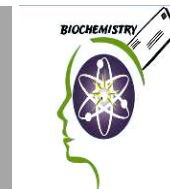




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Combination of Fibrotic Markers accurately predict liver fibrosis in Hepatitis C Patients

Faten Zahran Mohamed¹, Ibrahim El-Sayed M. El-Deen², Dina Mohamed Mohamed Ismail³

¹Chemistry Department, Faculty of Science, Zagazig University, Egypt.

²Chemistry Department, Faculty of Science, Port Said University, Egypt.

³Chemistry Department, Faculty of Science, Port Said University, Egypt.

ARTICLE INFO

ABSTRACT

Liver biopsy is invasive and prone to complications but it stills the gold standard for assessing hepatitis C virus (HCV)-related fibrosis. This study was designed to study non-invasive methods for the assessment of liver fibrosis. Our aim was to validate and compare the performance of MMP-8, upA in addition to GAL-3 as simple blood markers for different stages of liver fibrosis in HCV patients. The study was carried out on 90 individuals divided into the following groups: group 1: normal control, group 2: F1 = portal fibrosis without septa, group 3: F2 = few septa, group 4: F3 = numerous septa without cirrhosis and group 5: F4 = cirrhosis. Our results showed a positive correlation between MMP-8, upA and GAL-3 level and degree of liver fibrosis. We can conclude that, using of MMP-8, upA in addition to GAL-3 as simple biochemical markers for the assessment of hepatic fibrosis as alternative to liver biopsy.

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INTRODUCTION

Chronic hepatitis C virus infection is important globally as a cause of liver-related morbidity and mortality with hepatic fibrosis, cirrhosis and hepatocellular carcinoma as the major clinical sequelae [1]. Liver fibrosis is a significant health problem, with a worldwide mortality attributable to cirrhosis and primary liver cancer of around 1.5 million deaths per year [2]. In patients with chronic viral hepatitis, precise definition of the hepatic fibrosis stage is the most important parameter to assess the risk of disease progression and to decide for an

immediate and appropriate antiviral therapy. In these patients liver biopsy represents the gold standard for valuating the presence, type and stage of liver fibrosis [3]. This procedure, however, is invasive, stressful for patients, costly, and difficult to standardize [4].

Collagenase-2 (MMP-8), a member of the MMP family which has recently emerged as a candidate to play a protective role during tumor progression. MMP-8 is mainly produced by neutrophils, and it has been implicated in a variety of tissue remodeling processes associated with inflammatory conditions [5].

Corresponding author: **Faten Zahran Mohamed**, Chemistry Department-Faculty of Science, Zagazig university, Egypt

Urokinase-type plasminogen activator (uPA) is a serine protease, and together with its membrane associated receptor uPAR, is part of the uPA/uPAR system, which is an important component of the fibrinolytic system. In addition, it plays an important role in cancer progression and metastasis. In addition, expression pattern, secretion, and function of uPA members have been observed in a broad range of human malignancies [6,7].

Galectin-3 (Gal-3), a multifunctional protein of an expanding family of β -galactoside-binding animal lectins, is mainly produced by macrophages, and is implicated in a variety of biologic events, such as inflammation, apoptosis, angiogenesis, adhesion, migration and fibrosis [8,9].

Subjects and Patients:

The present study involved 75 patients (HCV) with different stages of liver fibrosis, have been selected from the Tropical Medicine Department, EL Ahrar Hospital, Zagazig, Egypt during the years 2016 and 2017, in addition to 15 negative control (Healthy volunteers).

Liver pathological examination:

Needle liver biopsy specimens (n = 75) were taken from the patients and examined by a pathologist unaware of the laboratory results. Biopsies were processed for diagnostic purposes, fixed in 10% neutral buffered formalin, embedded in paraffin, cut into 4 μ m thick and routinely stained with hematoxyline and eosin stain. Fibrosis was assessed according to the Metavir scoring system on a five-point scale (F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = few septa, F3 = numerous septa without cirrhosis and F4 = cirrhosis). Activity grading by the Metavir system (based on the intensity of periportal and lobular necro-inflammation) was scored as follows: A0 = no histological activity, A1 = mild activity, A2 = moderate activity and A3 = severe activity. The presence of stage F2, F3 or F4 was termed 'significant fibrosis', whereas the term 'advanced fibrosis' was reserved for stage F3 or F4. The presence of stage F4 was termed 'liver cirrhosis' [10].

Blood samples:

Blood samples were collected from all healthy and patients by vein-puncture within 2 weeks of liver biopsy. Sera were separated from the blood samples and tested fresh for the tested biochemical marker matrix metalloproteinase-8 according to **Monaghan et al.** [11], in addition to urokinase plasminogen activator according to the method of **Finckh et al.**[12] and Gal-3 according to the method of **Christenson et al.** [13] by using a sandwich Enzyme- Linked Immunosorbent Assay (ELISA) Kit method.

Statistical Analysis:

All statistical analyses were done by a statistical software package (Statistical Package for Social Sciences (SPSS 15.0) for Microsoft Windows, SPSS Inc.). Descriptive results were expressed as mean \pm SD and range or number (percentage) of patients with a condition. Differences in continuous variables were assessed using Student's t-test or analysis of variance (ANOVA) and χ^2 test for categorical variables [14].

RESULTS

Diagnosis of samples:

The present study involved 75 patients with clinically and laboratory confirmed chronic hepatitis C and liver fibrosis in addition to 15 negative controls (healthy volunteers). Positive patients with liver fibrosis were divided into different degrees according to METAVIR system as: 22 patients were categorized as F1 by (29%), 26 were F2 by (35%), 9 were F3 by (12%) and 18 patients were F4 by (24%).

MMP-8 activity was extremely significant elevated gradually with the degree of liver fibrosis, to be 279.38 ± 27.13 , 454.75 ± 28.58 , 544.55 ± 25.11 and 846.11 ± 130.3 in F1, F2, F3, and F4 by 226.44%, 431.31%, 536.23% and 885.56%; respectively; ($p < 0.001$), compared to healthy control individuals (F0), illustrated in (Fig.1). uPA levels were significantly increased to be 181.41 ± 15.27 , 321.34 ± 72.60 , 577.50 ± 37.21 and 690.04 ± 24.52 in F1, F2, F3, and F4 by 178.28%, 392.93%, 785.87% and 958.51%; respectively; ($p < 0.001$), compared to healthy control individuals (F0), as shown in (Fig. 2). Finally, Gal-3 concentrations was extremely

significant increased gradually with the degree of liver fibrosis, to be 12.46 ± 1.36 , 23.46 ± 3.9 , 46.15 ± 5.28 and 61.37 ± 2.6 in F1, F2, F3, and F4 by 219.49%, 501.54%, 1083.33%, and 1473.58%; respectively; ($p < 0.001$), compared to healthy control individuals (F0), illustrated in (Fig. 3).

Correlations between different studied parameters among studied groups:

There were significant positive correlations between MMP-8, uPA and Gal-3 to each other according to the degree of liver fibrosis.

Discussion:

Liver biopsy, the gold standard, is still recommended in the majority of patients [15]. However, liver biopsy is invasive, requires an experienced gastroenterologist, examination is required by a professional histopathologist, adds expense and is associated with complications and mortality patients with chronic hepatitis C [16, 17]. Biomarkers are being developed as alternatives to liver biopsy for predicting liver fibrosis in patients with chronic hepatitis C [18].

In the current study, Matrix metalloproteinase-8 (MMP-8) activity was extremely significant elevated gradually with the degree of liver fibrosis, to be 279.38 ± 27.13 , 454.75 ± 28.58 , 544.55 ± 25.11 and 846.11 ± 130.3 in F1, F2, F3, and F4 by 226.44%, 431.31%, 536.23% and 885.56%; respectively; ($p < 0.001$), compared to healthy control individuals (F0). MMP-8, a member of the MMP family which has recently emerged as a candidate to play a protective role during tumor progression. MMP-8 is mainly produced by neutrophils, and it has been implicated in a variety of tissue remodeling processes associated with inflammatory conditions [5]. The predominant role of matrix metalloproteinase 8 in extracellular matrix turnover, modulation of inflammatory responses and other physiological processes is well documented. Several recent studies highlight the involvement of MMP8 in a wide range of pathologies and as a disease marker in some inflammatory disorders and in cancer progression [19]. Under physiological condition, inactive MMP-8 is expressed at low levels in tissues and body fluids, but its level

and activation increase significantly under various pathological conditions, e.g., inflammatory, fibrosis diseases and cancers [20, 21].

Urokinase plasminogen activator (uPA) levels were significantly increased to be 181.41 ± 15.27 , 321.34 ± 72.60 , 577.50 ± 37.21 and 690.04 ± 24.52 in F1, F2, F3, and F4 by 178.28%, 392.93%, 785.87% and 958.51%; respectively; ($p < 0.001$), compared to healthy control individuals (F0). Urokinase plasminogen activator (uPA) is a serine protease, and together with its membrane associated receptor uPAR, is part of the uPA/uPAR system, which is an important component of the fibrinolytic system. In addition, it plays an important role in cancer progression and metastasis. In addition, expression pattern, secretion, and function of uPA members have been observed in a broad range of human malignancies [6, 7]. A number of investigations have indicated that uPA, uPAR, and PAI-1 are elevated in hepatocellular carcinoma (HCC) in comparison to normal liver tissues [22, 23]. Moreover, the uPA system has been suggested to be operative in HCC and cirrhotic liver, more than fibrolamellar HCC [24]. Further, uPAR- and PAI-1-elevated levels were correlated to poor survival in HCC patients [23]. In comparison to liver cirrhosis, higher levels of uPA and uPAR were found in malignancy and tuberculosis of the liver [25, 26]. **Berres et al.**, [27] reported that soluble urokinase plasminogen activator receptor (suPAR) is associated with progressive liver fibrosis in hepatitis C Infection, as they assessed suPAR serum levels in 146 chronically HCV infected patients by enzyme-linked immunosorbent assay and correlated them with biopsy-proven histologic stage of liver fibrosis and noninvasive liver fibrosis markers (aspartate transaminase to platelets ratio index score, transient elastography). Their study revealed that suPAR serum levels were strongly associated with the histologic stage of liver fibrosis.

Finally, Gal-3 concentrations was extremely significant increased gradually with the degree of liver fibrosis, to be $12.46 \pm$

1.36, 23.46 ± 3.9 , 46.15 ± 5.28 and 61.37 ± 2.6 in F1, F2, F3, and F4 by 219.49%, 501.54%, 1083.33%, and 1473.58%; respectively; ($p < 0.001$), compared to healthy control individuals (F0). Recently, mounting evidence has demonstrated that Gal-3 is activated in fibrotic models and is abnormally increased in fibrotic patients, and that Gal-3 inhibitors protect against fibrotic disorders [28, 29]. The role of Gal-3 in fibrotic diseases and the antifibrotic effect of Gal-3 inhibition in fibrogenesis raise the possibility that Gal-3 inhibition may be a novel potent therapeutic strategy for treating tissue fibrosis. Moreover, Gal-3 level may be a prominent and reliable biomarker in patients with fibrotic diseases [8, 9].

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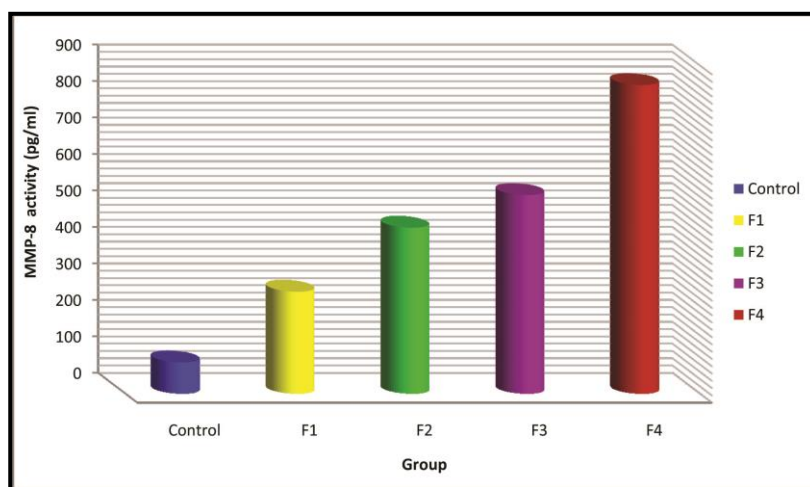


Fig. 1. MMP-8 activity in all studied groups.

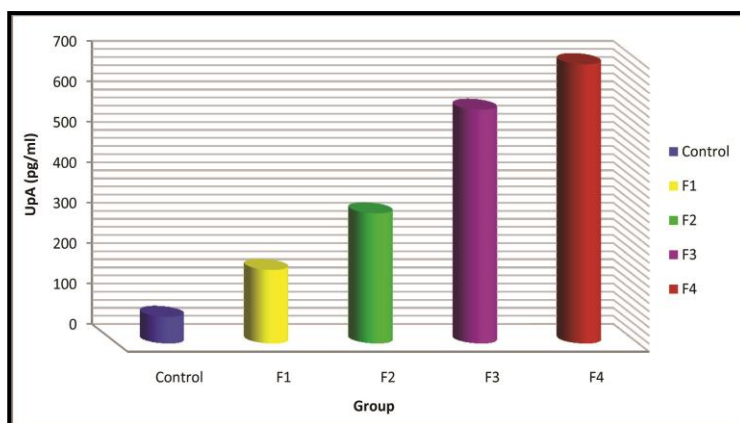


Fig. 2. uPA levels in all studied groups.

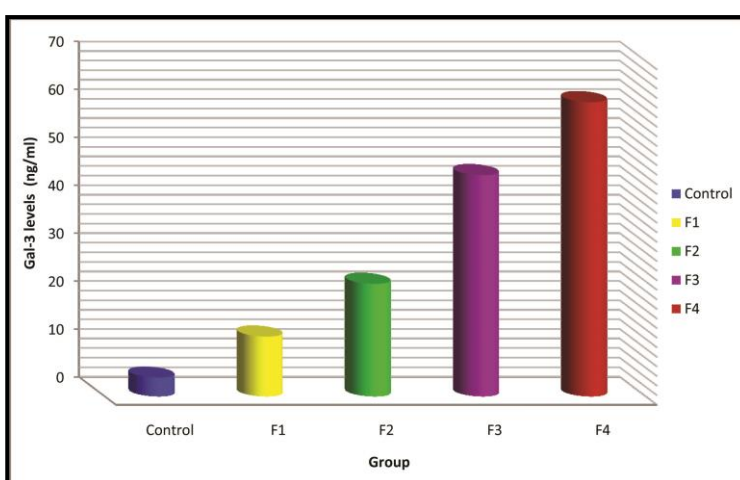


Fig. 3. Galectin-3 levels in all studied groups.

Table 1

Pearson's correlations analysis between different studied parameters in patients studied groups

**Correlation is significant at $p < 0.001$.

Parameter		uPA	MMP-8	Gal-3
uPA	r	-----	0.927**	0.978**
MMP-8	r	0.927**	-----	0.937**
Gal-3	r	0.978**	0.937**	-----

Table 2: The laboratory data of liver fibrosis and non liver fibrosis, advanced liver fibrosis and liver cirrhosis

Marker	Significant N=53	Non significant N=37	P value
MMP-8 (pg/ml)	529.3±287.8	85.1±9.3	< 0.0001
uPA(pg/ml)	458.4±228.4	63.9±7.8	< 0.0001
Galctin-3 (ng/ml)	36.7±22.2	3.6±0.11	< 0.0001

Levels of liver fibrosis markers in significant liver fibrosis (F2, F3, F4) and non significant liver fibrosis (F0, F1)

Table 3

Levels of liver fibrosis markers in advanced liver fibrosis (F3, F4) and non advanced liver fibrosis (F0, F1, F2)

Marker	Advanced N=27	Non advanced N=63	P value
MMP-8 (pg/ml)	745±179	175.8±162	< 0.0001
uPA(pg/ml)	652.5±61.1	143.6 ±134.1	< 0.0001
Galctin-3 (ng/ml)	56.3±8.1	9.2± 8.9	< 0.0001

Table 4

Levels of liver cirrhosis markers in liver cirrhosis (F4) and non liver cirrhosis (F0, F1, F2, F3).

Marker	cirrhosis N=18	Non cirrhosis N=72	P value
MMP-8 (pg/ml)	846.1±130	221.8± 195.4	< 0.0001
uPA(pg/ml)	690.0±24.5	197.8 ± 191	< 0.0001
Galctin-3 (ng/ml)	61.4±2.7	13.5± 9.2	< 0.0001