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COULD SERUM LAMININ REPLACE LIVER BIOPSY AS GOLD STANDARD FOR PREDICTING SIGNIFICANT FIBROSIS IN PATIENTS WITH CHRONIC HEPATITIS B? CLINICAL AND HISTOPATHOLOGICAL STUDY

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ABSTRACT

Background: *The prognosis and clinical treatment of chronic liver disease depends greatly on the progression of liver fibrosis, which has resulted from the loss of normal liver cell function due to disorganized over-accumulation of extra-cellular matrix (ECM) components in the liver. Liver biopsy has been considered the gold standard for staging and grading hepatic fibrosis and inflammation. However, the procedure is associated with complications such as bleeding, infection, damage to liver tissue, and it is difficult to put into practice.* ***Aim of the work:*** *To evaluate the diagnostic performance of serum laminin (LN) as serum markers for predicting significant fibrosis in chronic hepatitis B (CHB) patients.* ***Subjects and methods:*** *The study included 50 subjects, selected to represent 2 groups: group (I) included 30 patients with chronic hepatitis B who were diagnosed by positive serologic tests for serum hepatitis B surface antigen for at least 6 months and group (II) included 20 blood donors subjects who had no symptoms suggestive of liver diseases and negative serologic tests for serum hepatitis B surface antigen served as control. All participants were subjected to thorough history taking, physical examination and routine laboratory investigations include liver function tests. Serum laminin levels were assayed by radio immune assay (RIA). Liver biopsies were done to all chronic hepatitis B patients. Liver fibrosis stages were determined according to the Metavir scoring-system* ***.Results:*** *Serum LN concentrations increased significantly with the stage of hepatic fibrosis, which showed positive correlation with the stages of liver fibrosis ($r = 0.591$, $p < 0.001$). There were significant differences of serum LN levels between F2-4 groups (patients with significant fibrosis) in comparison with those in F0-F1 groups (mean values ± SD were 156.4 ± 53.8 vs 90.9 ± 20.1 ,p <*

0.001). Serum LN at value 107.5ng/ml was the optimal cut-off value for diagnosis of significant fibrosis (sensitivity, specificity, positive predictive and negative predictive values were 84.2%, 63.6%, 80%, and 70.0%, respectively). ***Conclusion:*** *Liver biopsy will remain gold standard for staging and grading hepatic fibrosis and inflammation. But LN could be clinically useful serum markers for predicting significant fibrosis in patients with chronic hepatitis B, especially when liver biopsy is contraindicated. Further studies are needed for determining its value in other chronic liver diseases such as chronic hepatitis C (CHC) and non alcoholic fatty liver disease (NAFLD), its use as marker of success of management and regression of fibrosis and clinical validity in comparison to other non invasive serum markers.*

Keywords: Serum laminin, Chronic hepatitis B, Radio immune assay.

INTRODUCTION

Significant progress has been made in the last two decades in our understanding of the pathogenesis of the wound healing response of the liver to chronic injury. The advent of chronic hepatitis B and C as major causes of end-stage liver disease has played a significant role in the drive to uncover pathogenic mechanisms involved in fibrogenesis. In hepatitis B disease, inflammation appears to be a key driving factor for the fibrogenic response, and the process in turn is likely to be influenced by an array of metabolic and genetic factors. We now appreciate that fibrogenesis is a dynamic process, reflecting a balance between matrix synthesis, deposition, and degradation. Extensive investigation currently indicates that the hepatic stellate cell is a key effector in the fibrogenic response (Feng *et al.*, 2012). Fibrosis is thought to lead to impaired synthetic function, portal hypertension, and, ultimately, reduced survival. Data are now available that indicate that the fibrogenic response is reversible; for example, antiviral therapy in chronic hepatitis B and C leads to histological improvement of fibrosis (Poynard *et al.*, 2002). Given the apparent importance of fibrosis in predicting prognosis and, moreover, data indicating that it is important to stage fibrosis prior to therapy (and to judge the effect of therapy); histological assessment of the liver has taken on a major role in the management of patients with liver disease (Keyur *et al.*, 2006). Liver biopsy provides useful information to the clinician for determining prognosis and the urgency of therapy, predicting response to treatment, and investigating the etiology of liver injury, as well as for providing a baseline to allow comparisons of future histological outcomes (Bravo *et al.*, 2001). However, percutaneous liver biopsy is an invasive procedure and may be associated with significant complications in 3% of recipients such as bleeding, infection, damage to liver tissue, with a mortality rate of 0.03% (The role of liver biopsy in hepatitis, 1997). Risk factors such as age and cirrhosis increase the likelihood of adverse events from liver biopsy (Poynard *et al.*, 2000). Currently, with the improvements in treatment modalities for chronic liver disease, there is an increasing need for rapid, safe and reliable noninvasive diagnostic methods to stage liver fibrosis, and some of which have been widely validated in patients with chronic hepatitis (Carey and Carey, 2010). Laminin is one of the main glycoproteins of the basement membrane and participates in a series of such biological phenomena as adhesion,

migration, cellular differentiation and growth, inflammatory response and the maintenance of the cytoskeleton upon its binding to several components of the matrix, such as collagen type Ⅳ, heparan-sulphate and entacin (Kershenobich and Weissbrod, 2003). In the liver, Laminin is thought to be synthesized by hepatocytes and sinusoidal cells. Among all cellular types in the sinusoids, special attention should be given to stellate cells or lipocytes, which produce the largest amount of serum laminin. (Timpl *et al.*, 1979). Laminin has an important role in the mechanism of fibrogenesis and is, thus, related to hepatic fibrosis (Leroy, 2008). Reports showed that serum fibrosis indices, including HA and LN, could reflect the activity of liver fibrosis to some extent (Friedrich-Rust *et al.*, 2010). In this study, we aimed to evaluate serum LN levels as potential indicators of significant fibrosis in patients with chronic hepatitis B according to the Metavir scoring-system**.**

Subjects and Methods

This study was carried out in the gastroenterology unit of Internal Medicine and histopathology departments, Faculty of Medicine, Zagazig and Ain Shams University in Egypt. Thirty patients with chronic hepatitis B, who were diagnosed by positive serologic tests for serum hepatitis B surface antigen for at least 6 months, including 19 men and 11 women, mean age ± standard deviation (SD) was (42.3 ± 11.2 in men, 39.8 ± 9.8 in women).These patients were included in this study with an indication for percutaneous liver biopsy, which was performed for assessment of the severity of liver fibrosis. Liver transplantation, gastrointestinal bleeding and other chronic liver diseases were excluded. Sera of 20 blood donors was used as a control group, including 12 men and 8 women, mean age ± (SD) was (33.9 ± 7.2 in men, 29.6 ± 5.4 in women). Healthy subjects had no symptoms suggestive of liver diseases, negative serologic tests for serum hepatitis B surface antigen and had normal serum levels of regular biochemical parameters such as alanine aminotransferase (ALT), aspartate aminotransferases (AST) and alkaline phosphatase (ALP), etc. All patients provided written informed consent to participate in the study and Ethical Committee in both institutes approved the study.

Histopathology

Liver biopsies were done to all chronic hepatitis patients and performed with a suction needle (18G, Angiomed Corporation – German). Ultrasound was routinely used to determine the percutaneous biopsy site. The size of liver biopsy specimen exceeded 1 cm. The liver tissue sections were fixed in 10% neutralized formadehyde, embedded in paraffin and stained with hematoxylin-eosin and Masson trichrome stain. All biopsy specimens were analyzed by an experienced pathologist blinded to the clinical results of the patients. Liver fibrosis stages were evaluated semi-quantitatively according to the Metavir scoring-system (Bedossa and Poynard, 1996). Fibrosis was staged on a scale of 0 to 4: F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = portal fibrosis and few septa, F3 = numerous septa without cirrhosis, F4 = cirrhosis.

Determination of Serum Specimens

All serum specimens from the participants were stored at -20oC. Levels of serum LN were analyzed by RIA and determined from a standard curve. Kits of LN were provided all by the Shanghai Navy Medical Institute. The procedures were performed according to the user's manual.

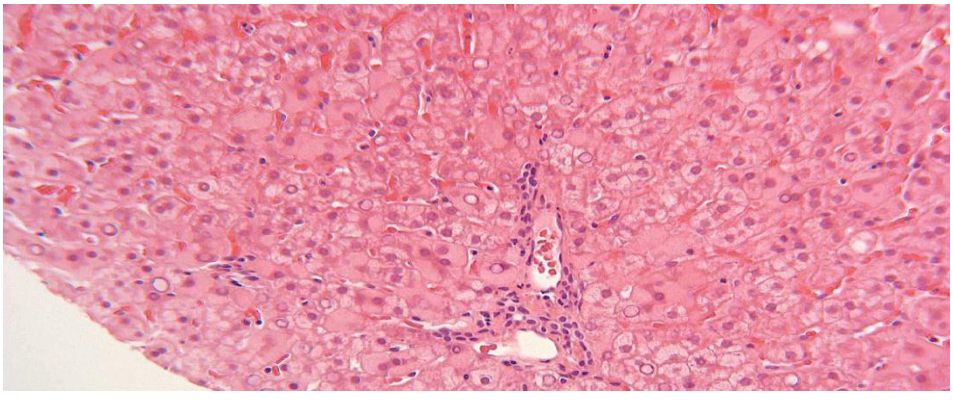
Statistical Analysis

The results were presented as mean ± standard deviation (S.D.). Statistical comparisons of individual groups were based on unpaired Student's t-test. The gender ratio was compared with $\chi 2$ test. Correlation between variables was done using correlation coefficient "r". P value is considered significant at ≤ 0.05 level, highly significant at ≤ 0.01 and non significant at > 0.05.

Results

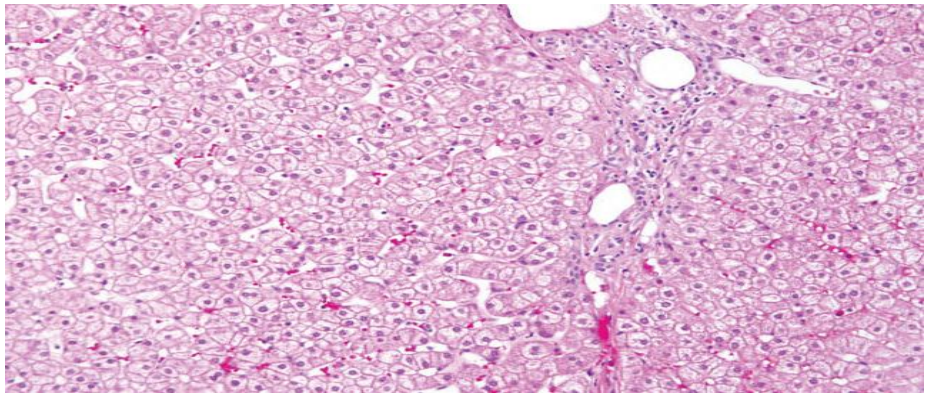
According to the Metavir scoring-system, the fibrosis stages on liver biopsy was F0 in 4 patients (13.3%) figure 1, F1 in 7(23.3%) figure 2-3, F2 in 6 (20%) figure 4-5, F3 in 8 (26.7%) figure 6-7, and F4 in 5(16.7%) figure 8-9. So in this study; a total of 19 patients (63.3%) had significant fibrosis ($\geq$ F2) figure 10.

Figure-1. (F0)



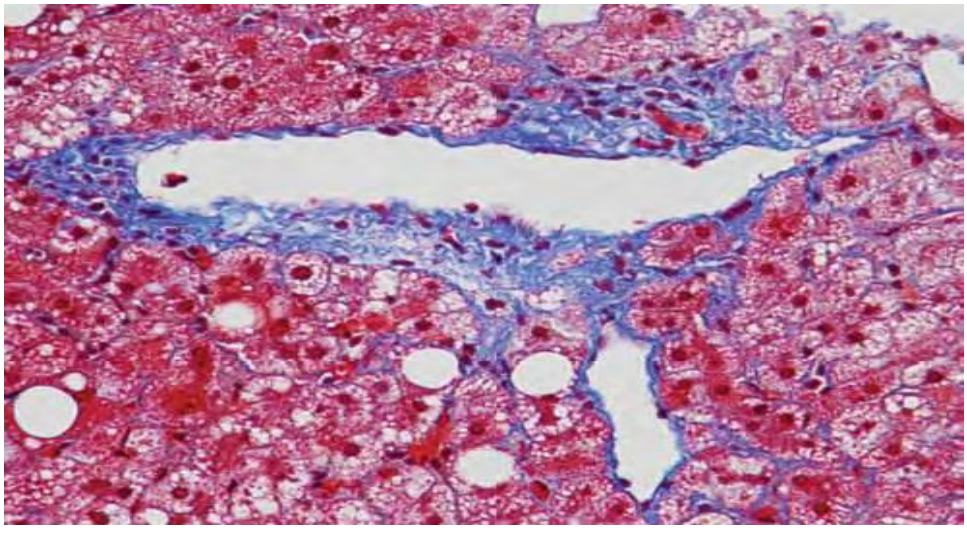
Mild portal inflammation showed infiltration of portal tract with few lymphocytes without fibrosis (x 10 H & E)

Figure-2. (F1)



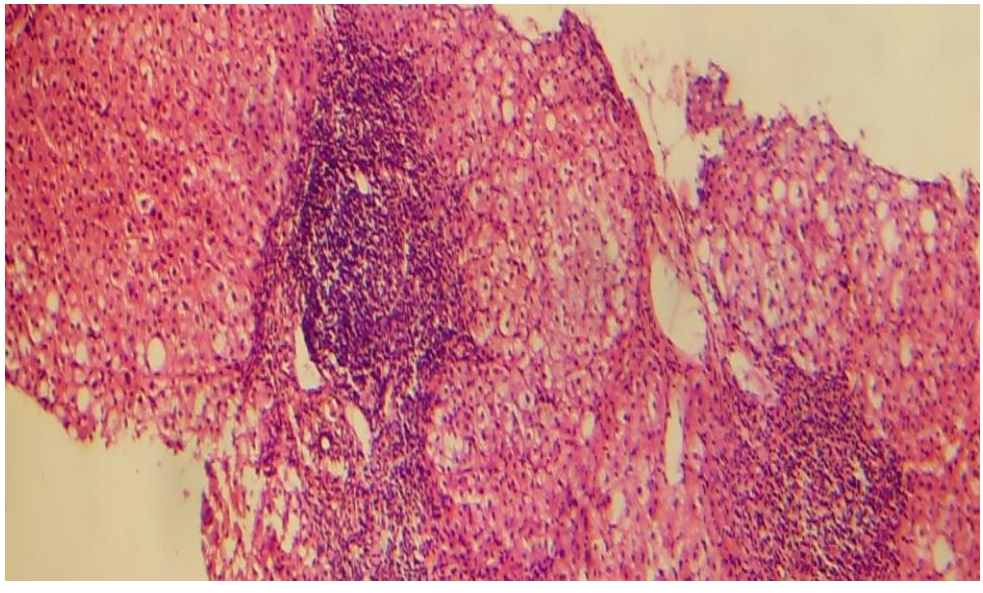
Chronic hepatitis of moderate activity showed expansion of portal tracts with some chronic inflammatory cells mainly lymphocytes, histocytes and fibrous tissue without septa (x 10 H & E).

Figure-3. (F1)



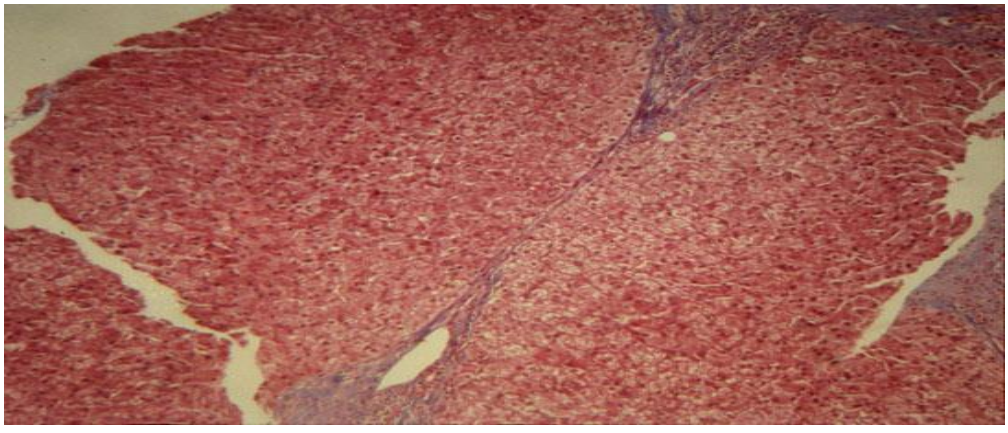
Trichrome stained liver showing fibrous tissue. The fibrous tissue is stained blue while the cytoplasm of hepatocytes is stained red. The nuclei can be seen as dark red to black structures within cells (x 20).

Figure-4. (F2)



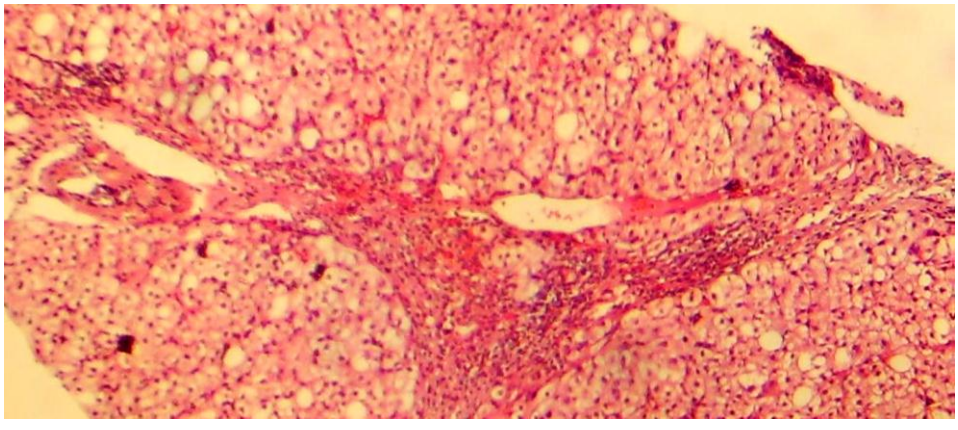
Chronic hepatitis of moderate activity showed expansion of portal tracts by lymphoid aggregates, histocytes and fibrous tissue with few septa (x 10 H & E).

Figure-5. (F2)



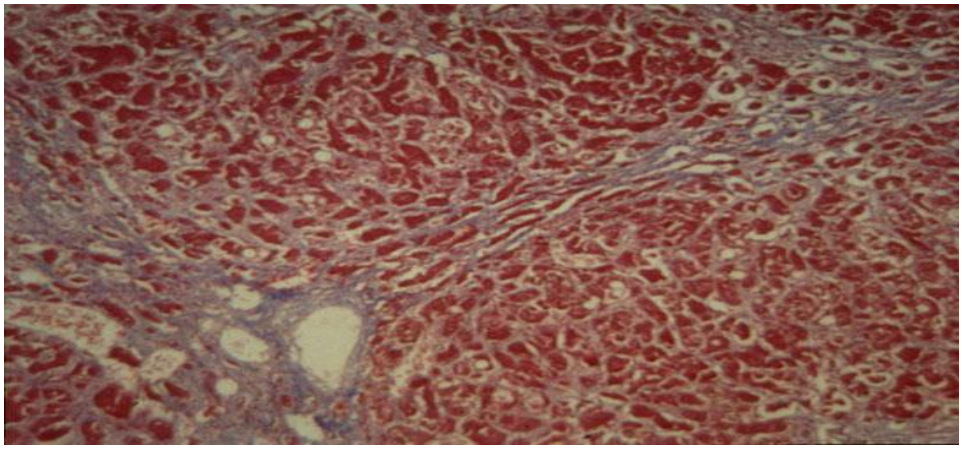
Trichrome stained liver showed chronic hepatitis of moderate activity with few fibrous tissue septa (stained blue) (x10).

Figure-6. (F3)



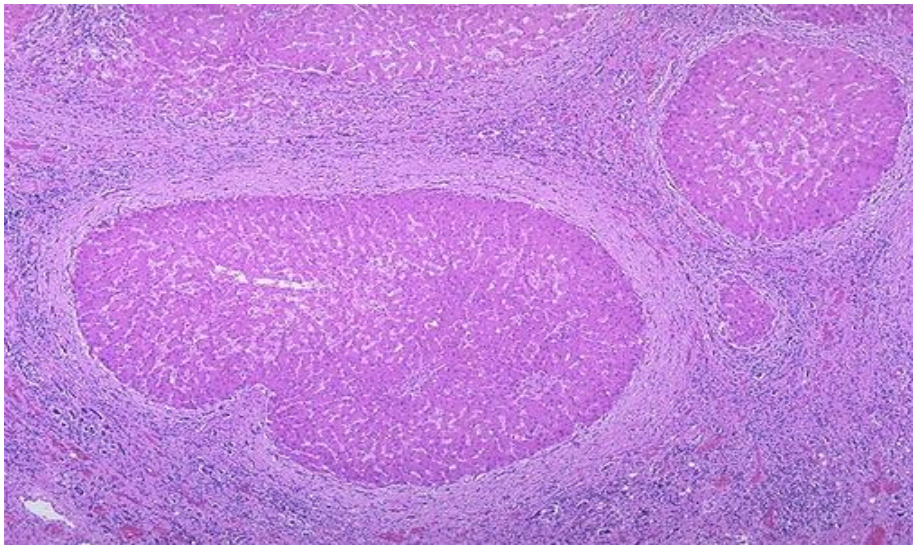
Chronic hepatitis of moderate activity showed expansion of portal tracts by chronic inflammatory cells mainly lymphocytes, histocytes and extensive fibrosis (x 10 H & E)

Figure-7. (F3)



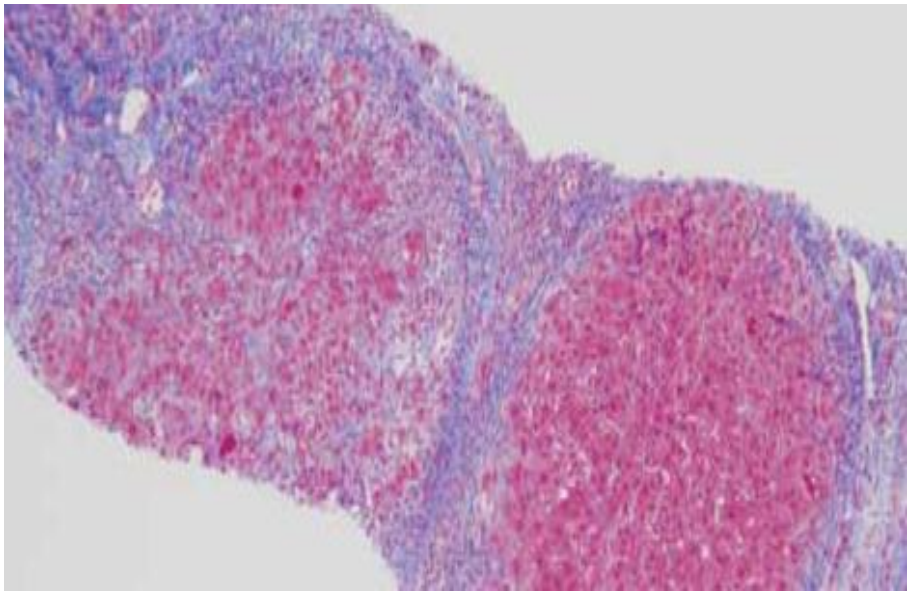
Trichrome stained liver showed chronic hepatitis of moderate activity with marked deposition of fibrous tissue (stained blue) with numerous fibrous tissue septa (x10)

Figure-8. (F4)



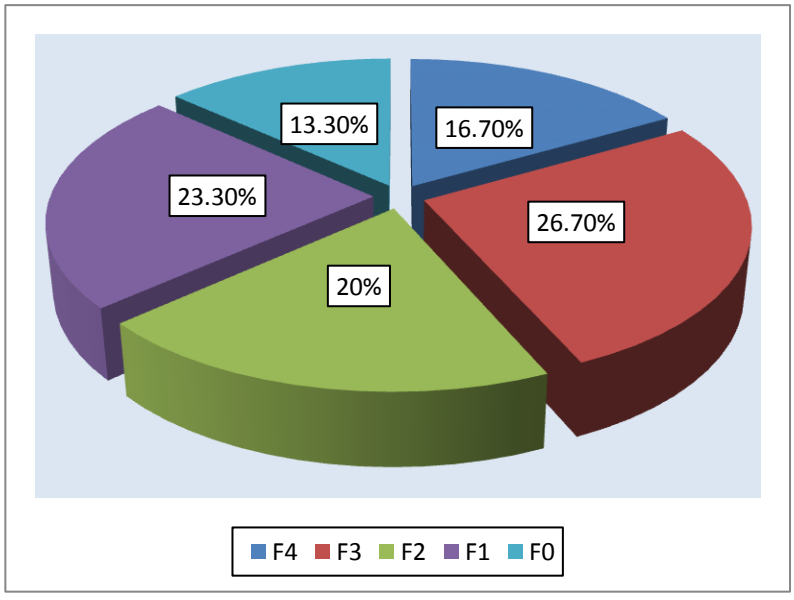
Advanced cirrhosis showed regenerative nodules separated by fibrous tissue bands containing inflammatory cells mainly lymphocytes with loss of normal liver architecture(x 10 H & E).

Figure-9. (F4)



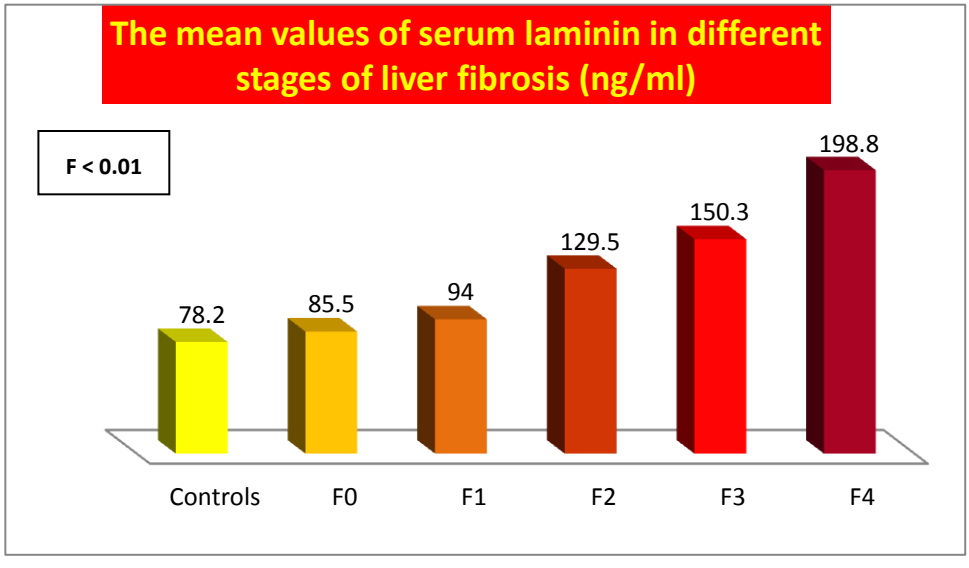
Trichrome stain showed cirrhotic liver with loss of normal liver architecture replaced by regenerative nodules surrounded by blue fibrous bands (x 10)

Figure-10. Distribution of chronic hepatitis patients according to Metavir scoring-system (grades of liver fibrosis)



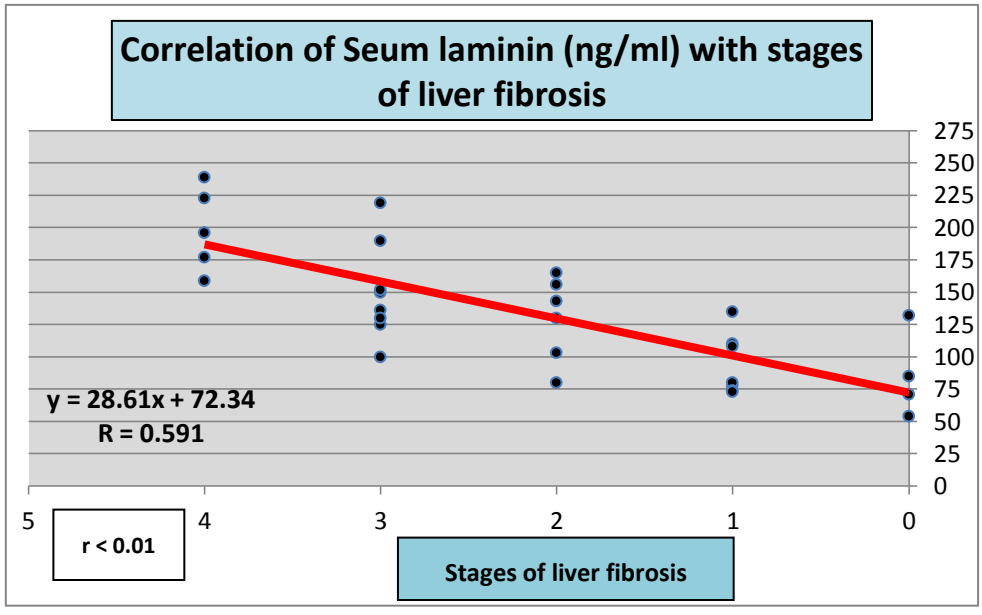
Our results showed that serum LN concentrations increased significantly with the stage of hepatic fibrosis and the highest values of LN were all found in cirrhotic patients (F < 0.01) figure 11.

Figure-11.



LN levels showed positive correlation with the stages of liver fibrosis ($r = 0.591$, $p < 0.01$) figure 12.

Figure-12.



The serum LN concentrations did not differ significantly between the control group and the F0 group (mean values ± SD were 78.2 ± 9.8 vs 85.5 ± 6.3 ($p > 0.05$). Significant differences of serum LN levels (ng/ml) were found in patients with significant fibrosis (F2-F4 groups) in comparison with those in F0-F1 groups (mean values ± SD were 156.4 ± 53.8 vs 90.9 ± 20.1 ($p < 0.001$). In the other hand Serum LN at value 107.5ng/ml was the optimal cut-off value for diagnosis of significant fibrosis (sensitivity, specificity, positive predictive and negative predictive values were shown in table 1.

Table-1. Sensitivity, Specificity, Positive predictive value and Negative predictive value of serum lamininn as a marker of significant liver fibrosis at cut off value 107.5 ng/ml

Sensitivity	Specificity	Positive predictive value	Negative predictive value
84.2%	63.6%	80%	70%

DISCUSSION

In Egypt, chronic hepatitis B is one of the main causes of chronic liver disease in addition to the main cause; chronic hepatitis C. Therefore, it is clinically important to assess the progression of liver fibrosis for guiding clinical therapy (National Institutes of Health Consensus Development Conference Statement., 2002). Liver biopsy provides useful information to the clinician for determining prognosis and the urgency of therapy, predicting response to treatment, and investigating the etiology of liver injury, as well as for providing a baseline to allow comparisons of future histological outcomes (Bravo *et al.*, 2001). However, percutaneous liver biopsy is an invasive procedure and may be associated with significant complications in 3% of recipients such as bleeding, infection, damage to liver tissue, with a mortality rate of 0.03% (The role of liver

biopsy in hepatitis, 1997). Actually liver biopsy fails to satisfy the more and more pronounced need for a rapid, safe and repeatable tool to monitor the fibrogenic progression of chronic liver disease. The ideal surrogate blood markers should enable repetitive measurement and be provided with other features, such as liver specificity, sensitivity for fibrogenesis/fibrolysis, known half-life, known excretion route, synthesis by an identified cell source, etc (Pinzani *et al.*, 2005). In this study, we aimed to assess the usefulness of serum LN as biomarker for predicting significant fibrosis in CHB patients. We found highly significant differences as regard serum LN in patients with chronic hepatitis B with different grades of liver fibrosis and control subjects. As liver fibrosis progresses, there is a significantly increase of serum LN concentrations correspondingly, and the highest values of LN were all found in cirrhotic group (F = 4). In addition, LN levels showed positive correlation with the stages of liver fibrosis in CHB patients ($r = 0.591$, $p < 0.001$). The results suggested that increased serum LN levels, which are components of ECM, might indicate a consequence of chronic liver injury, leading to architectural changes in the liver parenchyma that causes liver fibrosis eventually. Same results were obtained by Feng et al; which suggested a positive role of hyaluronic acid (HA) and LA as serum markers for predicting significant fibrosis in patients with chronic hepatitis B (Feng *et al.*, 2012). In the same way Keyur et al, recommended the use of clinical biomarkers of liver fibrosis for its simplicity and accepted correlation with different stages of liver fibrosis (Keyur *et al.*, 2006). In recent years, detention of serum LN was usually as a member of combined analysis of several fibrosis indexes rather than assay of only LN levels in liver fibrosis (Santos *et al.*, 2005; Bolarin and Azinge, 2007). Our results showed significant differences as regard serum LN in patients with significant fibrosis (F2-4) when compared to patients with mild or absent fibrosis (F0-1) and its sensitivity for significant fibrosis (F2-4) in patients with chronic hepatitis B (cut off value: 107.5 ng/ml) was 84.2% which considered more than accepted. However its specificity was to some extent lower; 63.3% .These results might be related to small number of the patients in the study but even though still in accepted figures. Combining of more than one biomarker might increases the sensitivity and specificity of these non invasive markers. This idea supported by Feng et al, which assessed the performance of combined HA and LN for predicting significant fibrosis and recorded better results. No significant differences in serum LN concentrations between patient with chronic hepatitis without fibrosis (F0) and control subjects and also between chronic hepatitis patients in grade F0 and grade F1. These results give impression that serum LN could be useful marker of significant liver fibrosis only but not marker of inflammation or mild fibrosis. Another impression, that we cannot obsolete liver biopsy as gold standard for staging and grading hepatic fibrosis and inflammation. Same concept supported in 2010 by Carey (Carey and Carey, 2010). But despite this superiority of liver biopsy still serum LN and other serum biomarkers should be kept in mind as a valuable, simple, cheap and non invasive tool in assessing liver fibrosis in chronic hepatitis patients especially if liver biopsy is contraindicated or at least cannot be repeated**.**

CONCLUSION

Liver biopsy well remains gold standard for staging and grading hepatic fibrosis and inflammation. But LN could be clinically useful serum markers for predicting significant fibrosis in patients with chronic hepatitis B, especially when liver biopsy is contraindicated.

Further large scale studies are needed for determining its value in other chronic liver diseases such as chronic hepatitis C (CHC) and non alcoholic fatty liver disease (NAFLD), its use as a marker of management success and regression of fibrosis and clinical validity in comparison to other non invasive serum markers.

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