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Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) Circulating Levels Are Related to LDL Coronary Artery Disease

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ABSTRACT

Background: Low-density lipoprotein receptors (LDL-R) in hepatocytes are degraded by the enzyme proprotein convertase subtilisin/kexin type-9 (PCSK9). A brand-new target for lipid-lowering treatment is PCSK9 inhibition. Three subsets of monocytes, which play a critical role in the pathophysiology of atherosclerosis, are known

Objectives: The aim of this study was to examine whether circulating levels of PCSK9 are associated with LDL liped subsets.

Materials and Methods: We included 70 patients with coronary artery disease. PCSK9 levels were mea- sured and LDL liped and 30 control health

Results: Eighty percent of the patients were men, with a mean age between 40 and 70. Patients increese displayed greater PCSK9-levels compared to the 30 male control group. PCSK9 levels in the blood were associated with CM treatment in patients, whereas NCM had the opposite effect. Patients whose levels of PCSK9 were greater than the median displayed a significantly higher

Introduction

Cardiovascular illnesses, such as coronary artery disease (CAD), are the main source causes of morbidity and mortality in the West. It is thought that atherosclerosis, the primary cause of CAD, is an inflammatory condition of the vessel wall1. It is believed that low-density lipoprotein, or LDL, particles enter the innermost layer of the artery wall, where they stick and oxidizing alter. It causes a major inflammatory reaction that leads to monocyte adhering and transmigration into the intimal layer [1][2].

Acute coronary syndrome (ACS) or stable angina pain are the two able ways of life of the lesion as it fuels with the keeps inflammatory processes involving cytokines and cell processes. A considerable body of research, including epidemiologic, genetic, clinical observational, and interventional investigations, has established a link of some sort between the presence of low-den and the development of atherosclerotic plaques. Because of this, lowering LDL cholesterol has emerged as a crucial element of secondary preventive care. Statins and ezetimibe are the cornerstones of pharmacological LDL-C lowering; much greater novel PCSK9-blocking

substance use are available for high-risk patients. PCSK9 is an enzyme that breaks down the LDL-receptor in the hepatocyte. It was first discovered in a family with familial hypercholesterolemia[3][2].

This increased LDL receptor recycling. As a result, the number of hepatocyte surface-bound LDL-receptors increases, lowering LDL cholesterol levels in the blood. There is minimal evidence to back up PCSK9's alleged role in inflammatory pathways. Inflammatory principles are fundamental to all stages of CAD, from disease development to plaque destabilization. Monocytes, the cellular signature of CAD, are a heterogeneous population of cells that may be classified into at least three subgroups with distinct functions. While IM were seen to have strong pro-inflammatory behavior, NCM appeared to have a distinct patrolling tendency, very close damaged vessel walls. CM were defined to show everyday monocyte behavior. Observational studies have found that different monocyte subsets play diverse roles atherogene-sis [4] in all stages The purpose of the current investigation was to assess the relationship between circulating PCSK9 levels and innate immune activity because it is critical for the development of atherosclerosis that LDL-cholesterol and the innate immune system interact.

Materials & methods

The subject group consists of 90 apparently healthy adult. Their age ranged from 30 to 79 years for male Samples were collected from the Karbala Center for Heart Diseases in Imam Hussein Medical City in the holy city of Karbala. The study included males (90) samples, which were divided into (60) patients with cardiovascular diseases and ischemic diseases of the heart, and they had a cardiac catheterization of the coronary arteries in the same center, and 30 people for the period from November 2022 to December 30, 2023, where sufficient information was taken from The patient, the patient's companion, the patient's drum, and the resident doctor in the center in terms of age, weight, hereditary diseases, and pressure according to the form. Blood was drawn after taking official approvals from the center management and the patient to perform the required analysis. Blood samples were obtained and separated in two fractions, a plasma fraction for biochemical analyses was centrifugal separated and stored at −20°C, and another fraction for total genomic DNA analyses was maintained at 4°C until processing

Blood sampling

Blood was drawn in the morning prior to coronary an- giography from an antecubital vein at fasting state. The first 3ml of blood were discarded and blood was drawn into an Ethylenediaminetetraacetic acid (EDTA) tube (Greiner Bio- One). 100 µL were used for immediate flow cytometry and the remaining blood was centrifuged at 3000 RPM at 4 °C and stored at -80 °C for later analysis and blood was sent to the central laboratory of the General Hospital of Vienna for standard laboratory parameters.

Measurement of PCSK9

For the measurement of PCSK9 serum levels, a specific ELISA (RND Systems, Minneapolis, MN, USA) was used according to the manufacturer’s instructions with an assay range between 0.6 and 40 ng/mL.

Laboratory measurements

High-sensitive C-reactive Protein (hsCRP), one common laboratory test, was examined in the General Hospital of Vienna's central lab. Particularly developed enzyme-linked immunosorbent assays (ELISAs) were used to quantify the levels of circulating interleukin-6 (IL-6) (Human IL-6 Quantikine high-sensitivity Immunoassay Kit, R&D Systems, which is Biotechne, Minneapolis, Minnesota, MN, USA, catalog number HS600B). Following the directions given by the manufacturer, a customized multiplex test (Luminex Assay, R&D Sys- tems, catalog number FCST03) was used to evaluate the concentrations of IL-4, IL-10, the monocyte chemoattractant

protein-1 (MCP-1), and tumor necrosis factor-alpha (TNF-) in circulating plasma

Lipid measurements

Enzymatic procedures were utilized to get standard lipid values such as total cholesterol, bad cholesterol (LDL), high density lipoprotein cholesterol (HDL), and triglycerides from the general laboratory of Krankenanstalten Dr. Dostal. For the quantification of lipid subfractions, the Quantimetrix LDL and HDL Lipoprotein Systems® (Quantimetrix company, Redondo Beach, CA, USA) were used in accordance with the manufacturer's instructions, as previously described. Using high resolution polyacrylamide gel electrophoresis, this approach determined very large volume lipoprotein (VLDL), divides HDL into 10 subfractions, and divides LDL into eight subfractions. Small dense LDL (LDL) particles are detected by LDL subfractions , whereas small HDL particles are identified by HDL subfractions .

Statistical analysis

Categorical variables are displayed as counts and percent- ages and were compared by the $\chi 2$ or by Fisher's exact test as deemed appropriate. Continuous variables are expressed as median and interquartile range (IQR). Data was compared by Mann-Whitney test and Spearman's correlation coeffi- cient was calculated. classical cardiovascu- lar risk factors (BMI, presence of hypertension and diabetes as well as smoking status). Two-sided p-values of < 0.05 in- dicated statistical significance. SPSS 2025 (IBM Corporation, Armonk, NY, USA) was used for all analyses[5].

Experimental design

This is a case-control study that included ninety patients divided into two groups with clinical evidence of coronary artery disease in the form of angina pectoris (60) This group divided into four groups as related diseases {smoker n = 36, non-smoker n = 24, hypertension blood = 40, mean blood pressure = 20} and subgroups divided into three groups by age { (40-49) n = 15, (50-59) n = 20, (60-69) n = 25} This subgroup is divided into Three groups as related diseases {stable angina n = 15 and unstable n = 15 and myocardial infarction n = 30. Body mass was measured as normal n = 20, obese n = 20 and high weight gain n = 20, as well as measuring the percentage of fat for patients compared to control and Figure (3-1), diagnosed with typical chest pain, positive change in ECG, angiography and estimation of positive cardiac indices. From the coronary care unit (CCU) of the heart in Imam Hussein Medical City / Karbala Center for Diseases from January to August 2023, the study also included thirty men as a control group, while the men had no history of infection. Chest pain, no history of admission to the intensive care unit and normal resting ECG, control men are collected from relatives and outpatient clinic.

All participants are exposed to fluid about age, chest pain, history of admission to the intensive care unit, history of high blood pressure, and diabetes mellitus, while they were subjected to weight measurement, then blood samples are sent for analysis, and both male and female control patients are informed about the study and consent is guaranteed

Ethical approval : Ethical approval : The study was conducted on animals, as no patients were included with the maintenance of all human rights. According to document number 6289, a local on the date of 6/12/2022 reviewed and authorized the study protocol.

The main problem

Dhukkubsattoota kateeterii onnee booda hospitaala galan irratti infarkii maayookaardiyaa dafanii adda baasuu fi tilmaamu

Result

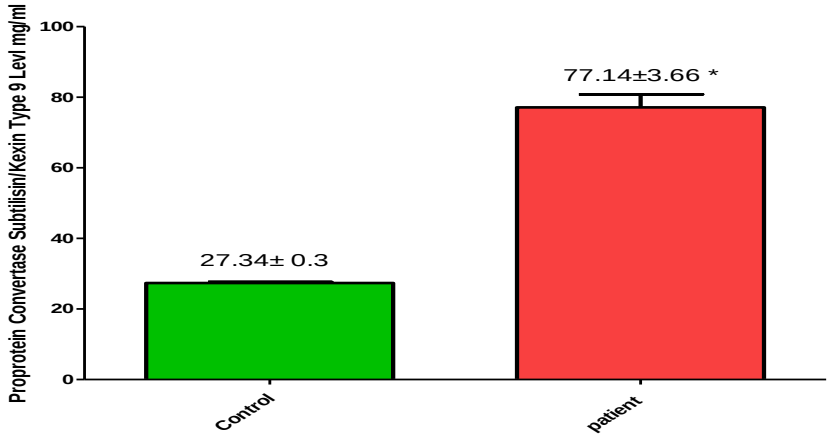

Figure (1) show level of Proprotein Convertase Subtilisin/Kexin Type 9 in patient compare with control number of samples control = 30 patients = 60 * denotes significant (P<0.05). The level of Proprotein Convertase Subtilisin/Kexin Type 9 showed a significant increase (P< 0.05) in patient compare with control

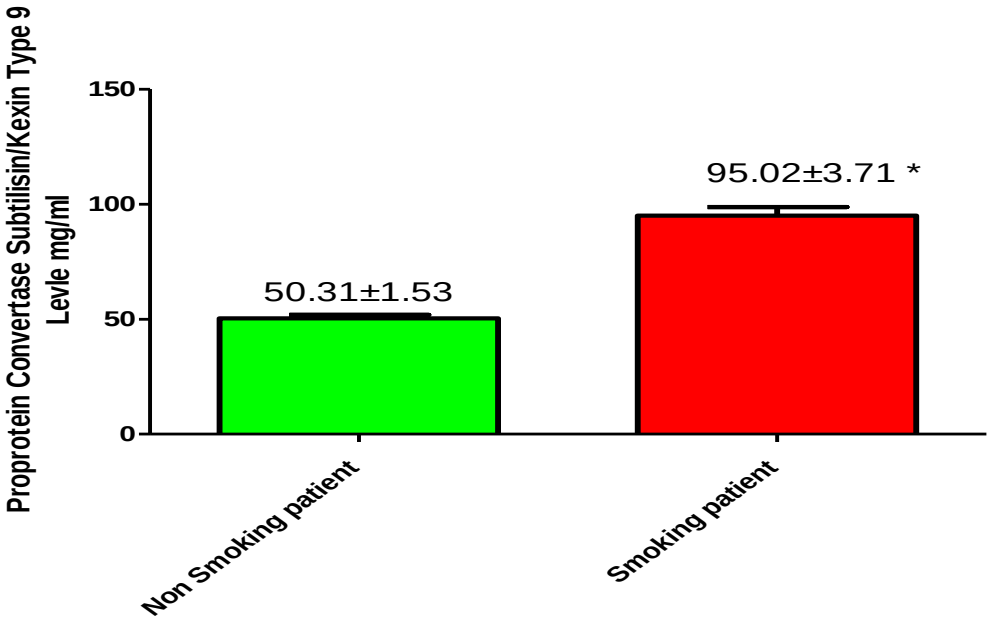

Figure (2)show The level of Proprotein Convertase Subtilisin/Kexin Type 9 in Non Smoking patient compare with Smoking patient. Number of samples Non Smoking patient = 24 Smoking patient = 36* denotes significant (P<0.05). The level of Proprotein Convertase Subtilisin/Kexin Type 9 showed a significant Decrease (P< 0.05) in Smoking patient compare with Non Smoking

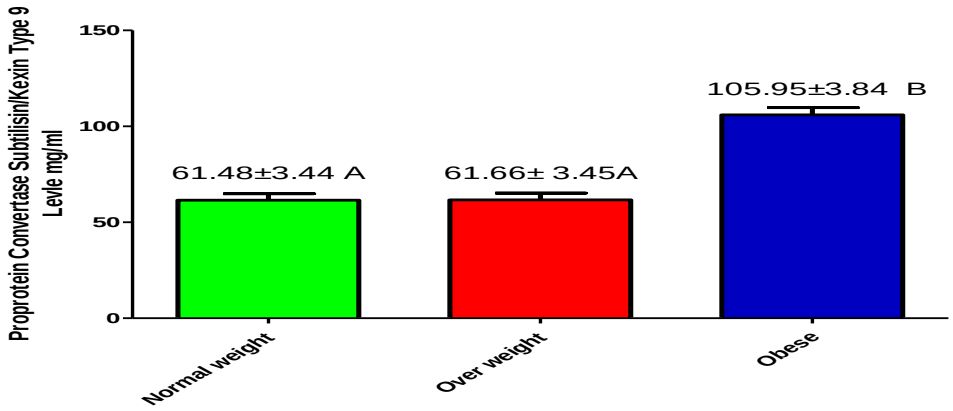

Figure (3)show The level of Proprotein Convertase Subtilisin/Kexin Type 9 in Normal weight patient compare with Overweight patient and Obese patient. Number of samples Normal weight= 20 Over weight=20 Obese =20 * Different letters denote significant (P<0.05). The level of Proprotein Convertase Subtilisin/Kexin Type 9 showed a significant Decrease (P< 0.05) in Normal weight patient and Overweight patient compare with Obese patient (figure 4-10).

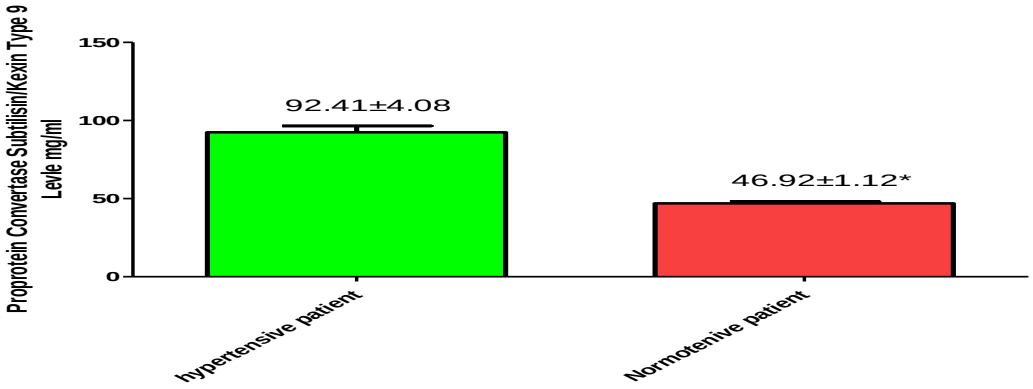

Figure (4)show The level of Proprotein Convertase Subtilisin/Kexin Type 9 in hypertensive patient compare with Normotenive patient. Number of samples Stable angina patient = 40 unstable angina patient =20* denotes significant (P<0.05) The level of Proprotein Convertase Subtilisin/Kexin Type 9 showed a significant Decrease (P< 0.05) in hypertensive patient compare with Normotenive patient

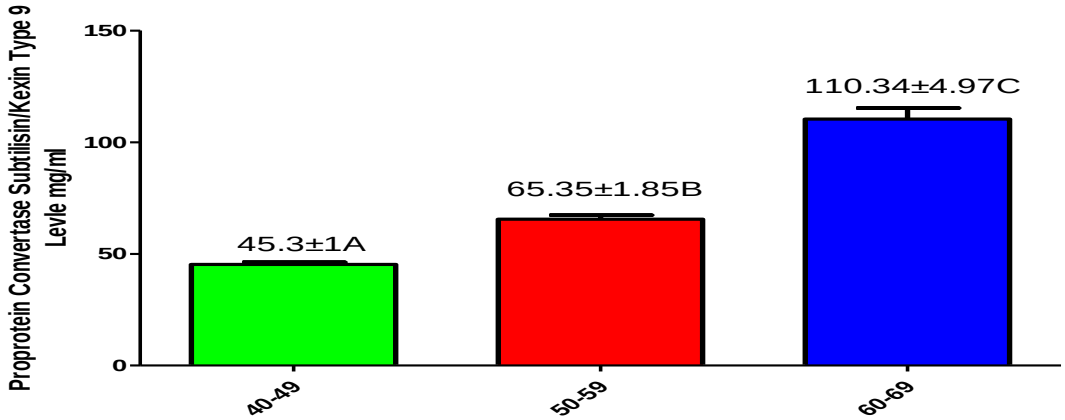

Figure (5)show The level of Proprotein Convertase Subtilisin/Kexin Type 9 in Normal weight patient compare with Overweight patient and Obese patient. Number of samples40-49 years n= 15 , 50-59 years n=20 ,60-69 yearsn =25 ,* Different letters denote significant (P<0.05). The level of Proprotein Convertase Subtilisin/Kexin Type 9 showed a significant Decrease (P< 0.05) in Normal weight patient and Overweight patient compare with Obese patient

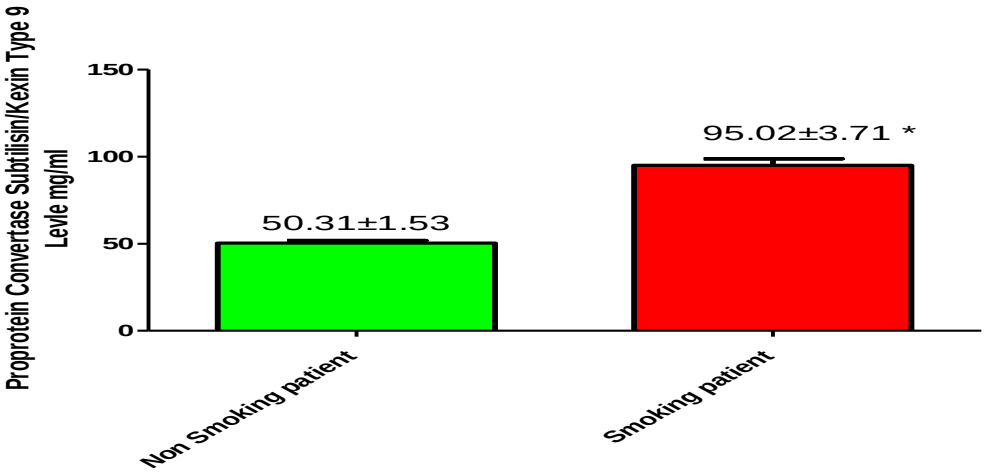

Figure (6)show The level of Proprotein Convertase Subtilisin/Kexin Type 9 in Non Smoking patient compare with Smoking patient. Number of samples Non Smoking patient = 24 Smoking patient = 36* denotes significant (P<0.05). The level of Proprotein Convertase Subtilisin/Kexin Type 9 showed a significant Decrease (P< 0.05) in Smoking patient compare with Non Smoking patient

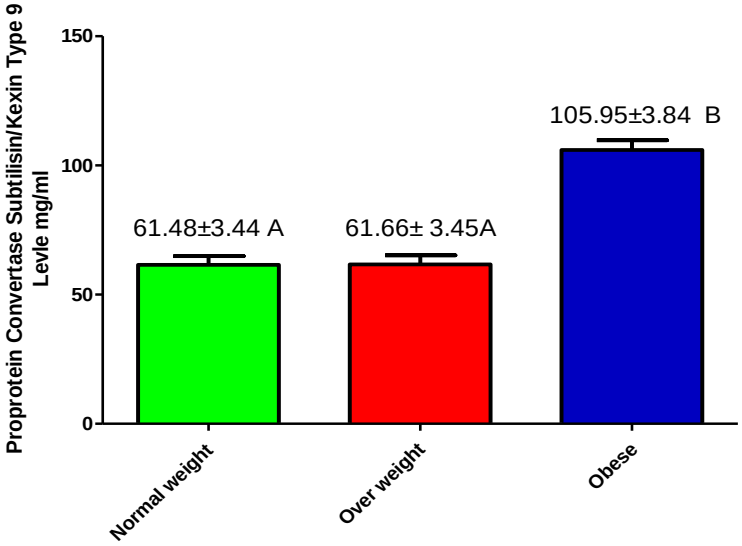

Figure (7)show The level of Proprotein Convertase Subtilisin/Kexin Type 9 in Normal weight patient compare with Overweight patient and Obese patient. Number of samples Normal weight= 20 Over weight=20 Obese =20* Different letters denote significant (P<0.05). The level of Proprotein Convertase Subtilisin/Kexin Type 9 showed a significant Decrease (P< 0.05) in Normal weight patient and Overweight patient compare with Obese patient

Discussion

In this single-center, cross-sectional study, we show for the first time an association between circulating monocyte subsets and PCSK9 levels in patients with stable CAD. We have included 90 patients undergoing diagnostic coronary angiography for known or suspected CAD. In total, 69 patients had stable CAD and both PCSK9 and flow cytometry data available.

Figure (6) The Result of the current study has shown elevated serum Study The effective of Smoking on the level of Proprotein Convertase Subtilisin/Kexin Type 9 in Non Smoking patient compare with Smoking patient. The level of Proprotein Convertase Subtilisin/Kexin Type 9 showed a significant Decrease(P< 0.05) in Smoking patient compare with Non Smoking patient (6).

In HepG2 cells, the current study shown that smokers increased PCSK9 expression and decreased LDLR expression. According to epidemiological research, exposure to CS is linked to dyslipidemia. Quitting smoking for more than 6 years can lessen the incidence of dyslipidemia. Smokers had higher blood cholesterol levels, higher plasma triglyceride concentrations, and lower HDL C concentrations than non-smokers. Additionally, exposure to smokers has been linked to dyslipidemia in animal trials. Without a doubt, LDL C causes atherosclerosis. It has been clarified that long exposure to smokers raises the levels of blood LDL C in experimental animals [6][2].

An earlier study suggested that the elevated levels of LDL C in the blood could be due to the lowered expression of LDLR on liver cells brought on by smoking's exposure. largely to the study, CSE increased the expression of PCSK9 mRNA and protein, which could provide an explanation for how CSE prevents the production of LDLR. PCSK9 is essential for maintaining cholesterol homeostasis. It attaches to LDLR on the surface of hepatocytes and triggers the liver's endosomal and lysosomal degradation of LDLR, raising serum LDL C levels. In line with lower LDLR expression, the smoker in this investigation stimulated PCSK9 expression in HepG2 cells in a time- and concentration-dependent manner. However, lowering LDLR on the hepatocyte surface is not the only significant[7].

Figure (6) The level of Proprotein Convertase Subtilisin/Kexin Type 9 showed a significant Decrease ($P< 0.05$) in Normal weight patient and Overweight patient compare with Obese patient (figure 4-10).

Obesity is widely spread around the world, especially among males due to its relationship with obesity Our study also confirmed that obesity represents a major burden on public health. Studies contain data on the effects of obesity and the degree of apnea on the development of dyslipidemia [8] .

The present study showed implicated biomarker of dyslipidemia, PCSK9, . The level of Proprotein Convertase Subtilisin/Kexin Type 9 showed a significant Decrease ($P< 0.05$) in Normal weight patient and Overweight patient compare with Obese patient Various mechanisms have been proposed for how the presence of obesity causes disturbances in metabolic homeostasis. The most important of them is CIH which promotes oxidative stress, systemic inflammation, and dyslipidemia through the induction of hypoxia-inducible factor-1 (HIF-1) synthesis in the liver, which activates the SREBP-1C transcription factor and increases PCSK9 [9]

It was found in a study that other reactive oxygen species seemed to activate nuclear factor kappa B (NF-κB) in hepatocytes, which in turn caused PCSK9 expression. One question arises from both the data in our study and the data that have been published: Does PCSK9 get affected by obesity? Despite their positive correlation, it was not sufficient for higher plasma PCSK9 levels. Studies on patients with stable angina, unstable angina, or myocardial infarction who are not on lipid-lowering medication have revealed a favorable correlation between circulating PCSK9 levels and LDL cholesterol levels increased SREBP-2 activity in patients leads to an increase in circulating PCSK9 protein Furthermore, patients with PCSK9 levels above average showed a higher proportion of CM and stable angina than patients with unstable angina and myocardial infarction and a lower proportion of NCM [10]

A previous study, suggesting an association between circulating PCSK9 and white blood cell counts, could only show an association with lymphocyte counts and a borderline association with neutrophils, while no association with absolute monocyte counts could be shown [10]

A growing body of evidence suggests an intriguing role for PCSK9 in sepsis. In this regard, an elegant study described PCSK9 as a critical regulator of the acute innate immune response in septic shock. In the future, it would be useful to study the cost-effectiveness of PCSK9 inhibition

in other healthcare systems, and in other patient groups with similar, or even higher, risk profiles than those with a history of MI included in the current analysis[11]

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some recent study groups on the basis of BMI for systolic blood pressure, but not for sex, age, height, diastolic blood pressure, heart rate, diagnosis, concomitant medications, or serum parameters analyzed . Cardiovascular risk increases with BMI The finding of cytoplasmic localization of serpinB8 in monocytes and its downregulation in macrophages does not in itself exclude its function as a furin inhibitor. BFA triggers the fusion of microtubules of the trans-Golgi network (TGN) and early endosomes, leading to their structural and functional dissection from the Golgi complex [11]

Figure (6) The level of Proprotein Convertase Subtilisin/Kexin Type 9 showed a significant Decrease ($P< 0.05$) in Stable angina patient compare with unstable angina patient and myocardial infarction patient (6).

Recent studies have PCSK9 is a well-established target for treating hypercholesterolemia and atherosclerosis progression Stable angina patient with unstable angina patient and myocardial infarction patient. Although the major source of PCSK9 is the liver, in a recent experimental study in mice and explanted human hearts, Ding et al. reported that PCSK9 is up-regulated in the zone bordering the infarct area and determines, at least in part, infarct size, cardiac function, and autophagyStable angina patient with unstable angina patient and myocardial infarction patient The same group reported that PCSK9 is highly expressed in vascular smooth muscle cells, and its expression and development of autophagy are regulated by well-known inflammation mediators, such as lipopolysaccharide, tumour necrosis factor α (TNFα), and reactive oxygen species[12].

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The current research Stable angina patients, unstable angina patients, and people who have undergone myocardial infarction are all likely to profit in targeting PCSK9 for the treatment of hypercholesterolemia and the advancement of atherosclerosis. Despite the fact that the liver is the primary source of PCSK9, Ding et al. recently observed that PCSK9 is upregulated in the area surrounding the infarct and that it influences infarct size, cardiac function, and autophagy in patients with stable angina, unstable angina, and myocardial infarction. The study was conducted in mice and on transplanted human hearts. According to the same group, PCSK9 is abundantly expressed in vascular smooth muscle cells, and recognized inflammatory mediators including lipopolysaccharide and tumor necrosis factor reactive mediators control PCSK9 expression and autophagy development [13]

Figure (6) The level of Proprotein Convertase Subtilisin/Kexin Type 9 showed a significant

Decrease (P< 0.05) in hypertensive patient compare with Normotenive patient (6).

In the current study, we found that PCSK9 level was positively associated with hypertension but such a positive association was absent after adjustment for age To be different from the study conducted by Participants in our study were not treated with lipid-lowering medications that could increase the level of PCSK9 Moreover, hypertensive patients were younger in our study compared to those in the study by Lee et al while correlates with age and hypertension. All of this could explain the difference in results[14].

Moreover, a significantly positive association between PCSK9 and was found in male hypertensive patients, while in female hypertensive patients there was a positive tendency for PCSK9 to be associated with Which may result from a smaller sample of females Interestingly, the positive association between PCSK9 and male hypertension was absent after adjustment for age. Likewise, the positive association between PCSK9 and hypertensive patients with blood pressure control after adjustment for age [15]

Similar to our findings, in a study conducted in the United Arab Emirates, hypertension and male gender were predictive factors for dyslipidemia Android fat distribution could explain the increased prevalence of dyslipidemia among males. Furthermore, 68.1% of male participants had high blood pressure and were overweight or obese. This may explain the high prevalence of dyslipidemia in our sample [16].

Another study showed a high percentage of patients with hyperlipidemia and high blood pressure among the Lebanese, a percentage that has increased in the past decades. Age and BMI were the main factors associated with abnormal lipid status and blood pressure, with age and body mass being the main causes of cardiovascular disease and atherosclerosis, with Lp(a) being higher in women and associated with higher blood pressure. Age, body mass. Index, not fat. PCSK9 was associated only with age, and HDL-C, and TG in both men and women was inversely associated with HDL-C in men. Because PCSK9 and Lp(a) levels are markers of coronary artery calcification in asymptomatic patients with familial hypercholesterolemia [17]

In the present study, we found that plasma PCSK9 levels were associated with BP in patients with and without PCSK9 Hypertension. Moreover, we also found that PCSK9 level was independently associated with hypertensive patients. this is the first study investigating the relationship between PCSK9 level and BP and hypertensive and normotensive patients [18]

Furthermore, reported that a positive association was found between PCSK9 concentrations and systolic blood pressure but BP in diabetic patients by univariate analysis, but the association was absent in multiple regression analysis that PCSK9 reduces epithelial Na+ uptake by reducing epithelial Na+ channel expression, and researchers speculated that PCSK9 could contribute to blood pressure control and that a decreased level of PCSK9 would increase the risk of hypertension [19]

Figure (6) The level of Proprotein Convertase Subtilisin/Kexin Type 9 showed a significant Decrease (P< 0.05) in Normal weight patient and Overweight patient compare with Obese patient (figure).

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All of this could explain the difference in results. positive association between PCSK9 and age was found in male hypertensive patients, while in female hypertensive patients there was a positive tendency for PCSK9 to be associated with age Which may r for age. the positive

association between PCSK9 and cIMT disappeared in hypertensive patients with blood pressure control after adjustment for age [21].

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Conclusion

From the previous results and discussion, we concluded the following:

- The current study documents the high prevalence of dyslipidemia CVD The patients population, which has increased in the last decades. Age and BMI are the major factors associated with abnormal lipid profile.

- correlated with age, BMI, nor lipid profile. PCSK9 was only correlated with age, non-HDL-C, and TG in both men and inversely correlated with HDL-C in men. Since PCSK9 and PCSK9 levels are two predictors of coronary artery calcification in asymptomatic patients with familial hypercholesterolemia

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