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BIOCHEMICAL STUDIES ON GALECTIN-3 AS FIBROTIC MARKER IN HEPATITIS C PATIENTS

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ABSTRACT

Although it is the gold standard for assessing hepatitis C virus (HCV)-related fibrosis, Liver biopsy is invasive and prone to complications. This study was designed to study non-invasive methods for the assessment of liver fibrosis where hepatitis C virus (HCV) is common in Egypt. Our aim was to validate and compare the performance of GAL-3 as simple blood marker for different stages of liver fibrosis in HCV patients in addition to GSH and NO. 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 GAL-3 level and degree of liver fibrosis. We can concluded that, using of GAL-3 a simple and non-invasive biochemical markers for the assessment of different stages of hepatic fibrosis as alternative to liver biopsy.

KEYWORD: Hepatitis C virus (HCV), GSH and NO.

INTRODUCTION

HCV and its long-term resultant consequences, is a major endemic medical health problem in Egypt.[1] In the Nile Delta and Upper Egypt, infection rates can be much higher at around 26% and 28%, respectively.[2] 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.[3] 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.[4] 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 fsor 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.[5] This procedure, however, is invasive, stressful for patients, costly, and difficult to standardize.[6] 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.[7,8]

Oxidative stress is a state of imbalance between the production and dismutation, or detoxification, of reactive oxygen species (ROS) by cellular mechanisms that can significantly affect signal transduction, gene expression, and functional responses of involved cells or cause cell damage. Evidence of oxidative stress has been detected in almost all the clinical and experimental conditions of chronic liver diseases with different etiology and fibrosis progression rate, and oxidative stress has been proposed as a major pro-fibrogenic mechanism.[9,10] Cellular glutathione (GSH) and related enzymes such as glutathione peroxidase (GSH-Px), glutathione S-transferase (GST) and glutathione reductase (GR) are among the principal protective mechanisms against endogenous and exogenous toxic substances and free radicals-mediated damage in liver tissue as well as in other tissues.[11,12] On the one hand, NO is important in the resolution of some viral infections; on the other hand, it could cause or potentiate deleterious effects on the host. Thus, advances in our knowledge of the role of NO in immunomodulation and in the pathogenesis of viral diseases could contribute to the development of vaccines and therapeutic strategies.[13,14]

SUBJECTS AND METHODS

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 µm 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'.[15]

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 Galectin-3 by using a sandwich Enzyme- Linked Immunosorbent Assay (ELISA) Kit method, according to the method of **Christenson et al.**[16], in addition to glutathione s-transferase activity by using Biodiagnostic kit method (Biodiagnostic company, Dokki, Giza, Egypt), according to the method of **Habig et al.**[17] and nitric oxide level by using Biodiagnostic kit method (Biodiagnostic company, Dokki, Giza, Egypt), according to the method of **Montgomery and Dymock.**[18]

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 ± 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 X^2 test for categorical variables.[19]

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%).

The mean values of NO, GST and Gal-3 levels in healthy control individuals (F0) were found

to be 17.91 ± 1.15 (µmol/l), 27.24 ± 3.2(U/L) and 3.90±0.11 (ng/ml) respectively. NO values were significantly increased gradually according to the progression of fibrosis degree to be 26.16 ± 2.8, 55.41 ± 7.8, 93.30 ± 4.6, 93.34 ± 10.23 in F1, F2, F3, and F4 by 46.06%, 209.55%, 420.94% and 421.16%; respectively; ($p<0.001$), compared to healthy control individuals (F0). While, there was no significant difference between F3 and F4 stages, (Fig. 1). Also, GST activity was extremely significant elevated gradually with the degree of liver fibrosis till F4, to be 169.43 ± 20.69, 394.43 ± 51.95, 565.44 ± 47.90, and 224.88 ± 22.76 in F1, F2, F3, and F4 by 521.98%, 1347.98%, 1975.77%, and 725.55; respectively; ($p<0.001$), compared to healthy control individuals (F0). Meanwhile, GST activity was reduced in F4 compared to other fibrotic degrees (F1, F2, and F3),(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).

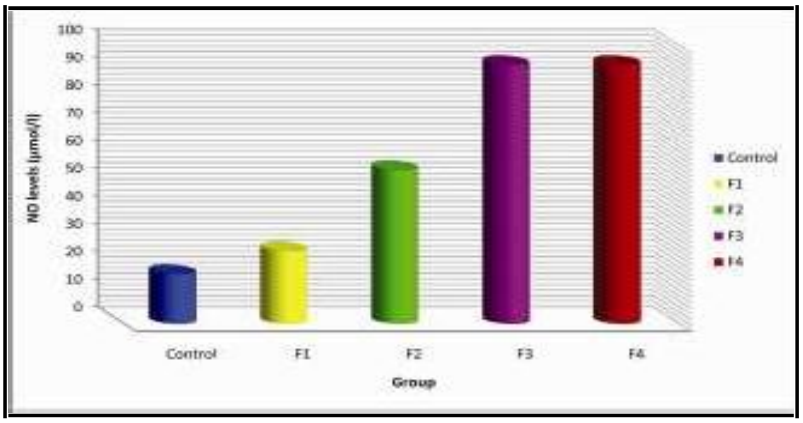


Fig. 1: NO levels in all studied groups.

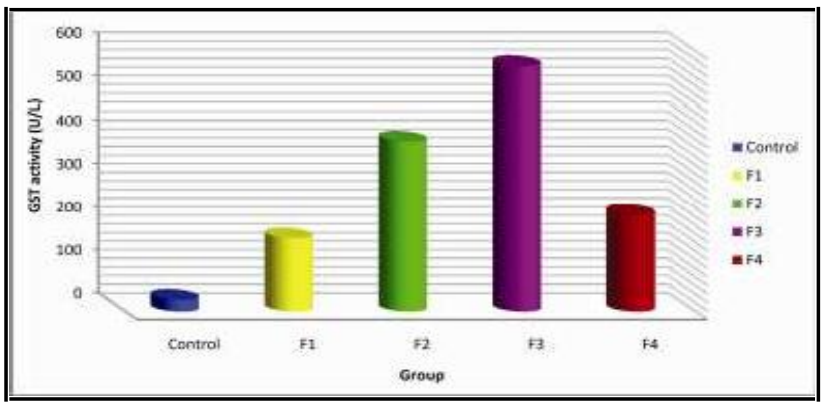


Fig. 2. GST activity in all studied groups.

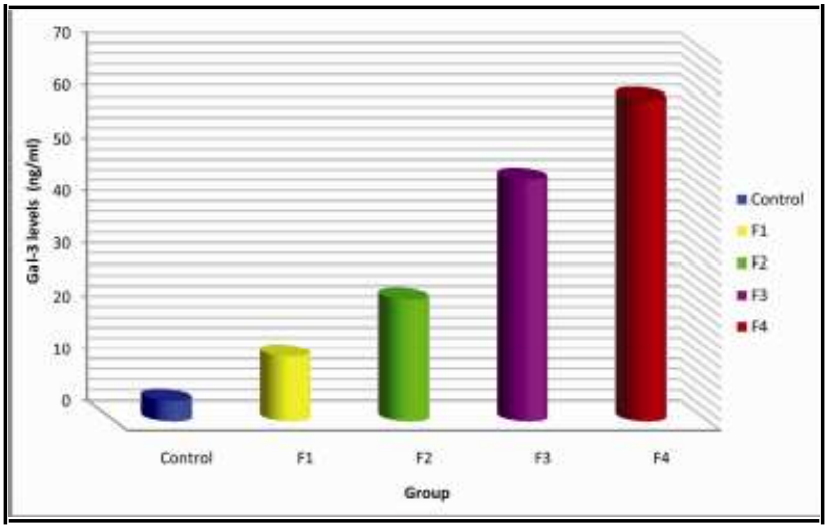


Fig. 3. Galectin-3 levels in all studied groups.

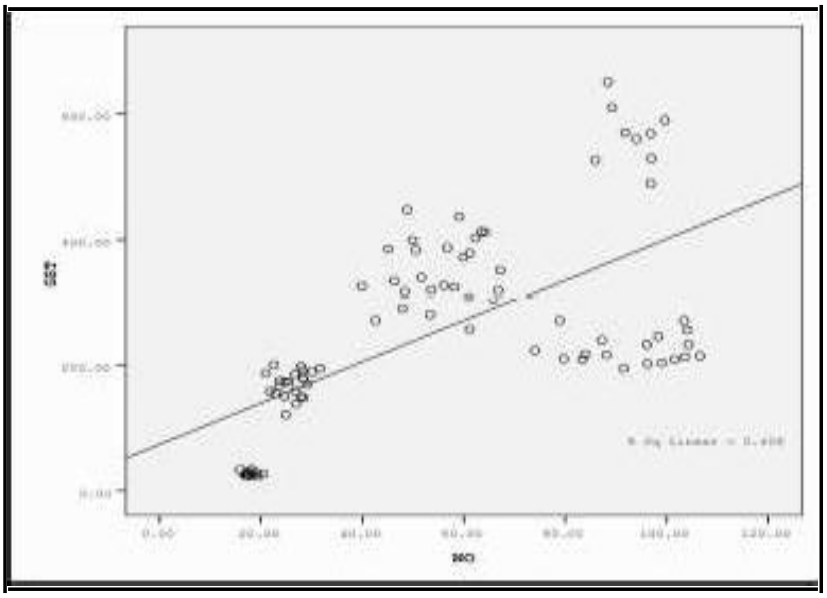
Correlations between different studied parameters among studied groups

There were signifaicant positive correlations between NO, GST and Gal-3 to each other according to the degree of liver fibrosis.

Table 1: Pearson's correlations analysis between different studied parameters in patients studied groups.

Parameter		NO	GST	Gal-3
NO	r	------	0.639**	0.940**
GST	r	0.639**	--------	0.462**
Gal-3	r	0.940**	0.462**	--------

**Correlation is significant at p<0.001



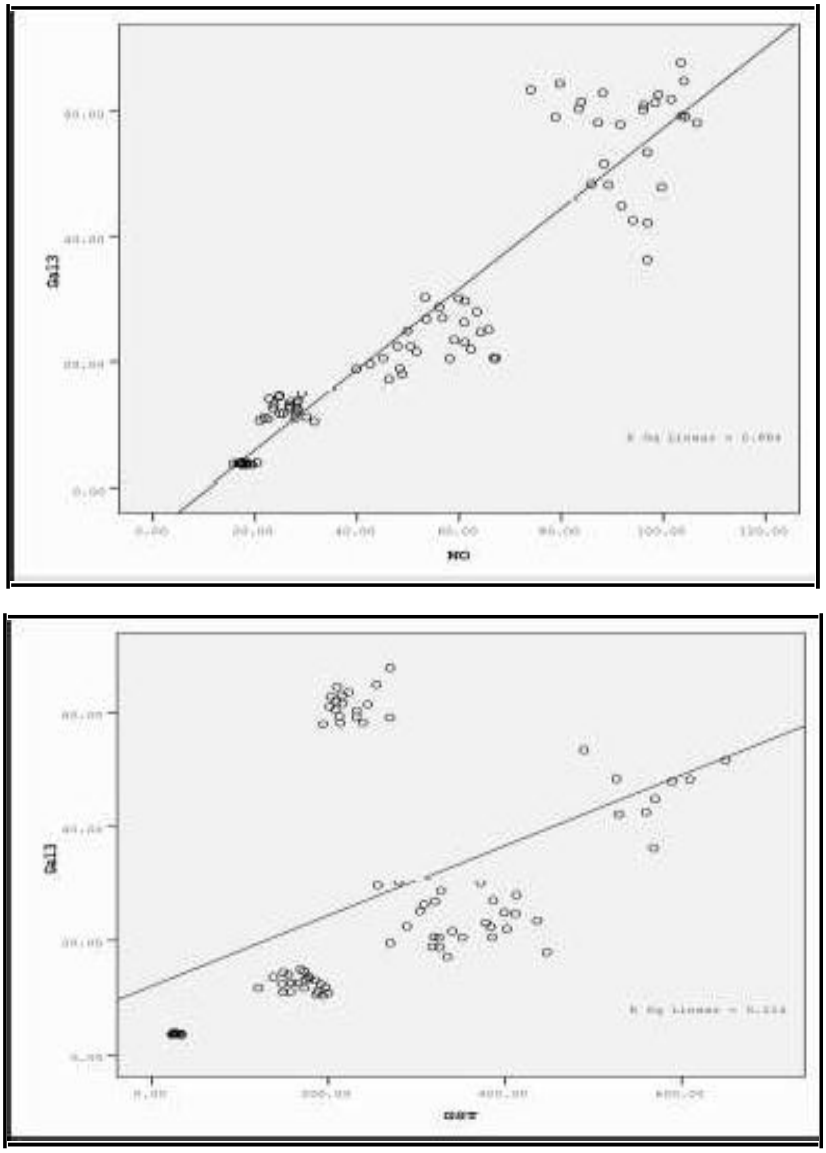


Fig. 4. Correlations between different parameters in all studied groups.

The laboratory data of liver fibrosis and non liver fibrosis, advanced liver fibrosis and liver cirrhosis

In the present study, patients with significant fibrosis were associated with higher mean NO, GST and Galectin-3 than those of non significant liver fibrosis with extremely significant difference ($p<0.0001$; table 2). Patients with advanced liver fibrosis were associated with higher mean NO, GST and Galectin-3 than those of non advanced liver fibrosis with extremely significant difference ($p<0.0001$; table 3). Also, patients with liver cirrhosis were associated with higher mean NO, GST and Galectin-3 than those of non liver cirrhosis with extremely significant difference ($p<0.0001$; table 4).

Table 2: Levels of liver fibrosis markers in significant liver fibrosis and non significant liver fibrosis.

Marker	Significant N=53	Non significant N=37	P value
NO(µmol/l)	56.9±35.5	17.9±1.2	< 0.0001
GST (U/l)	343.8±123.4	111.1±72.5	< 0.0001
Galctin-3 (ng/ml)	36.7±22.2	3.6±0.11	< 0.0001

Table 3: Levels of liver fibrosis markers in advanced liver fibrosis and non advanced liver fibrosis.

Marker	Advanced N=27	Non advanced N=63	P value
NO(µmol/l)	82.1 ±28.6	23.2± 13.9	< 0.0001
GST (U/l)	338.4±166.8	209.8 ±134.3	< 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 and non liver cirrhosis.

Marker	Cirrhosis N=18	Non cirrhosis N=72	P value
NO(µmol/l)	93.3± 10.2	27.2±22.1	< 0.0001
GST (U/l)	224.8± 22.7	254.3±173	< 0.0001
Galctin-3 (ng/ml)	61.4±2.7	13.5± 9.2	< 0.0001

DISCUSSION

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.[20,21] Moreover, liver fibrosis is evaluated by histological scores, which have inter-observer variability especially among non-expert pathologists.[22] Biomarkers are being developed as alternatives to liver biopsy for predicting liver fibrosis in patients with chronic hepatitis C.[23] A simple, reproducible, low-cost and non-invasive tool that can follow the evolution of the disease overtime would be beneficial for the testing physician and is desired by the patients.[24]

In the present study: nitric oxide (NO), glutathione s-transferase (GST) and Galectin-3 (Gal-3) were measured using standard methodologies in 75 patients with clinically and laboratory confirmed chronic hepatitis C and liver fibrosis in addition to 15 negative controls (healthy volunteers). Nitric oxide (NO) values were significantly increased gradually according to the progression of fibrosis degree to be 26.16 ± 2.8, 55.41 ± 7.8, 93.30 ± 4.6, 93.34 ± 10.23 in F1, F2, F3, and F4 by 46.06%, 209.55%, 420.94% and 421.16%; respectively; ($p<0.001$), compared to healthy control individuals (F0). While, there was no significant difference between F3 and F4 stages. Hepatic fibrosis in patients chronically infected with hepatitis B and C also appears to be correlated with increased expression of iNOS. Although the

molecular mechanisms have not been well elucidated, it was shown that fibrosis levels were correlated positively with iNOS expression, as well as that of TGF-b, which is an oxidative stress inducer and profibrogenic cytokine.[25] There is a controversy regarding the production of NO in chronic HCV patients with studies reporting an increase.[26,27], a decrease[28], or no change[29,30] in its level. During this study, Glutathione s- transferase (GST) activity was extremely significant elevated gradually with the degree of liver fibrosis till F4, to be 169.43 ± 20.69, 394.43 ± 51.95, 565.44 ± 47.90, and 224.88 ± 22.76 in F1, F2, F3, and F4 by 521.98%, 1347.98%, 1975.77%, and 725.55; respectively; ($p<0.001$), compared to healthy control individuals (F0). Meanwhile, GST activity was reduced in F4 compared to other fibrotic degrees (F1, F2, and F3). An increased GSH level in chronic liver diseases has been reported in many reports.[31,32] GST is a sensitive marker in the diagnosis of alcoholic liver disease[33] as well a reliable marker in monitoring the response to chronic liver disease treatment.[34] In both clinical and experimental HCC, reduced global activity of GST has been observed within tumors[11], and the specific isoforms GSTπ1 and GSTα1[35,36] have been shown to be over expressed and have been used as biomarkers in experimental models of HCC.

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). 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.[37,38] 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.[7,8]

From this study we conclude the following: It was shown that fibrosis levels were correlated positively with Nitric oxide (NO) concentration. Glutathione s-transferase (GST) activity was a reliable monitoring the response to chronic liver disease treatment. Addition of (NO) and (GST) to (Gal-3) gives a significant improvement in detection of different stages of fibrosis in patients with HCV. Therefore, combination of multiple markers may be more valuable in the diagnosis of liver fibrosis. **Finally,** There were significant positive correlations between NO levels, activity of GST with Gal-3 to each other according to the

degree of liver fibrosis.

We can recommended that, using of Galectin-3 a simple and non-invasive biochemical marker for the assessment of different stages of hepatic fibrosis as alternatives to liver biopsy which is invasive, expensive, painful and in some settings impossible to do in patients with chronic HCV infection in addition of glutathione s-transferase activity and level of nitric oxide.

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