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Evaluation of Plasma Urokinase-Type Plasminogen Activator Receptor and Interleukin 34 in Patients with Chronic Hepatitis C as Serological Fibrosis Markers

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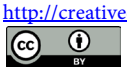

Abstract

Background: Hepatitis C Virus (HCV) infection is a progressive disease that may result in chronic hepatitis, fibrosis and cirrhosis. Assessment of liver fibrosis is an essential factor in the management of chronic HCV. **Objective:** To evaluate plasma soluble Urokinase Plasminogen Activator Receptor (sUPAR) and interleukin-34 (IL-34) as serological markers of liver fibrosis in patients with chronic HCV. **Methods:** This case-control study enrolled 60 chronic HCV patients who were subdivided into three groups of mild, moderate and severe hepatic fibrosis depending on Fibrosis-4 score (FIB-4). Patients were compared with 20 age and sex-matched controls. Plasma sUPAR and IL-34 levels were measured by Enzyme Linked Immunosorbent Assay (ELISA). **Results:** Plasma sUPAR and IL-34 were significantly increased in HCV patients when compared with controls, and their increase was positively correlated with the progression of hepatic fibrosis. Plasma sUPAR and IL-34 positively correlated with Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT), and negatively correlated with hemoglobin concentration and platelet count. The output data of Receiver Operating Characteristic (ROC) curve to differentiate patients from controls revealed that sUPAR at cut-off > 186.2 ng/L and Area Under Curve (AUC) of 0.944 had (85%) sensitivity and (100%) specificity, and IL-34 at cut off > 16.4 ng/L and AUC of 0.942 had (75%) sensitivity and (100%) specificity. The output data of ROC curve to differentiate severe from mild to moderate hepatic fibrosis patients revealed that sUPAR at cut-off > 510 ng/L and AUC of 0.837 had (80%)

sensitivity and (90%) specificity. While IL-34 at cut off > 55.3 ng/L and AUC of 0.844 had (85%) sensitivity and (80%) specificity. **Conclusions**: Increased plasma levels of sUPAR and IL-34 in chronic HCV patients with liver fibrosis and their increase was parallel to the degree of liver fibrosis. Plasma sUPAR and IL-34 can be used as serological markers of liver fibrosis in chronic HCV patients.

Keywords

HCV, Liver Fibrosis, Serological Markers, sUPAR, IL-34

1. Introduction

Hepatitis C Virus (HCV) infection represents a significant cause of chronic liver disease, with approximately 70 million chronically infected individuals worldwide. The persistent infection of HCV will lead to the development of hepatic fibrosis and cirrhosis due to the perpetual and impaired wound-healing process [1].

Assessment of liver disease progression and liver fibrosis staging is of particular importance, as the choice of the treatment regimen, post-treatment prognosis and follow-up depend on the stage of fibrosis [2].

Liver biopsy is considered the gold standard for diagnosing and staging liver fibrosis because it provides information on both the grade (degree of inflammation that reflects ongoing liver disease injury) and the stage (amount of currently established fibrosis). The procedure is particularly useful for diagnosing the early stages of fibrosis and identifying patients at high risk of progressing to fibrosis. However, it has some limitations [3]. Liver biopsy is invasive and occasionally causes severe complications. Pain appears in about one-quarter of patients, and other complications include bleeding, biliary peritonitis, pneumothorax and a mortality rate of about 0.01%, in addition to the need for frequent liver evaluation [4].

It is also prone to sampling error usually because of specimen fragmentation or inadequate length, and even in the ideal situation may incorrectly stage fibrosis in 20% of patients [5]. The interpretation might be unreliable, because the distribution of necro-inflammation and fibrosis is not homogeneous and a liver biopsy samples only 0.00002 of the mass of the liver, in addition to, intra or inter observer variability [6]. The rapid development of new medications for the treatment of chronic HCV, increases the requirement for more frequent evaluation of liver fibrosis to assess treatment response. Liver biopsy is not ideal for frequent evaluation. Consequently, there is a need for non-invasive reliable methods for diagnosing, grading hepatic fibrosis and monitoring the outcome of HCV infection treatment [7].

Various non-invasive tests for the evaluation of liver fibrosis have been developed over the past decade (such as aspartate aminotransferase platelet ratio

index (APRI) and Forns index). These tests are based on routine biochemical and clinical parameters. However, these tests reflect alterations in hepatic function rather than in Extracellular Matrix (ECM) metabolism [8].

Membrane-bound Urokinase Plasminogen Activator Receptor (UPAR) is expressed on various immunologically active cells such as macrophages, neutrophils and activated T lymphocytes, as well as on endothelial cells and podocytes. However, its expression on intrahepatic leucocytes is largely obscure. UPAR is cleaved from the cell surface by proteases upon inflammatory stimulation to the soluble form of the receptor, sUPAR [9].

It has been found that sUPAR has an important role in inflammation, proteolysis and tissue remodeling. Accordingly, sUPAR level is strongly elevated in acute liver failure and advanced chronic liver diseases, especially in decompensated cirrhosis, indicating that hepatic inflammation in principle is linked to hepatic UPAR expression and sUPAR release [10].

Interleukin-34 (IL-34) is a cytokine released in response to inflammatory stimuli and affects various immune cells, including monocytes, macrophages, and regulatory T cells that shape the immune microenvironment [11].

There is growing evidence that IL-34 contributes to the etiology of various diseases including autoimmune disorders, infections, inflammation and cancer [11]. IL-34 and its receptor are highly expressed in hepatocytes in patients with liver fibrosis induced by hepatitis B or C infection, mainly in hepatocytes located around fibrotic and inflammatory lesions. IL-34 regulates the profibrogenic functions of macrophages [12].

Our aim was to find out if levels of plasma sUPAR and IL-34 levels may serve as non-invasive serological biomarkers of liver fibrosis in chronic HCV infection.

2. Subjects and Methods

2.1. Subjects

This case-control study was conducted on 60 patients with chronic HCV infection who attended to Gastroenterology, Hepatology and Infectious Diseases, outpatient's clinic at Al-Zahraa University Hospital, Cairo, Egypt during the period from August 2019 to February 2020. The stage of liver fibrosis was assessed according to FIB-4 score and patients were subdivided into three groups of mild fibrosis (n = 20) (FIB-4 score < 1.45), moderate fibrosis (n = 20) (FIB-4 score: 1.45 - 3.25) and severe fibrosis (n = 20) (FIB-4 score > 3.25). 20 age and sex matched apparently healthy individuals were included as a control group.

Informed consent was taken from all participants before enrollment in the study. The study was done after approval from Research Ethics Committee of Faculty of Medicine for Girls Al-Azhar University (ethical approval number 20042019).

Inclusion criteria

Adult patients > 18 years suffered from chronic HCV infection and hepatic

fibrosis.

Exclusion criteria

Pregnancy, lactation, alcohol abusers, other types of viral hepatitis such as Hepatitis B Virus (HBV) and HAV, Human Immunodeficiency Virus (HIV), autoimmune hepatitis, malignancy, cancer liver, surgical operation for the liver, antiviral therapy and renal impairment.

Assessment of subjects

I: Full history taking and thorough clinical examination.

II: Abdominal and pelvic ultrasonography.

III: Laboratory investigations:

- Complete Blood Count (CBC).
- Liver enzymes: Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST).
- Hepatitis markers: (HCVAb, HBsAg) and HIVAb.
- Calculation of FIB-4 score:

$$\text{FIB-4 score} = \frac{\text{Age(years)} \times \text{AST(U/L)}}{\text{Platelet count(PLT)}\left(\times 10^{9}/\text{L}\right) \times \text{ALT(U/L)}}$$ [13].

2.2. Measurement of sUPAR and IL-34 Plasma Levels

Sample collection and preparation

About 6 ml of venous blood were drawn from each subject and divided into three aliquots: the first aliquot was 2 ml blood transferred to an EDTA tube for CBC using automated hematology cell counter (Cell dyne Ruby, Germany), the second aliquot was 2 ml blood transferred to plain tube for liver enzymes using fully automated chemistry analyzer (Cobas c 311, Roche Diagnostics kits, Germany), hepatitis markers and HIV using enzyme-linked immunosorbent assay (ELISA), the third aliquot was 2 ml blood transferred to an EDTA tube, centrifuged at 3000 rpm for 20 minutes, then plasma was separated and stored at −20˚C for measurement of sUPAR and IL-34 by ELISA in one assay to avoid repeated freeze-thaw cycles.

Measurement of sUPAR and IL-34

The concentration of sUPAR and IL-34 in plasma was analyzed according to the manufacturer's instructions by quantitative sandwich ELISA technique, with a complete set of ELISA reader (das 1851), using human soluble urokinase plasminogen activator receptor ELISA kit (Catalog number: E3759Hu) and human interleukin 34 ELISA kit (Catalog number: E0043Hu), supplied by Bioassay Technology Laboratory, China.

2.3. Statistical Analysis

Data were coded and entered to the Statistical Package for Social Science (SPSS) version 23. Data were summarized using numbers and percentages for qualitative data; mean ± Standard Deviations (SD) for quantitative parametric data and median with Inter-Quartile Range (IQR) for quantitative non parametric data. The comparison between qualitative data was done using Chi-square test, while

the comparison between quantitative data was done using independent t-test for parametric and Mann-Whitney test for non-parametric data. The comparison between more than two groups was done by using One Way ANOVA followed by post hoc analysis by Least Significant Difference (LSD) for quantitative parametric data. The correlations between quantitative data were done using Spearman correlation coefficients. Receiver Operating Characteristic (ROC) curve was performed with Area Under Curve (AUC) analysis to detect the best cut off value, sensitivity and specificity of sUPAR, IL-34 and FIB-4 score for differentiation between patients and controls, and also between patients with severe and mild to moderate hepatic fibrosis. P-values less than 0.05 were considered statistically significant.

3. Results

Our study included 60 patients with chronic HCV and 20 controls. The patients were divided into three groups according to stage of hepatic fibrosis: group 1 included mild fibrosis (n = 20), group 2 moderate fibrosis (n = 20) and group 3 severe fibrosis (n = 20). Comparison between patients and controls demonstrated a significant increase in sUPAR ($P < 0.001$), IL-34 ($P < 0.001$), FIB-4 score ($P < 0.001$), ALT ($P = 0.003$) and AST ($P < 0.001$) in patients than controls. There was a significant decrease in hemoglobin ($P = 0.002$) and platelets ($P < 0.001$) in patients than controls. While age ($P = 0.585$), sex ($P = 0.302$) and WBCs ($P = 0.231$) showed a non-significant difference between patients and controls (Table 1).

Comparison between patients groups with different stages of hepatic fibrosis reviled that plasma sUPAR and IL-34 levels and FIB-4 score were significantly different among these groups ($P < 0.001$). Post Hoc analysis showed that plasma sUPAR level was significantly increased in moderate ($P < 0.001$) and severe fibrosis ($P < 0.001$) in comparison to mild fibrosis, and in severe fibrosis in comparison to moderate fibrosis ($P = 0.004$). Plasma IL-34 level was significantly increased in moderate ($P < 0.001$) and severe fibrosis ($P < 0.001$) in comparison to mild fibrosis, and in severe fibrosis in comparison to moderate fibrosis ($P = 0.027$).Finally, FIB-4 score was significantly increased in moderate ($P < 0.001$) and severe fibrosis ($P < 0.001$) in comparison to mild fibrosis, and in severe fibrosis in comparison to moderate fibrosis ($P < 0.001$) (Table 2).

The correlation of sUPAR with other parameters revealed a significant positive correlations with IL-34 ($r = 0.722$, $P < 0.001$), FIB-4 score ($r = 0.799$, $P < 0.001$), ALT ($r = 0.452$, $P < 0.001$) and AST ($r = 0.496$, $P < 0.001$), and negative correlations with WBCs ($r = -0.359$, $P = 0.005$), hemoglobin ($r = -0.670$, $P < 0.001$) and platelets ($r = -0.647$, $P < 0.001$). While no correlation was found with age ($r = 0.202$, $P = 0.072$). In addition, IL-34 showed a significant positive correlations with FIB-4 score ($r = 0.806$, $P < 0.001$), ALT ($r = 0.571$, $P < 0.001$) and AST ($r = 0.605$, $P < 0.001$), negative correlations with hemoglobin ($r = -0.628$, $P < 0.001$) and platelets ($r = -0.617$, $P < 0.001$) and no correlation with age ($r = 0.202$, $P = 0.072$) and WBCs ($r = -0.359$, $P = 0.005$) (Table 3).

Table 1. Comparative data between patients and controls.

Variables		Patients	Controls	Test value	P-value
		n = 60	n = 20		
Age (years)	Mean ± SD	52.32 ± 7.23	51.25 ± 8.39	−0.549•	0.585
	Range	36 - 68	32 - 65		
Sex (n, %)	Female	28 (46.7%)	12 (60.0%)	1.067*	0.302
	Male	32 (53.3%)	8 (40.0%)		
sUPAR (ng/L)	Median IQR	178.35 152.45 - 240.7	132.70 110.3 - 160.3	−3.889¥	<0.001
IL-34 (ng/L)	Median IQR	22.80 16.35 - 112.8	14.15 12.65 - 16.35	−4.439¥	<0.001
WBCs ($\times 10^9$/L)	Mean ± SD	6.35 ± 2.20	6.99 ± 1.59	1.208•	0.231
	Range	2.3 - 10.9	4.9 - 10		
Hb (g/dL)	Mean ± SD	12.34 ± 1.81	13.83 ± 1.60	3.255•	0.002
	Range	8.2 - 17	10.3 - 15.9		
PLT ($\times 10^9$/L)	Mean ± SD	181.62 ± 86.61	272.10 ± 59.16	4.338•	<0.001
	Range	59 - 469	191 - 410		
ALT (U/L)	Median	43.00	31.00	−2.973¥	0.003
	IQR	28.5 - 69.5	26.5 - 34		
AST (U/L)	Median	47.25	19.00	−5.857¥	<0.001
	IQR	30 - 66	15.5 - 21.5		
FIB-4 score	Median	2.26	0.66		
	IQR	1.15 - 3.58	0.44 - 0.82	−5.822¥	<0.001

•Independent t-test; *Chi-square test; ¥Mann Whitney test. P-value < 0.05: Significant. IQR: inter-quartile range; sUPAR: soluble urokinase plasminogen activator receptor; IL-34, interleukin-34; WBCs: white blood cells; Hb: hemoglobin; PLT: platelate; ALT: alanine aminotransferase; AST: aspartate aminotransferase. FIB-4: fibrosis-4.

Table 2. Comparative data between mild, moderate and severe fibrosis groups.

Variables		Mild fibrosis	Moderate fibrosis	Severe fibrosis	Test value	P-value
		n = 20	n = 20	n = 20		
sUPAR (ng/L)	Median	199.45	399.75	860.8	17.585¥	<0.001
	IQR	150.8 - 250.8	256.5 - 501.05	694.7 - 981.5		
IL-34 (ng/L)	Median	15.35	30.55	114.35	51.462¥	<0.001
	IQR	13.8 - 18.3	19.45 - 132.95	111.05 - 166.85		
FIB-4 score	Median	1.06	2.26	4.29	52.463¥	<0.001
	IQR	0.77 - 1.15	1.87 - 2.86	3.58 - 6.30		

¥Kruskal Wallis test. P-value < 0.05: Significant. IQR: inter-quartile range; sUPAR: soluble urokinase plasminogen activator receptor; IL-34: interleukin-34; FIB-4: fibrosis-4.

Post Hoc analysis by LSD test

Variables	P1	P2	P3
sUPAR (ng/L)	<0.001	<0.001	0.004
IL-34 (ng/L)	<0.001	<0.001	0.027
FIB-4 score	<0.001	<0.001	<0.001

LSD: least significant difference. P-value < 0.05: Significant. P1: Moderate Vs Mild, P2: Severe Vs Mild, P3: Severe Vs Moderate.

The output data of ROC curve to differentiate between patients and controls revealed that sUPAR at a cut off > 186.2 ng/L and AUC of 0.944 yielded a sensitivity of 85% and a specificity of 100%. IL-34 at a cut off > 16.4 ng/L and AUC of 0.942 yielded a sensitivity of 75% and a specificity of 100%. FIB-4 score at a cutoff > 1.01 and AUC 0.937 yielded a sensitivity of 85% and a specificity of 90% (Table 4, Figure 1).

The output data of ROC curve to differentiate sever from mild to moderate liver fibrosis revealed that sUPAR at a cut off > 510 ng/L and AUC of 0.837 yielded a sensitivity of 80% and a specificity of 90%. IL-34 at a cut off > 55.3 ng/L and AUC of 0.844 yielded a sensitivity of 85% and a specificity of 80%. FIB-4 score at a cutoff > 3.25 and AUC 1.0 yielded a sensitivity of 100% and a specificity of 100% (Table 5, Figure 2).

Table 3. Correlation of sUPAR and IL-34 with other study parameters.

Parameters	sUPAR (ng/L)		IL-34 (ng/L)	
	r	P-value	r	P-value
Age (years)	0.202	0.072	0.200	0.075
IL-34 (ng/L)	0.722	<0.001	-	-
sUPAR (ng/L)	-	-	0.722	<0.001
FIB-4 score	0.799	<0.001	0.806	<0.001
WBCs ($\times 10^9$/L)	−0.359	0.005	−0.243	0.062
Hb (g/dL)	−0.670	<0.001	−0.628	<0.001
PLT ($\times 10^9$/L)	−0.647	<0.001	−0.617	<0.001
ALT (U/L)	0.452	<0.001	0.571	<0.001
AST (U/L)	0.496	<0.001	0.605	<0.001

P-value < 0.05: Significant. sUPAR: soluble urokinase plasminogen activator receptor; IL-34: interleukin-34; FIB-4: fibrosis-4; WBCs: white blood cells; Hb: hemoglobin; PLT: platelet; ALT: alanine aminotransferase; AST: aspartate aminotransferase.

Table 4. The output data of ROC curve for discriminative power of sUPAR, IL-34 and FIB-4 score to differentiate between HCV patients with liver fibrosis and controls.

Parameters	AUC	Cut-off	Sensitivity %	Specificity %
sUPAR (ng/L)	0.944	>186.2	85	100
IL-34 (ng/L)	0.942	>16.4	75	100
FIB-4 score	0.937	>1.01	85	90

AUC: area under curve; sUPAR: soluble urokinase plasminogen activator receptor; IL-34: interleukin-34; FIB-4: fibrosis-4.

Table 5. The output data of ROC curve for discriminative power of sUPAR, IL-34 and FIB-4 score to differentiate between sever and mild to moderate liver fibrosis.

Parameters	AUC	Cut-off	Sensitivity %	Specificity %
sUPAR (ng/L)	0.837	>510	80	90
IL-34 (ng/L)	0.844	>55.3	85	80
FIB-4 score	1.000	>3.25	100	100

AUC: area under curve; sUPAR: soluble urokinase plasminogen activator receptor; IL-34: interleukin-34; FIB-4: fibrosis-4.

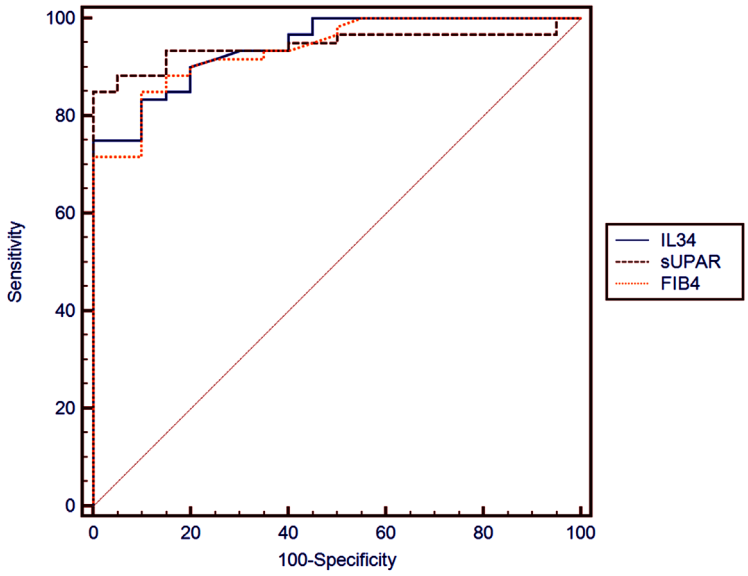

Figure 1. The output data of ROC curve for discriminative power of sUPAR, IL-34 and FIB-4 score to differentiate between HCV patients with liver fibrosis and controls.

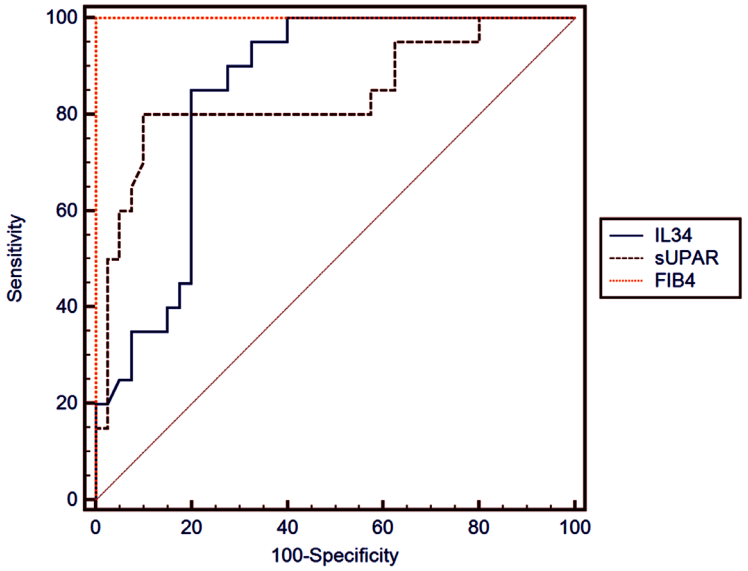

Figure 2. The output data of ROC curve for discriminative power of sUPAR, IL-34 and FIB-4 score to differentiate between sever and mild to moderate liver fibrosis.

4. Discussion

Egypt has managed to implement a successful nationwide HCV screening and treatment program. By late 2017, the number of persons with new cases who presented for treatment decreased to less than 5000 a month. Identifying and treating all infected patients is the major step toward disease elimination in the country that used to have the highest global prevalence [14].

Detection and quantification of hepatic fibrosis in chronic HCV infection have become increasingly important in order to make therapeutic decisions, determine prognosis and to follow up disease progression [14].

The current methods used to assess liver fibrosis in chronic HCV patients have some limitations that highlight the importance of different biomarkers in predicting liver fibrosis for early treatment and prevention of complications. This justifies our aim to investigate the utility of plasma sUPAR and IL-34 as non-invasive serological biomarkers of liver fibrosis in chronic HCV infection.

The current study showed a significant increase in sUPAR among HCV patients with hepatic fibrosis as compared with controls. This was in agreement with Akdogan *et al*., who reported that sUPAR was higher in chronic HCV patients with hepatic fibrosis as compared with controls [15].

This can be explained by sUPAR that is expressed on macrophages and is cleaved by proteases upon inflammation. The constitutive up regulation of liver macrophage as a result of the underlying liver disease leads to increased sUPAR level [16].

We also reported a significant increase in sUPAR in patients with moderate and severe hepatic fibrosis as compared with mild fibrosis, and in patients with severe fibrosis as compared with moderate fibrosis. This was in agreement with Saraiva *et al*., who found that sUPAR was significantly higher in patients with severe hepatic fibrosis than those with mild to moderate fibrosis [17].

This can be explained by the association of increased sUPAR level with hepatic inflammation and fibrosis in chronic HCV infection. Earlier studies have suggested that activated hepatic leucocytes are responsible for the sUPAR generation in HCV infection [18].

In addition, the current study showed a significant increase in IL-34 among HCV patients with hepatic fibrosis as compared with controls. This was in line with Wang *et al*., who reported that IL-34 was higher in chronic HCV patients with hepatic fibrosis as compared with controls [19].

This can be explained by HCV that induces the production of IL-34 by hepatocytes and this increases macrophage proliferation and differentiation with profibrogenic properties [20].

We also reported a significant increase in IL-34 in patients with moderate and severe hepatic fibrosis as compared with mild fibrosis, and in patients with severe fibrosis as compared with moderate fibrosis. This was in line with Kanto and Yoshio, who found that IL-34 was significantly higher in patients with advanced hepatic fibrosis than those with mild to moderate fibrosis [21].

This can be explained by IL-34 that has a central role in the pathogenesis of liver fibrosis and IL-34 differentiated monocytes up regulate profibrotic factors and induce collagen I production in hepatic stellate cells [22].

There was a significant increase in liver enzymes in patients when compared with controls which was in agreement with Khairy *et al*. [23].

Huynh *et al*., reported that liver enzymes have been considered as biochemical surrogates for liver injury, including HCV-mediated hepatocytic injury [24].

Hemoglobin concentration and platelet count were significantly decreased in patients compared with controls. This was in agreement with Abd Elbaser *et al*., who found that patients with HCV and liver fibrosis had a decreased hemoglo-

bin level and platelets count compared with controls [25].

This can be explained by hypoferremia and iron-restricted erythropoiesis that occurs in chronic inflammation despite normal iron stores (functional iron deficiency). Hypersplenism secondary to portal hypertension is another mechanism of anemia in patients with chronic liver disease. Hemolytic anemia occurs because of intrasplenic destruction of erythrocytes [26].

Shao *et al*., stated that the pathological mechanisms of thrombocytopenia in chronic HCV with hepatic fibrosis included hypersplenism, bone marrow suppression by HCV, immune dysfunction, decreased thrombopoietin levels and activity and direct infection of platelets and megakaryocytes by HCV [27].

A comparison of WBCs showed a non-significant difference between patients and controls. This was in line with Abd Elbaser *et al*. [25]. In addition; there was a non-significant difference between patients and controls as regards age and sex. This was in agreement with Zayed *et al*. [28].

The correlation studies revealed that sUPAR was positively correlated with FIB-4 score of the patients. This result was in agreement with Berres *et al*. and Saraiva *et al*., who stated that sUPAR was strongly associated with the degree of liver fibrosis in chronic HCV [17] [29].

sUPAR was positively correlated with liver enzymes. This was in agreement with Udomsinprasert *et al*., who found that sUPAR has positive correlation with aminotransferases in patients with HCV and hepatic fibrosis [30].

Our study revealed a negative correlation of sUPAR with WBCs, hemoglobin and platelets. This can be explained by chronic inflammation that has a central role in the change in hematological indices in liver disease and is also the trigger of cleavage of the cellular UPAR [18]. While, there was no correlation of sUPAR with age or sex of the patients. This was in line with Huang *et al*., who did not find any correlation of sUPAR with age or gender of patients with HCV with hepatic fibrosis [10].

Our study revealed that IL-34 was positively correlated with FIB-4 score. This was in agreement with Shoji *et al*., and Kanto and Yoshio who reported a positive correlation of IL-34 with FIB-4 score in patients with HCV with hepatic fibrosis [21] [31]. IL-34 was positively correlated with liver enzymes. This was in agreement with Wang *et al*., who stated that IL-34 had a positive correlation with aminotransferase in HCV patients with hepatic fibrosis [19].

There was a significant negative correlation of IL-34 with hemoglobin and platelets, while no correlation was seen with WBCs. In consistence with our results, Wang *et al*., stated that IL-34 was negatively correlated with platelet count in HCV patients with hepatic fibrosis.

This can be explained by proinflammatory cytokines released as a response to chronic inflammation in liver disease that trigger secretion of IL-34 and cause suppression of hematopoiesis [32]. IL-34 was positively correlated with sUPAR and not correlated with age or sex of the patients.

The output data of ROC curve showed that sUPAR and IL-34 have an accepted discriminative power to differentiate between HCV patients with liver fi-

brosis and controls with AUC values higher than FIB-4 score, proposing them as serological markers of hepatic fibrosis in chronic HCV. This was in agreement with Wang *et al*., who stated that IL-34 has higher AUC value than FIB-4 score in predicting hepatic fibrosis [19].

In addition, our study reviled that sUPAR and IL-34 have the ability to discriminate severity of hepatic fibrosis but with AUC values lower than FIB-4 score proposing them as monitoring markers.

The relatively limited number of cases in the study may not be enough to generalize our findings; thus further studies with more comprehensive investigations are needed to confirm our observations. Additionally, the possible role of sUPAR and IL-34 in the development of hepatic fibrosis in HCV increases the need for many studies to evaluate them as potential therapeutic targets.

5. Conclusion

The plasma level of sUPAR and IL-34 was increased in chronic HCV with hepatic fibrosis and their increase was parallel to the degree of fibrosis. These findings propose sUPAR and IL-34 as serological biomarkers for hepatic fibrosis in chronic HCV infection and monitoring biomarkers for fibrosis severity.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Giuffrè, M., Fouraki, S., Comar, M., Masutti, F. and Crocè, L.S. (2020) The Importance of Transaminases Flares in Liver Elastography. Characterization of the Probability of Liver Fibrosis Overestimation by Hepatitis C Virus-Induced Cytolysis. *Microorganisms*, **8**, 348. https://doi.org/10.3390/microorganisms8030348

[2] Redman, J. and Sterling, R. (2020) Non-Invasive Assessment of Liver Fibrosis. *Current Treatment Options in Gastroenterology*, **18**, 255-269. https://doi.org/10.1007/s11938-020-00285-z

[3] Catanzaro, R., Sciuto, M., Lanzafame, C., Balakrishnan, B. and Marotta, F. (2020) Platelet to Lymphocyte Ratio as a Predictive Biomarker of Liver Fibrosis (on Elastography) in Patients with Hepatitis C Virus (HCV)-Related Liver Disease. *Indian Journal of Gastroenterology*, **39**, 253-260. https://doi.org/10.1007/s12664-020-01038-7

[4] Fallatah, H. (2014) Noninvasive Biomarkers of Liver Fibrosis: An Overview. *Advances in Hepatology*, **2014**, Article ID: e357287. https://doi.org/10.1155/2014/357287

[5] Tsochatzis, E., Bruno, S., Isgro, G., Hall, A., Theocharidou, E., Manousou, P., Dhil-

lon, A.P., Burroughs, A.K. and Luong, T.V. (2014) Collagen Proportionate Area Is Superior to Other Histological Methods for Subclassifying Cirrhosis and Determining Prognosis. *Journal of Hepatology*, **2014**, 948-954. https://doi.org/10.1016/j.jhep.2013.12.023

[6] Zhou, K. and Lu, G. (2009) Assessment of Fibrosis in Chronic Liver Diseases. *Journal of Digestive Diseases*, **10**, 7-14. https://doi.org/10.1111/j.1751-2980.2008.00356.x

[7] Sadeghi, A., Amiri, R., Akbarpour, E., *et al*. (2021) Changes in Liver Fibrosis in Patients with Chronic Hepatitis C after Successful Direct-Acting Antiviral Therapy. *International Journal of Clinical Practice*, **75**, e14145. https://doi.org/10.22541/au.159986388.88453135

[8] Udompap, P., Sukonrut, K., Suvannarerg, V., Pongpaibul, A. and Charatcharoenwitthaya, P. (2020) Prospective Comparison of Transient Elastography, Point Shear Wave Elastography, APRI and FIB-4 for Staging Liver Fibrosis in Chronic Viral Hepatitis. *Journal of Viral Hepatitis*, **27**, 437-448. https://doi.org/10.1111/jvh.13246

[9] Koch, A., Zimmermann, W., Gassler, N., Jochum, C., Weiskirchen, R., Bruensing, J., Buendgens, L., Dückers, H., Bruns, T., Gerken, G., Neumann, U.P., Adams, D.H., Trautwein, C., Canbay, A. and Tacke, F. (2014) Clinical Relevance and Cellular Source of Elevated Soluble Urokinase Plasminogen Activator Receptor (sUPAR) in Acute Liver Failure. *Liver International*, **34**, 1330-1339. https://doi.org/10.1111/liv.12512

[10] Huang, F., Li, Y., Xu, R., Cheng, A., Lv, Y. and Liu, Q. (2020) The Plasma Soluble Urokinase Plasminogen Activator Receptor Is Related to Disease Activity of Patients with ANCA-Associated Vasculitis. *Mediators of Inflammation*, **2020**, Article ID: 7850179. https://doi.org/10.1155/2020/7850179

[11] Ge, Y., Huang, M. and Yao, Y. (2019) Immunomodulation of Interleukin-34 and Its Potential Significance as a Disease Biomarker and Therapeutic Target. *International Journal of Biological Sciences*, **15**, 1835-1845. https://doi.org/10.7150/ijbs.35070

[12] Kawagishi, N., Suda, G., Kimura, M., *et al*. (2020) High Serum Angiopoietin-2 Level Predicts Non-Regression of Liver Stiffness Measurement-Based Liver Fibrosis Stage after Direct-Acting Antiviral Therapy for Hepatitis C. *Hepatology Research*, **50**, 671-681. https://doi.org/10.1111/hepr.13490

[13] Taneja, S., Tohra, S., Duseja, A., *et al*. (2016) Noninvasive Assessment of Liver Fibrosis by Transient Elastography and FIB4/APRI for Prediction of Treatment Response in Chronic Hepatitis C—An Experience from a Tertiary Care Hospital. *Journal of Clinical and Experimental Hepatology*, **6**, 282-290. https://doi.org/10.1016/j.jceh.2016.08.001

[14] Waked, I., Esmat, G., Elsharkawy, A., *et al*. (2020) Screening and Treatment Program to Eliminate Hepatitis C in Egypt. *New England Journal of Medicine*, **382**, 1166-1174. https://doi.org/10.1056/NEJMsr1912628

[15] Akdoğan, Ö., Yücel, A., Sargin, Z., Sönmez, C., Yilmaz, G.E. and Özenirler, S. (2019) Evaluation of Plasma Urokinase-Type Plasminogen Activator Receptor (UPAR) in Patients with Chronic Hepatitis B, C and Non-Alcoholic Fatty Liver Disease (NAFLD) as Serological Fibrosis Marker. *Journal of Clinical and Experimental Hepatology*, **9**, 29-33. https://doi.org/10.1016/j.jceh.2018.02.001

[16] Grønbæk, H., Gantzel, R., Laursen, T., Kazankov, K. and Møller, H.J. (2020) Macrophage Markers and Innate Immunity in Cirrhosis. *Journal of Hepatology*, **73**, 1586-1588. https://doi.org/10.1016/j.jhep.2020.07.033

[17] Saraiva, G., do Rosário, N., Medeiros, T., Medeiros, T., Leite, P.E.C., de Souza La-

cerda, G., de Andrade, T.G., de Azeredo, E.L., Ancuta, P., Almeida, J.R., Xavier, A.R. and Silva, A.A. (2018) Restoring Inflammatory Mediator Balance after Sofosbuvir-Induced Viral Clearance in Patients with Chronic Hepatitis C. *Mediators of Inflammation*, **2018**, Article ID: 8578051. https://doi.org/10.1155/2018/8578051

[18] Garnæs, E., Mortensen, C., Hobolth, L., Andersen, O., Nehlin, J. and Møller, S. (2019) Kinetics of the Soluble Urokinase Plasminogen Activator Receptor (suPAR) in Cirrhosis. *PLoS ONE*, **14**, e0220697. https://doi.org/10.1371/journal.pone.0220697

[19] Wang, Y., Cao, W., Gao, Y., Ye, J. and Zou, G.-Z. (2018) Serum Interleukin-34 Level Can Be an Indicator of Liver Fibrosis in Patients with Chronic Hepatitis B Virus Infection. *World Journal of Gastroenterology*, **24**, 1312-1320. https://doi.org/10.3748/wjg.v24.i12.1312

[20] Muñoz-Garcia, J., Cochonneau, D., Télétchéa, S., *et al*. (2021) The Twin Cytokines Interleukin-34 and CSF-1: Masterful Conductors of Macrophage Homeostasis. *Theranostics*, **11**, 1568-1593. https://doi.org/10.7150/thno.50683

[21] Kanto, T. and Yoshio, S. (2017) Hepatitis Action Plan and Changing Trend of Liver Disease in Japan: Viral Hepatitis and Nonalcoholic Fatty Liver Disease. *European Journal of Hepato-Gastroenterology*, **7**, 60-64. https://doi.org/10.5005/jp-journals-10018-1213

[22] Preisser, L., Miot, C., Le Guillou-Guillemette, H., *et al*. (2014) IL-34 and Macrophage Colony-Stimulating Factor Are Overexpressed in Hepatitis C Virus Fibrosis and Induce Profibrotic Macrophages That Promote Collagen Synthesis by Hepatic Stellate Cells. *Hepatology*, **60**, 1879-1890. https://doi.org/10.1002/hep.27328

[23] Khairy, R., Hmmad, S., Sayed, M., *et al*. (2020) Serum MicroRNAs as Predictors for Fibrosis Progression and Response to Direct-Acting Antivirals Treatment in Hepatitis C Virus Genotype-4 Egyptian Patients. *International Journal of Clinical Practice*, **75**, e13954. https://doi.org/10.1111/ijcp.13954

[24] Huynh, T., Zhang, J. and Hu, Q. (2018) Hepatitis C Virus Clearance by Direct-Acting Antiviral Results in Rapid Resolution of Hepatocytic Injury as Indicated by Both Alanine Aminotransferase and Aspartate Aminotransferase Normalization. *Journal of Clinical and Translational Hepatology*, **6**, 258-263. https://doi.org/10.14218/JCTH.2018.00014

[25] Abd Elbaser, S., Abdelkader, A. and Afifi, S. (2020) IL-1α and TGF-β1 as Non-Invasive Liver Fibrosis Markers of Chronic Liver Injury among Chronic Liver Diseases Patients in Sharkia Governorate, Egypt. *Afro-Egyptian Journal of Infectious and Endemic Diseases*, **10**, 108-113. https://doi.org/10.21608/aeji.2020.23397.1050

[26] Zaki, N., El-Naggar, A., Ghallab, O., *et al*. (2017) Anemia in Egyptian Chronic Hepatitis C Patients: Study of the Causes Prior to and during Interferon/Ribavirin versus Sofosbuvir Therapy. *World Journal of Pharmaceutical and Medical Research*, **3**, 20-28.

[27] Shao, N., Zhang, T., Wang, N., *et al*. (2020) Platelet Indices Significantly Correlate with Liver Fibrosis in HCV-Infected Patients. *PLoS ONE*, **15**, e0227544. https://doi.org/10.1371/journal.pone.0227544

[28] Zayed, R., Omran, D., Mokhtar, D., *et al*. (2017) Association of Toll-Like Receptor 3 and Toll-Like Receptor 9 Single Nucleotide Polymorphisms with Hepatitis c Virus Infection and Hepatic Fibrosis in Egyptian Patients. *The American Journal of Tropical Medicine and Hygiene*, **96**, 720-726. https://doi.org/10.4269/ajtmh.16-0644

[29] Berres, M., Schlosser, B., Berg, T., *et al*. (2012) Soluble Urokinase Plasminogen Activator Receptor Is Associated with Progressive Liver Fibrosis in Hepatitis C Infec-

tion. *Journal of Clinical Gastroenterology*, **46**, 334-338. https://doi.org/10.1097/MCG.0b013e31822da19d

[30] Udomsinprasert, W., Honsawek, S., Jirathanathornnukul, N., *et al*. (2016) Elevation of Serum Urokinase Plasminogen Activator Receptor and Liver Stiffness in Post-operative Biliary Atresia. *World Journal of Hepatology*, **8**, 1471-1477. https://doi.org/10.4254/wjh.v8.i33.1471

[31] Shoji, H., Yoshio, S., Mano, Y., *et al*. (2016) Interleukin-34 as a Fibroblast-Derived Marker of Liver Fibrosis in Patients with Non-Alcoholic Fatty Liver Disease. *Scientific Reports*, **6**, Article No. 28814. https://doi.org/10.1038/srep28814

[32] Baghdadi, M., Ishikawa, K., Endo, H., *et al*. (2018) Enhanced Expression of IL-34 in an Inflammatory Cyst of the Submandibular Gland: A Case Report. *Inflammation and Regeneration*, **38**, Article No. 12. https://doi.org/10.1186/s41232-018-0069-6