختلاف كبير (p0.001) في المجموعة الثالثة مقارنةً بـ المجموعة الاولى. بالإضافة إلى ذلك، لاحظت زيادة ملحوظة (P <0.05) في مؤشر تصلب الشرايين من البلازما في المجموعة الثانية عند مقارنتها بـ المجموعة الاولى وكان هناك ارتفاع كبير (p0.001) في المجموعة الثالثة بالمقارنة مع المجموعة الاولى. لاحظت النتائج زيادة ملحوظة (P <0.05) في التركيز المئوية للهيموغلوبين وحجم الخلية في المجموعة الثالثة مقارنة مع المجموعة الاولى.

الاستنتاجات: من الدراسة الحالية نستنتج ما يلي:

مستويات الميلوبيروكسيديز في مجموعة مدخني النركيلة أعلى منها في مجموعة مدخني السجائر. وقد أثبتت الدراسة ارتفاعًا كبيرًا في مستويات الهوموسستين في مجموعة مدخني السجائر مقارنة بمجموعة مدخني النركيلة.

تظهر من خلال البيانات الحالية أن مستويات (الدهون الثلاثية، الكوليسترول الكلي، البروتين الدهني منخفض الكثافة، البروتين الدهني منخفض الكثافة جدا، حجم الخلية، الهيموغلوبين ومؤشر تصلب الشرايين من البلازما) بمجموعة مدخني النركيلة أعلى من مجموعة مدخني السجائر، بينما مستويات الهوموسستين والبروتين الدهني عالي الكثافة في مجموعة مدخني السجائر أعلى من تلك بمجموعة مدخني النركيلة. بسبب الزيادة في مستويات الميلوبيروكسيديز والهوموسستين في مجموعة مدخني السجائر والنركيلة عند مقارنتها بالمجموعة الضابطة، قد يكون هؤلاء الأفراد أكثر عرضة لأمراض القلب والأوعية الدموية وتصلب الشرايين في المستقبل.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بغداد
كلية التربية للعلوم الصرفة -ابن الهيثم
قسم الكيمياء

دراسة مقارنة مستويات الميلوبيروكسيديز و الهوموسستين على مدخني السيجارة والنركيلة للعراقيين الذكور البالغين

رسالة مقدمة إلى

مجلس كلية التربية للعلوم الصرفة/ ابن الهيثم، جامعة بغداد وهي جزء من متطلبات نيل درجة الماجستير في علوم الكيمياء

تقدمت بها

سارة كريم سامي

(بكالوريوس علوم في الكيمياء 2015)

بأشراف

أ.د. بشرى حميد علي

محرم 1441هـ أيلول 2019 م