Evaluation of proprotein convertase subtilisin /kexin 9 (PCSK9) in serum of men hypertensive patients

Yousra Abdul Hussein M Al-mohtaser, Arshad Noori G. Al-Dujaili*

Biology Department, College of Science, University of Kufa, Iraq.

* Corresponding author
yosraa.almohtaser@student.uokufa.edu.iq
another author: arshad.aldujaili@uokufa.edu.iq

Abstract
High blood pressure is considered a major factor for the development of heart disease and vascular disease in elderly people, due to higher tension in arteries leading to hypertension. PCSK9 is a proprotein convertase that increases circulating LDL levels by directing hepatic LDL receptors into lysosomes for degradation. The effects of PCSK9 on hepatic LDL receptors and contribution to atherosclerosis via the induction of hyperlipidemia are well defined.

Methods: The case-control study included (90) subjects divided into sixty (60) male patients. Samples were collected for patients with high blood pressure in Al-Sadr Medical City in Najaf Al-Ashraf / Iraq, and laboratory tests were conducted to measure the lipid profile in Al-Sadr Medical City laboratories. Height and weight were measured, and other information was also collected. The ELISA test was performed in the advanced animal laboratory in the college’s Department of Biology. Department of Science/University of Kufa. The study was conducted by collecting samples and measuring all factors in the period from 1/11/2023 to 2/2/2024. A control group study of 30 men was also conducted. All groups that appeared healthy were matched in age, and patients with diabetes, kidney disease and heart disease were excluded from the study, as well as any other systemic diseases. Conclusion: The present study concluded that PCSK9 considered as a prognostic marker for prediction of hypertension. Also, PCSK9 was very related in hypertensive patients with hyperlipidemia (cholesterol, TG, LDL, & HDL). High biomarker level associated with ages especially at new diagnosis without treatment and with short duration of disease. Smoking plays important roles with high a level of PCSK9 in hypertensive patients. The genetic may play a role in present study by high level of This biomarker in familial hypertensive patients. Obesity has a crucial role in in hypertensive patients with high PCSK9 level.

Keywords
Proprotein Convertase Subtilisin /kexin 9 (PCSK9), Cholesterol (Cho), Triglyceride (TG), High density lipoprotein (HDL), & Low-density lipoprotein (LDL).

Imprint

INTRODUCTION
High blood pressure is considered a major factor for the development of heart disease and vascular disease in elderly people, due to higher tension in arteries leading to hypertension. Elderly is often affected by hypertension caused by stiffness in the arteries so that blood pressure tends to increase. Hypertension is often referred as a silent killer disease, because it is often hypertensive patients for years without feeling any disturbance or symptoms[1]. The PCSK9 site comprised by amino acid residues 367–381 which is situated on the PCSK9/LDLR binding interface, was considered to be targeted by inhibitors.

After the removal of the EGFA domain, [2, 3]. PCSK9 is a proprotein convertase that increases circulating LDL levels by directing hepatic LDL receptors into lysosomes for degradation. The effects of PCSK9 on hepatic LDL receptors and contribution to atherosclerosis via the induction of hyperlipidemia are well defined. Monoclonal PCSK9 antibodies that block the effects of PCSK9 on LDL receptors demonstrated beneficial results in cardiovascular outcome trials[4, 5]. The structure and binding receptors of PCSK9 are shown in Figure (1).

Figure (1) (A) The domain structure of human PCSK9 in its zymogen form. The numbering throughout indicates the position of significant amino acid residues including D186, H226 and S386, which form the catalytic triad of PCSK9. D374Y indicates an amino acid substitution that has been characterized as a gain-of-function mutation. (B)
A three-dimensional cartoon representation of the X-ray crystal structure of human PCSK9 lacking its C-terminal domain.

The Prodo main is colored in red, the catalytic domain in green and the catalytic residues D186, H226 and S386 in pink. The region of PCSK9 that directly interacts with the EGF-(A) domain of LDLR is shown in blue. Adapted from PDB file code 4NMX[2, 4].

METHODS

Subject population

The present study was designed to investigate an important biomarker Proprotein Convertase Subtilisin/kexin 9 (PCSK9) as a prediction or prognostic biomarker in hypertensive patients and relation with lipid profile (cholesterol, triglyceride, HDL, and LDL)

The following criteria were dependent in a current study:
1. Four biomarkers (PCSK9, Cholesterol, TG, and HDL) level in serum
2. Ages
3. Body mass index
4. Hypertensive patients with and without treatment
5. Sex (only male)
6. Duration of disease
7. Smoking or no
8. Familial hypertensive or no

Exclusion criteria

The current study excluded many criteria related to diabetic nephropathy, kidney disease, or any complications such as liver disease, heart disease, and anemia, also excluded.

Blood collection

Each patient and control group undergo a drawn of blood samples from venous at 5 milliliters and left at room temperature for 10 minutes for collecting and then centrifuged at 3000 rpm for 15 minutes for given a serum then 1.5 milliliters was transferred to Eppendorf tube for PCSK9 and lipid profile measurement.

The serum transported to new Eppendorf tubes and stored at-20°C unlit until was used.

Body Mass Index (BMI)

Body mass index was calculated by following formula BMI = kg/m² where kg is a person’s weight in kilograms and m² is their height in meters squared

- Underweight ≤ 18.5 kg/m²
- Normal weight = 18.5–24.9 kg/m²
- Overweight = 25–29.9 kg/m²
- Obesity = BMI of 30 kg/m² or greater

DIAGNOSIS

Hypertension is diagnosed if, when it is measured on two different days, the systolic blood pressure readings on both days is ≥140 mmHg and/or the diastolic blood pressure readings on both days is ≥90 mmHg.

Biomarker measurement

The assessment of serum lipid profile of cholesterol, triglyceride and HDL, which uses an automated microtitre plate ELISA reader and an enzyme-linked immunosorbent assay Biotech, USA) is provided by Human Proprotein Convertase Subtilisin/kexin 9 (PCSK9) ELISA Kit (Pars Biochem).

Statistical analysis

The results were expressed as (Mean ± Standard Error). correlation coefficients were calculated to estimate the correlation between parameters.

The descriptive statistics and correlation coefficients were performed by using IBM SPSS Statistics version 26.2019.

Unpaired sample t-test was used for the comparison between the patients and control groups and multiple comparison for observed mean test (by Tukey multiple comparison test) was used for the comparison among subdivided groups, while the figures constructed by using excel program. p < 0.001 was used as a level of statically significant.
RESULTS

Blood pressure of hypertensive patients in comparison with control group.

In Figure (2) and Figure (3) show significant increase (p-value < 0.01) in hypertensive patients in both Diastolic (10.350 ± 1.0508 mmHg) and systolic pressure (16.23 ± 1.226358 mmHg). Comparison with control group (systolic 12 mmHg and diastolic 8 mmHg).

Lipid profile in hypertensive patients in compare with control group.

The results of the current study Figure (4) showed that there are significant increase (p<0.01) in cholesterol level between patients (263.53 ± 33.693 mg/dL) with high blood pressure and control group (154.067 ± 2.99 mg/dL), also significant increase (p<0.001) in Triglyceride level of patients (275.67 ± 275.67 mg/dL) in compare with control group 112.17 ± 18.45 mg/dL, as in Figure (5) in addition Figure (6) indicate significant decrease (p<0.05) in HDL-level in patients (15.5 ± 7.17 mg/dL) in compare with control group 45.067 ± 3.41 mg/dL as well as Figure (7) revealed a significant...
increase in LDL-Level in patients (143.8±12.15mg/dL) in compare with control group (80.167±9.24mg/dL).

Comparison between Hypertensive patients and control group with PCSK9.

From Figure (7) refer to significant differences of p-value < 0.01 in PCSK9 level in patients (33.16±13.8ng/L) in compare with control group (5.64±0.34ng/L).

Comparison between Hypertensive patients according to age.

From Figure (8) appear significant increase (p<0.001) in PCSK9 level (48.46±11.9ng/L) in age group (40-49) years higher than age group (50-59) years and age (60-69) with mean (30.76±2.62ng/L) & (20.57±3.43ng/L) respectively.

Comparison between hypertensive patients according to duration of disease.

In Figure (9) document that significant increase (p<0.01) in PCSK9 level in duration of disease, duration (one month-one year) in mean (51.99±11.82ng/L) in compare with duration (1-10) years in mean (33.24±3.43ng/L) and duration (11-20) years in mean (21.79±4.68ng/L).

Comparison between hypertensive patients according to treatment.

From Figure (10) indicate significant increase (p<0.01) in PCSK9 level indicate significant increase (p<0.01) in PCSK9 level in patients without treatment (45.65±12.09ng/L) in compare with treatment (24.24±5.59ng/L).

Comparison according to Smoking.

From Figure (11) show significant increase (p<0.05) in PCSK9 level in Smoking group (41.27±12.37ng/L) higher than non-Smoking (21.79±4.68ng/L).

Comparison between hypertensive patients according to body mass index.

From Figure (12) appear significant increase (p<0.01) in PCSK9 level in obese hypertensive patients (45.65±12.09ng/L) in PCSK9 level higher than over-
Figure (9) Comparison between Hypertensive patients according to duration of disease. N=15 (one month-one year), N=20 duration (1-10) years, &N=35 duration (11-20) years. Different letters refer to significant differences between duration groups.

Figure (10) Comparison between Hypertensive patients according to treatment. N=25 (without treatment), N=35 (with treatment). (*) refer to significant differences of p-value < 0.01.

Figure (11) Comparison between Hypertensive patients according to Smoking. N=35 (Smoking), N=25 (non-Smoking). (*) refer to significant differences of p-value < 0.01.

Figure (12) Comparison between Hypertensive patients according to body mass, N=15 (normal weight), N=20 (overweight), &N=25 (obese) different letters refer to significant differences between body mass index groups.
weight (28.34± 2.57ng/L) and normal weight (18.77± 3.31ng/L).

Comparison between Hypertensive patients according to familial hypertensive.

Results of Figure (13) show significant increase (p<0.01) in PCSK9 level in familial hypertensive patients (43.59± 12.41ng/L) in compare with patients non familial (23.29± 5.19 ng/L).

![Figure (13) Comparison according to familial hypertensive. N=30(familial hypertensive), N=30(non-familial). (*) refer to significant differences of p- value < 0.01.](image)

PCSK9 level and lipid profile.

From figure (15), (16) and (18) indicate a positive significant correlation between PCSK9 and cholesterol, triglyceride and LDL-levels also negative significant correlate between PCSK9 and HDL-level significant in figure (17).

Correlation between PCSK9 and cholesterol level in hypertensive patients and control group.

The results of correlation and liner regression between PCSK9 level and cholesterol levels are indicated by the person of a significant positive correlation (p< 0.001) between PCSK9 and cholesterol levels of hypertensive patients (R”=0.892), Y=1.5E2+3.22*X, Figure (15).

Correlation between PCSK9 and triglyceride level in hypertensive patients and control group.

The results of correlation and liner regression between PCSK9 level and triglyceride levels are indicated by the person of a significant positive correlation (p< 0.01) between PCSK9 and triglyceride levels of hypertensive patients (R”=0.906), Y=1E2+5.04*X, Figure (16).

Correlation between PCSK9 and High-density lipoprotein level in hypertensive patients and control group.

The results of correlation and liner regression between PCSK9 level and High-density lipoprotein levels are indicated by the person of a significant positive correlation (p< 0.01) between PCSK9 and High-density lipoprotein levels.

![Figure (15) Relation between PCSK9 and cholesterol level in hypertensive patients.](image)
lipoprotein levels of hypertensive patients ($R^2=0.812)$, $Y=44.56-0.8*X$, Figure (17).

**Correlation between PCSK9 and Low-density lipoprotein level in hypertensive patients and control group.**

The results of correlation and liner regression between PCSK9 level and low-density lipoprotein levels are indicated by the person of a significant positive correlation ($p<0.01$) between PCSK9 and low-density lipoprotein levels of hypertensive patients ($R^2=0.801$), $Y=82.49+1.67*X$, Figure (18).

**DISCUSSION**

From the results of Figure (7) found that a significant increase $p$-value $<0.05$ in PCSK9 level
in Hypertensive patients compared to the control group.

The present study agrees with several studies that have suggested a relation between PCSK9 level and hypertension (systolic and diastolic) and discusses the relation depend on that decrease (PCSK9) considered as a main regulator of LDL-C by binding with hepatic LDL-receptors which lead to enhance in LDL-degradation and promoting LDL-c conception and may effect on development of hypertension [3, 6, 7].

An important mechanism has been postulated to show a role of PCSK9 in hypertension. In vitro appears that increase level of PCSK9 may result in downregulated epithelial Na+ absorption by reducing the epithelial Na+ channel expression, and the researchers speculated that PCSK9 could contribute to BP control [8].

The current as in figure (8) indicates a significant increase in PCSK9 level in age (40-49) years as compared with other ages (50-59) and (60-69) years old.

Some studies have been revealed that a level of PCSK9 decrease with age in men but increase in age in women and this result due to effect of testosterone level in men and estrogen level in women in other words increase testosterone associated with high PCSK9 level whereas low level at advanced age negatively associated with PCSK9 and considered as a risk factor for initiation hypertension or risk factor [9]. The age (40-49) years in present study has higher PCSK9 level in compare with other ages may result due to that these ages have new diagnosed or and do not administrate to any types of therapy.

From figure (9) also show a significant increase in duration of disease one month to one year in compare with another duration of diseases and this result may support our view that a hypertensive patients in present study that do not administrated any type of therapy or checking a blood pressure newly after suffering a some of symptoms associated with highly PCSK9 level. Also figure (10) proved that a patients of present that newly diagnosed with any treatment has a highly a level of PCSK9 and this may related with high LDL-C, VLDL, TG concentration without any therapy lacking (hypolipidemic or hypertensive drug may result to occurrence of blood pressure in these patients highly in compare with treated patients [10-13].

Figure (11) revealed a significant increase in PCSK9 level in smoker patients in compare with non-smoker.

The current results agreement with several studies that showed that cigarette smoking extract in hance a level of PCSK9 by a mechanism including stimulation of various harmful substances such as high concentrations of oxidant and include Reactive oxygen species production (ROS) which is considered one of the major cause of arterial hypertension and atherosclerosis in carotid arteries also produce a proinflammatory response.
factor such as oxidized LDL, Tumor necrosis factor TNF-α through a signaling ROS/Nuclear factor Kappa-β (NFK-β) with high LDL-C level all these may be associated with high PCSK9 level[14, 15].

A present result in figure (12) documented high level of PCSK9 in obese hypertensive patients in compare with overweight and normal weight.

In a previous study has been showed that high level of PCSK9 in obese individuals after exposure to pollution as a result of increase in proinflammatory mediator such as cytokines/adipokines in adipose tissue also over production of ROS therefore obesity it’s a strong risk factor for hypertension atherosclerosis with high LDL-C level [16-19].

In current results as in figure (4-13) indicate a high level of PCSK9 level in familial hypertensive patients in compare with non-familial previous study has been found that defect in the epithelial Na-channel (ENaC) has a crucial role in Na⁺-homeostasis and control of blood pressure and found that defection a regulation of ENaC lead to form a inherited form of hypertensive and hypotension and show that PCSK9 as a proteases regulate a ENaC gating channel and reduce a hypertension or CVD[20, 21].

In several research has been suggested that PCSK9 has a critical role in regulation by trafficking ENaC in pathway of synthesis by reducing ENaC channel numbers and Na⁺ absorption therefore and disruption of PCSK9 lead to high expression of Na⁺ by excessive renal Na⁺ and these defects known as genetic hypertension forms[22, 23].

The figure (15), (16), and (18) show a significant positive correlation between PCSK9 and cholesterol, TG and LDL whereas significant negative correlation with HDL as in figure (17).

Previous study Abi faded et al, [24] has been described that cholesterol metabolism and hypercholesterolemia linking with serine protease producing by the liver such as PCSK9 also with LDL-C through Mediate LDL-R, receptors degradation therefore lowering and fewer LDL-receptors on membrane and decrease clearance from circulation so that high PCSK9 associated with high LDL-C, cholesterol, and triglyceride [25] [26].

In other demographic studies on several metabolic parament has been document a correlation between PCSK9 and other parameters such as LDL-C, HDL-C, Cholesterol, Triglyceride, apolipoprotein n-B, Insulin and glucose [27] [28].

In a study of [29] has been indicated that both secretion and degradation of TG-rich lipoprotein so that hypertriglyceridemia with high PCSK9 linked with multiple mechanism such as increased secretion of APO-B containing lipoprotein in liver and stimulate degradation of LDL-R receptors.

Some former studies have been linked between as present of mature PCSK9 and furin cleaved in circulation with predicts of atherosclerosis and coronary heart disease with high LDL-C and cholesterol [30] [31] [32] [30].

Another studies evident that PCSK9 associated with blood pressure, age, smoking, obesity and correlation with level of apo-B, LDL-C and triglyceride [33] [32] [34].

**CONCLUSION**

1. The present study concluded that PCSK9 considered as a progestcr marker for prediction of hypertension.
2. PCSK9 was very related in hypertensive patients with hyperlipidemia (cholesterol, TG, LDL, & HDL)
3. High biomarker level associated with ages especially at new diagnosis without treatment and with short duration of disease.
4. smoking play important roles with high a level of PCSK9 in hypertensive patients.
5. The genetic may play a role in present study by high level of This biomarker in familial hypertensive patients.
6. Obesity have a crucial role in in hypertensive patients with high PCSK9 level.

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