Physiological and Immunological Study of Biomarkers in Non-Alcoholic Fatty Liver Disease for Predicting Chronic Kidney Diseases

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Keywords: Chronic Kidney Diseases (CKD), FABP-4, Non-Alcoholic Fatty Liver Disease (NAFLD), TNF-α.

Abstract: Non-alcoholic fatty liver disease (NAFLD) is characterized by the accumulation of lipids in macrovesicular hepatic vesicles in about ≥5% of hepatocytes in patients who do not have a secondary cause for steatosis, such as alcohol consumption, hepatitis C, medications, protein malnutrition, or parenteral nutrition. The current research was designed to evaluate the association between non-alcoholic fatty liver disease and chronic kidney disease in patients with NAFLD. This study was conducted from December 2023 until March 2024 at the following main locations: AL-Nasriya Technical Hospital, Nasiriya Governorate, Iraq; Mohammed AL-Mousawi Children′s Hospital, Nasiriya Governorate, Iraq; and AL-Imam AL-Hussain Hospital, Nasiriya Governorate, Iraq. The samples consisted of sixty patients diagnosed with NAFLD and twenty-eight patients without NAFLD. Patient samples included 30 males aged between 28 and 75 years and 30 females aged between 20 and 70 years. The control group's ages ranged between 28 and 65 years. The creatinine test results showed a non-significant increase in patients with NAFLD compared to the control group; in contrast, the glomerular filtration rate (GFR) decreased significantly in the patients group compared to the control. The results also showed significant decreases in FABP-4, PAI-1, and TNF-α levels in patients with NAFLD compared to the control group. Based on the results of the current study, FABP-4 and PAI-1 are considered excellent markers for predicting chronic kidney disease (CKD), and TNF-α can also be considered a reliable marker for predicting CKD.

1 INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD): it's characterized by accumulation of the fat in macro-vesicular hepatic about ≥ 5% of hepatocytes in patients who don't have a secondary caused for steatosis as e.g. alcohol consumption, hepatitis C, medications, protein malnutrition and parenteral nutrition [1].

The alarming increase in the prevalence in NAFLD worldwide is attributed to the increased the population who have obese and diabetic people. The prevalence rate of NAFLD is higher in men compared to women and tends to be rise in women after menopause. The globally prevalence of NAFLD is beginning to raising from 25.3% in 1990-2006 to 38.0% in 2016-2019. The causes for this increasing trend are multifactorial and predominantly blamed on urbanization, unhealthy diet habits and sedentary lifestyles [2].

NAFLD and metabolic syndrome (MS) are two different entity share common clinical and physiopathological attributes with insulin resistance (IR) as the most related [3].

Although the NAFLD is a complex and multifactorial disease that involves several genetic, epigenetic and environmental factors, But, isn't fully understood yet pathogenesis for this disease due to sensitivity to the accumulation of fat via a second pathogenic insult, the liver prompts inflammation and cell death that consequently interprets into oxidative stress (OS), which finally leads to non-alcoholic steatohepatitis NASH and fibrosis [4].

Most patients with NAFLD may remain asymptomatic, but about 5-10% of them are evaluated for development of the complication cirrhosis with a high risk of death. The existence of T2DM may be the most important clinical predictor of liver-relative morbidity and mortality in the NAFLD. [5].

Prevalence of NAFLD is about 80% to 90% in obese adults, 30% to 50% in patients with diabetes mellitus (DM), 90% or more than that in the patient with hyperlipidemia, 3-10% in children, and as high as 40% to 70% between children with obesity [6], [7].

Chronic Kidney Disease: CKD is a complex, progressive chronic condition that is defined either by abnormalities in structures or functions of the kidney and lasts for ≥ 3 months. CKD has a significant effect on global health, both as a direct cause of global mortality and as an important risk factor to CVD, identifying novel modifiable risk factor for CKD is critical to reducing the burden of disease. Globally, about 697.5 million statuses in all stages of CKD were recorded, with a global prevalence of 9.1% and 1.2 million deaths from CKD in 2017 [8].

CKD tends to associated with MS, likeT2DM, obesity, and hypertension. While in countries with low and middle income, the contagious diseases and environmental toxins are also commonly associated with CKD [9], [10]. Interestingly, the cellular phenomena, metabolic pathways and molecular mediators included in NAFLD and CKD are similar to each other and include IR, ectopic fats depositions, and activations of insulin and transformation growth factor pathway [11].

Accurate assessment of renal functions in the patients with LD is of central importance, particularly in the patients with cirrhosis. Where estimated Glomerular Filtration Rate (eGFR) is one of the monitoring indicators of the kidney functions in these patients and one the guiding criteria for simultaneous liver and kidney transplantation [12]. The most common methods used for estimating the GFR in these populations are depend on creatinine, which is affected by a decrease in creatinine production, a reduction of skeletal mass, and potentially by raising serum bilirubin. Creatinine is a product of the breakdown of creatine, which is manufactured in the liver and primarily stored in muscle tissues. Therefore, serum creatinine is closely related to body mass and is therefore affected by a number of variables such as age, gender, muscle mass, and ethnicity [13].

2 MATERIALS AND METHODS

The current study was conducted through duration from December 2023 and continued until March 2024. It has been carried out at the following main location:

- AL-Nasriya Technical Hospital, Nasiriya Government, Iraq.
- Mohammed AL-Mousawi Children′s Hospital, Nasiriya Government, Iraq.
- AL-Imam AL-Hussain Hospital, Nasiriya Government, Iraq.

2.1 Subjects

In this study included 88 participants. This participant divided into 60 participants infected with NAFLD and 28 participants non-infected with NAFLD. The infected participant divided into 30 patient man their ages range between (28-75) years and 30 patient women their ages range between (20-70) years. control group their ages range between (28-65) years, the information collected from them included the following:(Name, age, height, weight, sex, address, marital status, educational level, work, type of treatment using) where patient with cardiac disease, kidney disease, hepatitis disease is excluded and them with a genetic history of diabetes were excluded as well.

2.2 Collection of Blood Samples

After obtaining official approvals from the Thi-Qar Health Department to facilitate mission to collect samples from patients with NAFLD visiting the above-mentioned hospitals, also the approval of the medical committees in the hospitals was obtained, and then the approval of the patients' consent for the purpose of taking blood samples from them.

The time for sample collection began from 8:30 a.m. to 1:00 p.m. when 5 ml of venous blood was drawn for both infected and non-infected with non-alcoholic fatty liver disease. 2 ml was transferred to tubes containing EDTA to measure the HbA1c. 3 ml of blood were placed in tubes containing the gel substance. It was discarded in a centrifuged at speed reaches to 5000 revolutions per minute for the period 5 minutes, where blood serum was separated from the other components and after they were separated and put in a tube to keep the serum at a temperature of -20 below zero until performing biochemical tests.

2.3 Parameters Measured

2.3.1 Creatinine

This reagent is designated for use in laboratory (automated method) where it allows the quantification of creatinine in human serum and

plasma or urines to screen its level, which was provided by a company Biolabo SAS.

2.3.2 Plasminogen Activator Inhibitor (PAI)

Sandwich kit is used to assay PAI levels in Human serum, which was provided by a company Sunlong Biotech.

2.3.3 Human Fatty Acid Binding Protein 4

Sandwich kit is used to assay FABP4 level in Human serum, which was provided by a company Sunlong Biotech.

2.3.4 Human Tumor Necrosis Factorα (TNF)

Sandwich kit is used to assay TNF-α level in Human serum, which was provided by a company Sunlong Biotech.

2.4 Statistical Analysis

The data of current study was statistically analysis by used of SPSS (Statistical Package of Social Science version 26), depends on using independent sample t test and LSD, Chi-square, person for correlation, and ROC curve analysis at p. value < 0.05.

3 RESULTS

The current study showed creatinine increased non-significantly in patients with NAFLD group compared to the control group, in contrast, the GFR decreased significantly in patients group compared with the control group at p. value < 0.05, as in Table 1.

The present study showed that the FABP-4, PAI-1, and TNF decreased significantly in the patient with NAFLD group compared to the control group at p-value < 0.05 as in Table 2.

The current study recorded that a Creatinine recorded a strong negative correlation with GFR. The FABP-4 scored a weak positive correlation with PAI-, and strong correlation with TNF. The PAI-1 scored weak positive correlation with TNF, at p. value < 0.05, as in Table 3.

Table 1: Evaluation of renal function test in patients with NAFLD and control groups.

Kidney function tests	Patients No. 60	Control No. 28	p. value
	Mean ± S. E		
Creatinine	0.95 ± 0.04	0.87 ± 0.05	0.230
GFR	86.9 ± 5.67	109.1 ± 10.7	0.039*

Table 2: Evaluation of immune parameters in patients with NAFLD and control groups.

	Patients No. 60	Control No. 28	p. value
	Mean ± S. E		
FABP-4	13.1 ± 0.30	21.7 ± 1.02	<0.001**
PAI-1	0.83 ± 0.02	0.92 ± 0.0.4	0.044
TNF	7.86 ± 0.37	16.7 ± 1.01	<0.001**

This study recorded that 56% of patients their creatinine concentration increased compared with control group with cut value for positive 0.6, while only 33% of patients their GFR mean within the range of control group at p. value < 0.05 with cut value for positive 47.3, in addition, the sensitivity of parameters 0.91% and 0.86 and the specificity 0.85% and 0.96% respectively at above cut value, as in Table 4 and Figure 1.

This study recorded that lees than 1% of patients their FABP-4 mean within the range of control group, regarding API-1 the study noted 28% of patients had API-1 mean more than the mean of control group, according to TNF less than 1% of patients their mean of TNF increases than mean of TNF in control group at p. value < 0.05 as in Table 5 and Figure 2.

Table 3: Person correlation between involved parameters.

		Creatinine	GFR	FABP	PAI	TNF
Creatinine	r. value		-0.809**	-0.045	-0.065	0.016
	p. value		0.000	0.734	0.620	0.904
GFR	r. value			-0.037	-0.020	-0.058
	p. value			0.778	0.882	0.660
FABP	r. value				0.388**	0.712**
	p. value				0.002	0.000
PAI	r. value					0.351**
	p. value					0.006

Table 4: Receiver operating characteristic curve for renal function test.

Variable	Cut point	Sensitivity	Specificity	Area	S. E	p. value	Asymptotic 95%C1	
							Lower bound	Upper bound
Creatinine	0.60	0.91	0.85	0.562	0.065	0.351	0.434	0.690
GFR	47.3	0.86	0.96	0.337	0.063	0.014	0.213	0.461

Table 5: Receiver operating characteristic curve for immune parameters.

Variable	Cut point	Sensitivity	Specificity	Area	S. E	p. value	Asymptotic 95% CI	
							Lower Bound	Upper Bound
FABP-4	9.43	1.000	1.000	0.954	0.020	0.000	0.916	0.992
API-1	0.805	0.964	0.817	0.711	0.063	0.001	0.588	0.834
TNF-α	3.86	1.000	1.000	0.934	0.024	0.000	0.886	0.981
Positive 28 Negative 60								

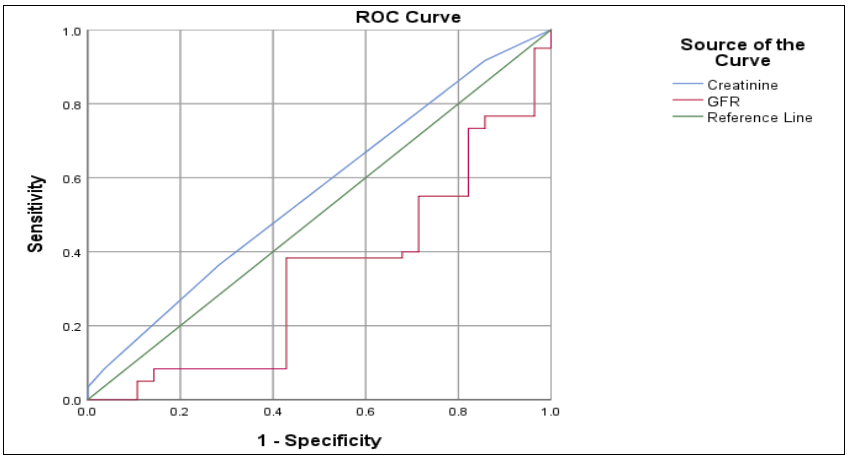

Figure 1: Receiver operating characteristic curve for renal function test.

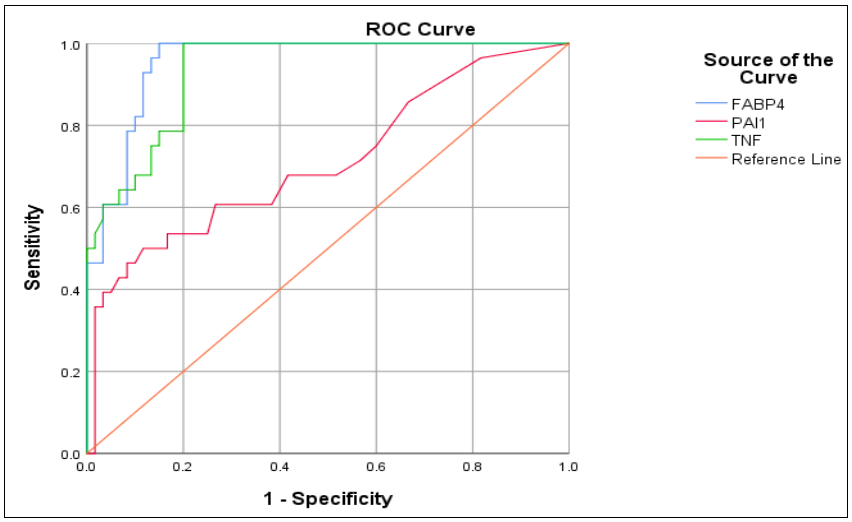

Figure 2: Receiver operating characteristic curve for immune and biochemical parameters.

4 DISCUSSION

Several studies in kidney disease have suggested that lower levels of PAI-1 protect against kidney fibrosis, while deletion of PAI-1 levels has been shown into promotes cardiac fibrosis, in vitro and vivo studies have suggested that the levels of PAI-1 may be have a pro-fibrotic and anti-fibrotic impacts. Studies in isolated and cultured astrocytes have indicate that anti-fibrotic impacts of the PAI-1 levels may be due to inhibition of interstitial collagenase during the onset of fibrosis [14].

Other study did agree with the result of current study, several years ago, the relationship between the levels of PAI-1 and (MS) with obesity was proven. It was found that patients with NAFLD have high levels of the PAI-1. This confirms the associated between thrombogenesis and MS [15].

The levels of TNF decreased significantly in the patients had disease less than one year than patients had disease duration more than one year at p-value < 0.05.

Also there is meta-analysis that does not agree with the result of our study, which included 27 studies including 698 healthy individuals and 1545 patients with NAFLD, circulating adiponectin levels were found to be decrease in patients with NAFLD compared to controls, they were also decreased more in patients with NASH than in patients with NAFLD, following the opposite direction to that of TNF-α. Interestingly, circulating adiponectin appears to be nonlinearly distributed in the NAFLD, being low in the NASH but increasing as the disease progresses from NASH to cirrhosis [16].

There is another study dis agreement with the result of current study, which some previous studies, it was noted that hypo-fibrinolysis seems to be associated with obesity, as the same changes were found in the obese control group. Some previous studies on the PAI-1 levels also indicated that altered fibrinolysis is more evident in the advance stages of disease [17].

In other study conducted, a possible relationship was discovered between levels of FABP4 and markers of liver injury in the serum, although there were no significant associations between them. Therefore, it is worth noting that these molecules are not reliable markers of disease, because about 75% of individuals with NAFLD don’t shows elevated levels of transaminase enzymes. However, the levels of FABP4 were positively correlated with GGT and CRP in the serum, indicating that FAPP in the serum is associated with liver injury and inflammation, which are features of advanced stages of the NAFLD [18].

Other studies that do not agree with the results obtained in current study, as it indicated that individuals with NAFLD have high levels of PAI-1, fibrinogen, von Willebrand factor, factor VII and C-reactive protein, which are known to be associated with an increased risk of strokes. This greatly supports in the vivo data on hypercoagulability in NAFLD. It also indicated a direct and highly significant relationship between PAI-1, fibrinogen, von Willebrand factor and VII factor with morphological criteria such as BMI and waist circumference (WC), but on the other hand, it also noted that there is a significant decrease in the levels of coagulating factors and a decrease in the levels of PAI-1 after losing weight induced by the exercise and dietary modification [15], [19].

5 CONCLUSIONS

This study provides compelling evidence that non-alcoholic fatty liver disease (NAFLD) is closely associated with changes in renal function and immunological biomarkers that may contribute to the early development of chronic kidney disease (CKD). Despite a non-significant elevation in serum creatinine levels among NAFLD patients, a statistically significant reduction in glomerular filtration rate (GFR) was observed, indicating early signs of renal impairment.

According to the ROC test, it was shown that all patients had negative results compared with the control group, where it was recorded that less than 1% of patients had their FABP-4 mean within the range of the control group. while API-1, the study noted 28% of patients had API-1 means more than or within the mean of the control group. Therefore, the FABP-4 and API-1 are considered excellent markers for predicting chronic kidney disease in the first place, followed by TNF.

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