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Alaa M. E. Antar
Department of Internal Medicine, Faculty of Medicine for girls, Al-Azhar University, Cairo, Egypt., alaamagdy2907@gmail.com

Karima Youssef Ahmed
Department of Internal Medicine, Faculty of Medicine for girls, Al-Azhar University, Cairo, Egypt.

Fatma El Zahraa AE Diab
Department of Clinical Pathology, Faculty of Medicine for girls, Al-Azhar University, Cairo, Egypt.

Fatma M. Kotb
Department of Internal Medicine, Faculty of Medicine for girls, Al-Azhar University, Cairo, Egypt.

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ORIGINAL ARTICLE

Evaluation of Serum Proprotein Convertase Subtilisin Kexin Type 9 (PCSK9) as a Diagnostic Biomarker of Hepatic Steatosis

Alaa M.E. Antar [a,*], Karima Youssef Ahmed [a], Fatma El Zahraa A.E. Diab [b], Fatma M. Kotb [a]

[a] Department of Internal Medicine, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt
[b] Department of Clinical Pathology, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt

Abstract

Background: **Triglyceride intake or synthesis increases and/or triglyceride outflow decreases in the liver, respectively, to cause hepatic steatosis.**

Aim of the work: **To evaluate serum levels of proprotein convertase subtilisin kexin type 9 (PCSK9) as a diagnostic biomarker of hepatic steatosis and their correlations with the severity of the disease.**

Subject and methods: **This case-control research was conducted in the Department of Internal Medicine, Al-Zahraa University Hospital, and included sixty Egyptian patients with hepatic steatosis (group I) diagnosed by pelvic-abdominal ultrasound with (group Ib includes 30 cases) or without (group Ia includes 30 cases) diabetes mellitus, as well as thirty apparently healthy individuals as a control group (group II).**

Results: **Differences in PCSK9, fasting insulin, and HOMA-IR across study groups are extremely statistically considerable (P value < 0.001). Differences in cholesterol, HDL, and LDL are extremely statistically considerable (P value < 0.001) between the research groups. There is statistically considerable variation (P value $= 0.001$) between research groups regarding TG. PCSK9, fasting insulin, HOMA-IR, cholesterol, TG, and LDL were higher in the group with hepatic steatosis with diabetes than without diabetes, and both of them were higher than the control group. On the other hand, HDL was lowest in steatosis with diabetes.**

Conclusion: **PCSK9 has a role in the development of hepatic steatosis, especially in diabetic patients.**

Keywords: High-density lipoprotein (HDL), Hepatic steatosis, Homeostasis model assessment of insulin resistance (HOMA-IR), Low density lipoprotein (LDL), Proprotein convertase subtilisin kexin type 9 (PCSK9)

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is described as the ectopic deposition of fat in the liver in the absence of any other causes of secondary liver fat accumulation (hepatic steatosis). Despite the fact that healthy people's livers might sometimes have modest fat deposition, pathologic fat deposition is defined as occurring in at least 5% of hepatocytes.[1]

The range of NAFLD includes basic adipose tissue deposition in the liver to more advanced steatosis with hepatitis, fibrosis, cirrhosis, and even hepatocellular cancer (HCC).[2]

Obesity, insulin resistance, food (especially an excess of carbohydrates or fructose), and a sedentary lifestyle are the main modifiable risk factors for the advancement of NAFLD.[3]

Hepatocytes secrete PCSK9, which targets the low-density lipoprotein receptor (LDLR) for degradation and possibly lipogenesis and prevents the absorption of low-density lipoproteins. Hepatic steatosis and liver biomarkers show a high correlation with PCSK9.[4]

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* Corresponding author.
E-mail address: alaamagdy2907@gmail.com (A.M.E. Antar).

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This work aimed to assess the serum level of PCSK9 as a diagnostic biomarker of hepatic steatosis and its correlation with the severity of the disease.

2. Subjects and methods

2.1. Subjects

The subjects of the current case-control study were ninety (90) subjects from April 2021 to October 2021 at Al-Zahraa University Hospital. Informed consent was obtained from all patients.

2.2. The subjects were classified into

Group 1(patient group): Include (60) patients chosen from the Internal Medicine department of Al-Zahraa University Hospital with hepatic steatosis diagnosed by pelvic-abdominal ultrasound with or without diabetes mellitus, and this group will be divided into 2 subgroups: **Group 1a**: Include 30 patients with hepatic steatosis without diabetes mellitus, males (15), females (15), and their mean age of 47.9 10.2 and **Group 1b**: Include 30 patients with hepatic steatosis and diabetes mellitus, males (14), females (16), and their mean age of 51.7 9.2. **Group 2 (control):** Include 30 apparently healthy volunteers whose age and sex were matched. Inclusion criteria: age more than 18 years, both sexes, and patients with hepatic steatosis with or without diabetes mellitus.

2.3. Exclusion criteria

Age below 18 years, pregnancy, malignancies, chronic inflammatory diseases, alcoholic use, viral hepatitis, and autoimmune liver disease.

2.4. Methods

Each and every research population underwent comprehensive medical history taking, including weight, blood pressure, and body mass index, and laboratory tests: postprandial blood sugar, fasting blood glucose, glycated hemoglobin (HBA1c), low density lipoprotein (LDL), total cholesterol, high density lipoprotein (HDL), triglycerides, alanine transaminase (ALT), serum creatinine, aspartate transaminase (AST), serum bilirubin, alkaline phosphatase (ALP), serum albumin, blood urea, serum PCSK9, and fasting insulin and HOMA-IR. Radiological: abdominal ultrasound for detection of hepatic steatosis (fatty liver).

2.4.1. Sample collection and preparation

A: The first example After a 9-h fast, 8 ml of peripheral venous blood were drawn from each individual in an entirely sterile environment utilizing a plastic disposable syringe and were divided as follows: The first tube contained 2 ml of anticoagulated blood with ethylenediaminetetraacetic acid (EDETA) for HBA1C. It was done using Biorad D 10, USA, high-performance liquid chromatography (HPLC). The second tube contained 4 ml of blood without anticoagulant that was left to clot for thirty minutes at room temperature prior to centrifugation for 20 min at 3000 rpm. The serum was separated and used for measurement of kidney function tests (urea, creatinine), liver function tests (ALT, AST, albumin, bilirubin), ALP, lipid profile (cholesterol, HDL, triglycerides, and LDL), and fasting blood glucose. They were done using a fully automated chemistry analyzer (Cobas C 311, Germany). The commercial kits were supplied by Roche Diagnostics (Germany). The third tube contained 2 ml of blood without anticoagulant that was left to clot for thirty minutes at room temperature before centrifugation for 20 min at 3000 rpm. The serum was separated and stored at −80 °C until the time of measurement of PCSK9 and fasting insulin.

B: Following two hours, all patients had a second sample of peripheral venous blood taken under perfect aseptic conditions using a plastic disposable syringe. This sample was used to assess blood glucose levels two hours after a meal.

2.4.2. Measurement of serum pcsk9

PCSK9 by enzyme-linked immunoassay (ELISA) kit, Sun Red Company, Shanghai, Lot No. 202111.

Table 1. Comparing research groups with reference to lipid profile.

	Groups			F	*P* value
	Group IA ($n = 30$)	Group IB ($n = 30$)	Group II ($n = 30$)		
CHOL (mg/dl) Mean ± SD	178.7 ± 59.3	197.0 ± 39.1	145.8 ± 34.5	**9.7**	<0.001 HS
TG (mg/dl) Mean ± SD	155.2 ± 74.9	193.3 ± 52.3	138.2 ± 27.7	**7.8**	0.001 S
HDL (mg/dl) Mean ± SD	38.7 ± 9.6	35.9 ± 2.6	44.5 ± 6.1	**12.7**	<0.001 HS
LDL (mg/dl) Mean ± SD	110.3 ± 53.2	131.2 ± 35.3	79.5 ± 17.6	**13.8**	<0.001 HS

CHOL, cholesterol; F, F value of ANOVA; HDL, high density lipoprotein; HS, highly significant; LDL, low density lipoprotein; S, significant; SD, standard deviation; TG, triglycerides.

Table 2. Comparing research groups with reference to liver function tests.

	Groups			KW	*P* value
	Group IA ($n = 30$)	Group IB ($n = 30$)	Group II ($n = 30$)		
ALT (U/l)					
Median	21	30	20	5.4	0.067 NS
IQR	18–37	20–54	18–36		
AST (U/l)					
Median	24	37	22	12.01	0.002 S
IQR	20–32	25–46	20–32		
ALP (U/l)					
Median	89	88	79	2.19	0.334 NS
IQR	60–115	66–99	61–96		
Bilirubin (mg/dl)					
Median	0.5	0.4	0.8	7.7	0.021 S
IQR	0.3–0.7	0.3–0.6	0.3–0.9		
ALB (g/dl)					
Median	3.8	3.9	3.8	4.8	0.091 NS
IQR	3.6–4.02	3.8–4.2	3.7–4.2		

ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; IQR, interquartile range; KW, Kruskal Willis test; NS, non-significant; S, significant.

2.4.3. Measurement of fasting insulin

By enzyme-linked immunoassay test (ELISA) kit, The Monocent, Inc.'s Insulin ELISA Test System, USA, Lot Number: INS6214.

2.4.4. Homeostasis model assessment of insulin resistance (HOMA-IR)

Is determined by applying the following formula to fasting insulin and glucose readings: fasting insulin (μU/ml) X fasting glucose (mg/dl)/405.

3. Results

This study included 90 participants, who were divided into G1a: hepatic steatosis without diabetes ($n = 30$), G1b: hepatic steatosis with diabetes ($n = 30$), and a healthy control group of 30.

As regards CHOL, HDL, and LDL, there was a highly statistically significant variation between studied groups and a statistically considerable

Table 3. Comparing research groups with reference to PCSK9 and fasting insulin.

	Groups			Test	*P* value
	Group IA ($n = 30$)	Group IB ($n = 30$)	Group II ($n = 30$)		
PCSK9 Mean ± SD	96.6 ± 27.03	121.6 ± 31.7	73.6 ± 12.4	F = 27.4	<0.001 HS
Fasting. insulin					
Median	18.5	39	8	KW = 44.3	<0.001 HS
IQR	9.07–38.4	26–57	4–15.3		
HOMI-IR					
Median	4.3	17.1	1.6	KW = 57.02	<0.001 HS
IQR	2–8.1	10.2–31.2	0.8–3.2		

F, F value of ANOVA test; HOMA-IR, Homeostatic Model Assessment For Insulin Resistance; IQR, interquartile range; KW, Kruskal Willis test; PCSK9, proprotein convertase subtilisin kexin type 9.
HS: *P*-value <0.001 is considered highly significant.

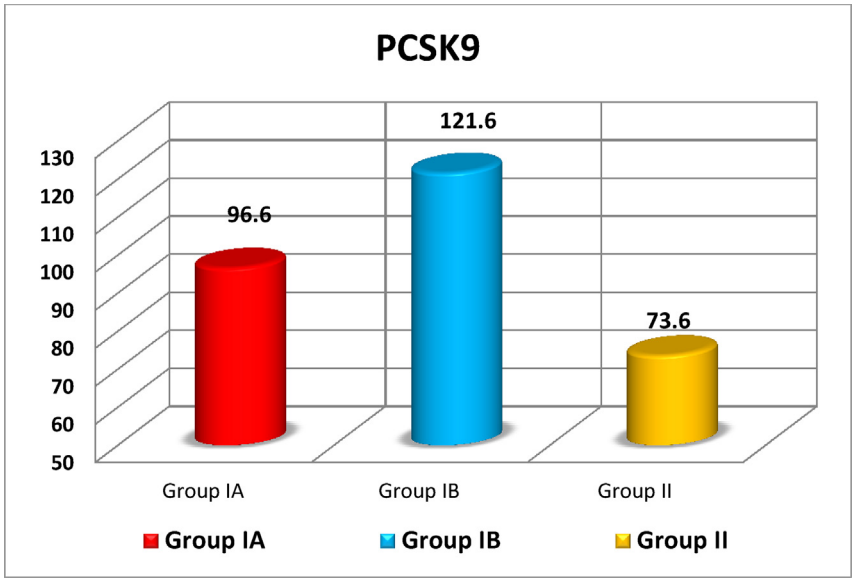

Fig. 1. Comparing research groups with reference to PCSK9.

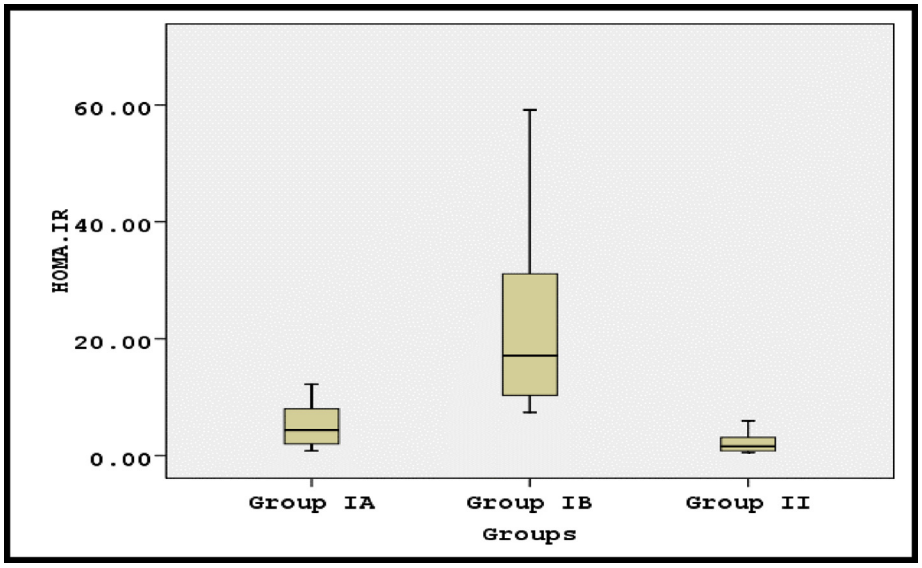

Fig. 2. Comparing research groups with reference to HOMA-IR.

Table 4. Study of the relationship between PCSK9 and lipid profiles in all groups under study.

Data	Group IA		Group IB		Group II	
	r	*P* value	r	*P* value	r	*P* value
PCSK9 vs CHOL	0.776	<0.001	0.614	<0.001	0.038	0.84
PCSK9 vs TG	0.384	0.036	0.366	0.046	0.04	0.835
PCSK9 vs HDL	−0.229	0.222	−0.539	0.002	−0.121	0.525
PCSK9 vs LDL	0.735	<0.001	0.761	<0.001	0.052	0.783

change between studied groups regarding TG (Table 1).

As regarding to AST and bilirubin there were statistical considerable changes between the studied groups & there were no statistical considerable changes as regard ALT, ALP, and ALB (Table 2).

As regards PCSK9, fasting insulin, and HOMA-IR, there were highly statistically significant changes between study groups (Table 3, Figs. 1 and 2).

As regards the IA group, there was a very statistically considerable positive connection between PCSK9 and CHOL and LDL, and a statistically considerable positive connection between PCSK9 and TG. Regarding the IB group, there was a very statistically considerable positive connection between PCSK9 and CHOL and LDL, and a statistically considerable positive connection between PCSK9 and TG and HDL. Regarding group II, there was no statistically considerable connection between PCSK9 and lipid profile (Tables 4 and 5).

As regards the IA group, there was a statistically considerable positive connection between PCSK9 and HOMA-IR; regarding the IB group: there was a statistically considerable positive connection between PCSK9 and F. insulin; and regarding group II: there was no statistically considerable connection between PCSK9 and HOMA-IR and F. insulin.

4. Discussion

As regard cholesterol, TG, HDL, and LDL, In line with our results, Paquette et al.[4] revealed that there was a statistically considerable change between patients with hepatic steatosis and controls.

As well, Kastberg et al.[5] revealed that there was considerable statistical variation between patients

Table 5. Correlation study between PCSK9 and HOMA-IR and F.

Variables	Group IA		Group IB		Group II	
	r	*P*-value	r	*P*-value	R	*P* value
PCSK9 vs HOMA-IR	0.38	0.036	0.14	0.453	−0.1	0.580
PCSK9 vs F. insulin	0.349	0.058	0.369	0.045	−0.13	0.495

with and without NAFLD as regards cholesterol, TG, HDL, and LDL.

While, the study by Silaghi et al.[6] revealed that there was statistically considerable change between T2DM patients with and without hepatic steatosis as regard cholesterol and HDL, there was no considerable change as regard LDl. The difference between our study and this study is due to First, their study has a retrospective design that could introduce bias concerning the selection of patients for hepatic ultrasound. Second, NAFLD was defined using the Fatty Liver Index (FLI), a steatosis biomarker validated against liver ultrasound. The performance of FLI for the prediction of steatosis was very low. Third, they could not completely exclude secondary causes of liver disease, and this limitation could have increased the number of included patients, which was 381.

However, the study by Ruscica et al.[7] revealed that LDL, but not cholesterol, TG, or HDL, was substantially connected with steatosis severity. This disagreement is due to the difference in sample size between our study and this study. The sample size in the Ruscica study was not sufficient to demonstrate the association between steatosis severity and lipid components and was conducted on 201 Italian patients who underwent liver biopsy for suspected non-alcoholic steatohepatitis (NASH), while our study was conducted on 90 participants without liver biopsy.

As regard AST, ALT, ALT, Albumin, Bilirubin and alkaline phosphatase, in agreement with our results, Paquette et al.[4] revealed that there were statistically considerable changes between patients with hepatic steatosis and controls as regards AST and bilirubin. But there were no statistically significant changes between the studied groups regarding ALT, albumin, or alkaline phosphatase.

In contrast, **Kahl *et al.***[8] revealed that there were statistically considerable changes between non-diabetic patients with and without steatosis as regards ALT, but there were no statistically considerable changes as regard AST. This difference is due to the Kahl study having a rather small sample size and a collective with low mean hepatocellular lipid contents and prevalence of steatosis, and the participants with significant consumption of alcohol were not omitted from the analysis of the whole collective.

Kastberg *et al.*[5] revealed that there were statistically considerable changes between patients with and without NAFLD as regards ALT, AST, and ALP, and this was due to the fact that neither an intra- nor inter observer validity ultrasound scan was carried out in this study (Kastberg study).

In the present research, we noted that there were no statistically significant changes between the studied groups as regards kidney function tests (creativity and urea).

This was corroborated by the research by **Jenks *et al.***[9] revealed that the rate of decrease in estimated glomerular filtration rate was 1.55 ml min11.73 m^2 per year for participants with liver steatosis compared to 1.84 ml min11.73 m^2 for those without steatosis ($P = 0.19$). Neither liver steatosis nor NAFLD were substantially connected with this rate of decrease in kidney function. When the analysis was limited to persons with and without NAFLD, similar outcomes were reported (−1.44 vs. −1.64 ml min 11.73 m^2 per year, respectively; $P = 0.44$).

Also, in accordance with our results, Ali et al.[10] revealed that there were no statistically significant changes between patients with and without NAFLD as regards S. creatinine.

As regards PCSK9, F. insulin, and HOMA-IR, In agreement with our results, Paquette et al.[4] revealed that there were statistically considerable changes between patients with and without hepatic steatosis.

Our results can be supported by Dongiovanni et al.[11] who reported that circulating PCSK9 rates were connected with both steatosis grade (forecast +0.18 ± 0.06 log ng/ml; $P = 0.0011$), and steatosis linked hepatic damage: hepatocellular ballooning (+0.28 ± 0.99 log ng/ml; $P = 0.005$), necro-inflammation (+0.27 ± 0.07; $P < 0.001$), and fibrosis stage (+0.16 ± 0.05; $P = 0.001$). PCSK9 values were related to the degree of steatosis at multivariate analysis (+0.13 ± 0.06 log ng/ml; $P = 0.03$).

As well, **Emma *et al.***[12] revealed that PCSK9 level was non-substantially connected with HbA1c and Glucose.

However, Ruscica et al.[7] stated that circulating PCSK9 was not substantially connected with levels of ALT, triglycerides, HDL and LDL cholesterol, or total cholesterol. And this disagreement is due to the difference in sample size and the lack of assessment of the relationship between circulating PCSK9 and lipoprotein composition in the Ruscica study.

While the research by Waluś-Miarka et al.[13] revealed that PCSK9 level was not substantially connected with cholesterol, TG, or LDL but not with HDL, this disagreement is due to the difference in the number of selected patients: 116 patients in Waluś-Miarka study and 60 patients and 30 controls in our study.

As well, Paquette et al.[14] reported that plasma PCSK9 values were substantially correlated with LDL cholesterol; however, even after several modifications, triglycerides still showed the highest correlation.

Regarding the connection between PCSK9 and HOMA-IR and F. insulin in all studied groups, in

agreement with our results, Hamamura et al.[15] stated that PCSK9 was substantially connected with HOMA-IR.

However, Emma et al.[12] stated that PCSK9 levels were not substantially connected with HOMA-IR and insulin. And this disagreement is due to the difference in sample size; their study was conducted on a selected population of morbidly obese subjects with non-NAFLD or NASH detected by liver biopsy performed during bariatric surgery with an average age of 39 years.

The limitation of our study is that we did not correlate the serum biomarkers with liver biopsies.

4.1. Conclusion

Our study's findings support the relationship between PCSK9 and liver steatosis that has previously been described in other investigations.

Disclosure

The authors have no financial interest to declare in relation to the content of this article.

Authorship

All authors have a substantial contribution to the article.

Conflicts of interest

The authors declared that there were NO conflicts of Interest.

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