Assessment and evaluation of serum laminin and interleukin-6 in schistosomiasis patients with chronic active hepatitis c

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Chronic hepatitis C infection (HCV) and Hepatic schistosomiasis are the most common agents causing liver fibrosis in humans. Laminin is related to hepatic fibrosis in chronic liver disease. Thus, this study's objective was to evaluate the ability laminin as serum biomarker of extra cellular matrix to predict hepatic fibrosis and compared to serum interleukin 6 (IL-6) in Egyptian patients which can assist the clinician in making prognostic decision. Patients and methods: The study had 4 groups: the first group contained 26 patients with schistosoma mansoni infection, the second group contained 40 HCV patients, the third group contained 45 patients with combined chronic viral hepatitis C and schistosomiasis and the last group contained 25 healthy individuals as control group. The following markers were assessed: Laminin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutamyltranspeptidase (GGT), Prothrombin time (PT), platelets, besides Interleukin6 (IL-6). Results: Serum Laminin and cytokine IL-6 concentrations were found in those patients with combined schistosomiasis and chronic viral hepatitis C significantly higher than in those with either schistosomiasis (P < 0.001) or chronic HCV (P <0.005) alone. Also, serum Laminin and IL-6 were significantly higher in the three patient groups rather than the healthy group (P < 0.001). The sensitivity, specificity, positive predictive value, negative predictive value and efficiency of serum Laminin and cytokine IL-6 were 92.5.0%, 86%, 90.8%, 85.1%, 90% and 89.8%, 81%, 88%, 82.2%, 85.9% respectively. The combined use of laminin and cytokine IL-6 achieved the highest sensitivity 96.8 %. Serum parameters measurements may reflect the intensity of liver disease. Serum laminin and IL-6 can differentiate HCV or schistosomiasis infected patients with liver fibrosis from those without. The combined use of LAMININ and IL-6 achieved the highest sensitivity to detect liver fibrosis due to combined infections so these biomarkers could assist the clinician in diagnosis, also they had a clinical value in making prognostic and therapeutic decisions for patients with combined HCV and schistosomiasis.

Keywords: laminin, interleukin-6, schistosomiasis & hepatitis c
INTRODUCTION
Liver fibrosis is due to chronic liver injury. Liver injury is from many causes as persistent viral, alcohol and helminthes infections. In Egypt, the most abundant form of Liver fibrosis is hepatic schistosomiasis. Advanced portal fibrosis is due to the final complements to schistosomal eggs that are trapped in the liver. Advanced portal fibrosis is accompanied with intensive deposits of connective tissue in portal tracts. Decreased degradation, increased biosynthesis or a combination of both lead to the increase in liver connective tissue (David, 2013; Mohamed et al., 2006). Chronic hepatitis C is a slow developed liver disease leading to cirrhosis and its complications. 10 to 30% of fibrotic patients develop cirrhotic liver (David, 2013; Mohamed et al., 2006).

The histological examination is the standard method to diagnose the liver fibrosis in chronic viral hepatitis and schistosomiasis patients. Histological examination is restricted because it is firstly, aggressive method, secondary, is hard to gain a liver biopsy due to the bleeding hazard and sampling error (Regev et al., 2002). Estimation of hepatic fibrogenesis would give important data about the ongoing process, especially under therapy and during follow-up (Pamela et al., 2016). Hepatic fibrogenesis is characterized by an excessive accumulation of extra cellular matrix components (Anna et al., 1998). It results from an imbalance between diminished matrix breakdown and enhanced connective tissue synthesis (Helen et al., 2013). The Extracellular Matrix composition in cirrhotic nodules consists mainly of non-collagenous proteins such as laminin and fibronectin, beside collagen types I and III (Bissell and Roll, 1990; Schuppan, 1990).

In 1979, Laminin was first identified by MARTIN and TIMPL, from a murine fibrosarcoma. Laminin molecule is a large molecule made up of three polypeptide chains called α1, β1 & γ1. α1 is with approximately 440 kDa, while β1 & γ1 each one is with approximately 200 kDa. These three chains are twisted by disulphide bridges, forming an asymmetrical structure[9]. Laminin is thought to be synthesized by hepatocytes and sinusoidal cells (Heitor and Edison, 2008).

Laminin is one of the basement membrane’s glycoproteins. Laminin participates in many of biological phenomena as growth, migration, adhesion and cellular differentiation (Stathakis et al., 1981).

Laminin plays important roles in hepatic fibrogenesis beside its roles in the development and pathogenesis of many disorders, including cancer (Gultekin et al., 2017; Andrew et al., 2011). The human IL-6 is a protein containing 160 amino acids. IL-6 is homodimer as functional unit. IL-6 is expressed by a set of cells, as T cells (TH1, TH2) and B cells derived from peripheral blood (Waal and Moore, 1998).

IL-6 plays an important role in immune responses and inflammation. IL-6 biological activities is included in both immuno-stimulatory effects and immunosuppressive. For example, IL-6 can improve the growth of B cells and mast cells, and inhibit or enhance the activities of T cells depending on their activation conditions. IL-6 can also inhibit the production of pro-inflammatory cytokines, and down regulates the expression of activating and co-stimulatory molecules on dendritic and monocytes cells (Erhard and Peter, 2013; Tsukamoto, 1998).

Human IL-6 inhibits the production of pro-inflammatory cytokines by T cells. It has both anti-inflammatory and immunosuppressive properties. IL-6 diminishes the intrahepatic inflammatory response and suppresses hepatotoxicity. Nelson et al. supported the hypothesis that IL-6 has an anti-fibrotic effect, where Nelson et al. reported that IL-6 has a part in normalizing ALT serum levels and reducing liver fibrosis (Brown et al., 2001; Nelson et al., 2000).

Data aren’t available for describing the pattern of laminin and IL-6 in combined hepatitis C and schistosoma-infected patients. So, this study was aimed to evaluate the ability of serum biomarkers of extra cellular matrix as laminin and routine laboratory tests to predict liver fibrosis and compared to serum cytokine (IL-6) in Egyptian patients and to determine if these parameters could assist the clinician in making prognostic decision.

MATERIALS AND METHODS
Patients and methods
111 patients (74 male (67%) and 37 female (33%); mean age = 40 ± 8.1 years) were recruited in this study. All patients were taken from Gastroenterology Department, Mansoura University and they had proven by histological assessment HCV and/or Schistosoma mansoni infection. They were matched for age, duration of HCV infection and stage of liver disease at presentation. Patients were classified into three...
groups. Group 1 included 26 patients with schistosomiasis alone; the inclusion criteria included history of schistosomiasis, detection of viable S. mansoni ova in stool or a rectal biopsy sample and seropositivity for schistosomal antibodies (indirect hemagglutination; Femouz laboratories). Group 2 included 40 patients infected with chronic HCV, and the inclusion criteria were: seropositivity for antibody to HCV (EIA 2; Abbott Laboratories), positively for HCV RNA by PCR and liver biopsy samples showing evidence of chronic hepatitis. Group 3 included 45 patients with combined chronic HCV and schistosomiasis, who were diagnosed by the above mentioned criteria. Beside the group 4 which included 25 healthy individuals who were matched for age and sex to serve as control subjects.

Complete history was taken from all patients; the duration of disease was defined as the interval between the probable time of acquisition of HCV infection (determined by the date of intravenous anti-schistosomal therapy and/or blood transfusion) and/or schistosomiasis (detected from the history of exposure and clinical presentation) and enrollment. Patients enrolled in the study had no serological markers for hepatitis A, B, D, cytomegalovirus infection or other hepatic or intestinal parasites. None of the patients or controls drank alcohol or was a smoker.

The entire participants were subjected to the following:

Determination of serum aspartateaminotransferase (AST), alanine aminotransferase (ALT), and glutamyltranspeptidase (GGT), they were measured with kinetic method. Upper normal limits (ULN), were: AST (up to 37 IU/L), ALT (up to 40 IU/L), and GGT (up to 50 IU/L).

Platelets were counted on an Advia 120® Hematology System (Bayer Diagnostic Division, Tarrytown, NY, USA); the normal range was 130–400 x 1000/μL.

Serum IL-6 was measured with an immunosorbent assay ELISA kit (Cat. No. EL10027, ANOGEN Immunotech; Ontario, Canada) (Florentino, 1991). Serum Laminin was measured in the different groups quantitatively by immunnoassay ELISA kit (Code No. MK 115, TAKARA) (Hynes, 1982).

Prothrombin time (PT) is a blood test that measures how long it takes blood to clot measured by (DiaMed, Cresier, Switzerland) (expressed as INR) (Matsui et al., 1997; Owren and Aas, 1951).

Statistical methods

Results were expressed as mean ± SD and were analyzed by using the Student’s t test, and ANOVA tests, as appropriate. Correlation between different parameters was performed using Pearson’s correlation test. P ≤ 0.05 was considered to be significant. All statistical procedures were performed using SPSS software, version 11 for Windows.

RESULTS

In table 1, laboratory measurements and patient demographics are shown. As shown in Fig. 1, LAMININ level expressed by mean ± SD, was the highest in group 3 (3.3 ± 1.05 units/ml) compared to both group 1 (1.92 ± 0.6 units/ml, P < 0.001) and group 2 (2.21 ± 0.75 units/ml, P < 0.001), while the three groups showed a highly significant increase in serum LAMININ compared with the healthy controls (0.7 ± 0.25 units/ml) (P < 0.001). High serum LAMININ concentrations were estimated in sera in 38 patients in group 3 with a high sensitivity (84.5%) compared to 21 patients in group 1 with 80.8% sensitivity and 36 patients in group 2 with 90% sensitivity. Also, this assay showed 90% specificity where a false-positive LAMININ result was found in 3 healthy samples.

Non invasive indirect markers of liver fibrosis as AST, ALT and GGT showed highly significance increase in group 2 and group 3 compared to group 1 and control group. The platelet counts decreases in all patients compared to control group. There was no significant difference in Prothrombine Time (PT) among the patients compared to control group.

As shown in Fig.2, IL-6 level (mean ± SD) was the highest level in third group (109.1± 34.2 units/ml) compared to both first group (84.4 ± 23.3 units/ml, P < 0.001) and second group (69.7 ± 21.2 units/ml, P = 0.005), while all three groups showed highly significant increase in serum IL-6 compared with the healthy group (33.3± 9.6 units/ml) (P< 0.001). High serum IL-6 concentrations were detected in serum in 40 patients in group 3 with a high sensitivity (88.8%) compared to 18 in group 1 with a 69.2% sensitivity and 34 in group 2 with a 85% sensitivity. In addition, this assay demonstrated a 81% specificity where a false-positive IL-6 result was detected in 4 samples of the healthy group.

The sensitivity, specificity, positive predictive value, negative predictive value and efficiency of
serum LAMININ and cytokine IL-6 were 92.5.0%, 86%, 90.8%, 85.1%, 90% and 89.8%, 81%, 88%, 82.2%, 85.9% respectively. The combined use of laminin and cytokine IL-6 achieved the highest sensitivity 96.8%.

The results showed that the cut off for LAMININ was 1.75 U/L, at sensitivity 92.5% and specificity 86%, while the sensitivity and specificity for IL-6 were 89.8% and 81% respectively.

Figure. (1): Mean ± SD of serum LAMININ levels in all tested group using ELISA technique

Table 1, Patient demographics and baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 26)</th>
<th>Group 2 (n = 40)</th>
<th>Group 3 (n = 45)</th>
<th>Group 4 (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex: Male</td>
<td>18 (69%)</td>
<td>30 (75%)</td>
<td>26 (58%)</td>
<td>16 (64%)</td>
</tr>
<tr>
<td>Female</td>
<td>8 (31%)</td>
<td>10 (25%)</td>
<td>19 (42%)</td>
<td>9 (36%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>44±9.1</td>
<td>40.5±4.6</td>
<td>45.8±7.9</td>
<td>36.3±5.2</td>
</tr>
<tr>
<td>ALT (up to 32 IU/L)</td>
<td>24.6±4.6</td>
<td>54±29.3*</td>
<td>73.2±32.5*</td>
<td>21±4.2</td>
</tr>
<tr>
<td>AST (up to 37 IU/L)</td>
<td>38.5±8.7</td>
<td>40.9±9.4*</td>
<td>45.5±13.8*</td>
<td>30.5±11.2</td>
</tr>
<tr>
<td>GGT (up to 50 IU/L)</td>
<td>53.7±13.4</td>
<td>65.8±18.1*</td>
<td>74.6±22.5*</td>
<td>33.6±21.7</td>
</tr>
<tr>
<td>Platelets counts (100-400×1000/Μl)</td>
<td>151.5±16.1*</td>
<td>144.5±33.7*</td>
<td>132.8±56.7*</td>
<td>173.8±25.3</td>
</tr>
<tr>
<td>ProthrombineINR (70-110 %)</td>
<td>77.3±5.2</td>
<td>73.6±7.9</td>
<td>69.8±8.8</td>
<td>84.5±4.7</td>
</tr>
<tr>
<td>Laminin (up to 1.1 U/mL)</td>
<td>1.92±0.6*</td>
<td>2.21±0.75*</td>
<td>3.3±1.05*</td>
<td>0.7±0.25</td>
</tr>
<tr>
<td>IL-6 (up to 45 ng/ml)</td>
<td>84.4±23.3*</td>
<td>69.7±21.2*</td>
<td>109.1±34.2*</td>
<td>33.3±9.6</td>
</tr>
</tbody>
</table>

Data expressed as mean ± standard deviation. ALT: alanine aminotransferase. AST: aspartate aminotransferase. GGT:gamma glutamyltransferase

Group 1: Schistosomiasis group. Group 2: HCV-infected group. Group 3: Combined group. Group 4: Control group. * = significance P<0.05 compared to control group.

Table 2: Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of LAMININ and IL-6 when tested independently or in combinations for patients with combined HCV and schistosomiasis.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Sensitivity%</th>
<th>Specificity%</th>
<th>PPV%</th>
<th>NPV%</th>
<th>Accuracy%</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAMININ</td>
<td>92.5</td>
<td>86.0</td>
<td>90.8</td>
<td>85.1</td>
<td>90.0</td>
</tr>
<tr>
<td>IL-6</td>
<td>89.8</td>
<td>81.0</td>
<td>88</td>
<td>82.2</td>
<td>85.9</td>
</tr>
<tr>
<td>LAMININ + IL-6</td>
<td>96.8</td>
<td>95.0</td>
<td>96.8</td>
<td>95.0</td>
<td>96.7</td>
</tr>
</tbody>
</table>
Figure. (2): Mean ± SD of serum IL-6 levels in all tested group using ELISA technique

Figure.(3). The receiver operating curve (ROC) for LAMININ, the area under curve (AUC) = 0.091. The healthy group was estimated against patient groups (hepatitis, schistosomiasis and combined)

The combined use of LAMININ (92.5%) as well as IL-6 (89.8%) achieved the highest sensitivity (96.8%) making them as useful tool for screening patients with combined HCV and Schistosomiasis. The overall combined sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy are shown in (Table 2). The area under the receiver operating characteristic (ROC) curve of laminin and IL-6 for discriminating patients with hepatic fibrosis from those with no fibrotic livers and its p value were 0.91 and 0.81 (P<0.001) respectively as shown in (fig. 3). Also indicate that these tests in discriminating the low and high concentrations of laminin and interleukin with very good validity.

DISCUSSION

In human liver, basement membranes (as laminin) are found in blood and lymphatic vessels and around bile ducts. Only a little antibodies reaction against laminin in the hepatic sinusoids can be observed (Heitor and Edison, 2008). With
the progression of cirrhotic liver, deposition of collagen and laminin occurs, both along the fibers of septal fibrosis and subendothelial sinusoids or Disse’s space

(Disse’s space phenomenon) (Gultekin et al., 2017). A significant correlation was found between laminin concentration and the degree of fibrotic liver.

Due to this relationship between advanced fibrosis and laminin tissue deposition, several authors determined the serum levels of laminin as a non-invasive biomarker to diagnose hepatic fibrosis in patients with viral hepatitis and hemochromatosis (Erhard and Peter, 2013).

Several connective tissue substances, as laminin, fibronectin, hyaluronic acid, type III procollagen N-terminal peptide and collagen type I, II, IV, have been studied as noninvasive biochemical markers for hepatic fibrosis and cirrhotic liver (Fortunato et al., 2001).

Several investigators studied the diagnostic power of serum LAMININ as an index of hepatic fibrotic patients with schistosomiasis (Han et al., 2015; Strickland et al., 2006) and with chronic HCV (Attallah et al., 2006; El-Masry et al., 2000; Lebensztejn et al., 2001). This is the first studies of pattern of laminin in combined schistosomiasis patients and viral hepatitis C in Egypt. Based on anti-LAMININ monoclonal antibody, several immunodiagnostic assays for assessment of serum LAMININ in hepatic fibrotic patients have been reported (Rockey, 2005).

The LAMININ concentrations were analyzed in the current study in the 111 patients’ sera with hepatic fibrotic patients due to schistosomiasis (n = 26), HCV (n = 40) and combined hepatitis C infections and schistosoma (n = 45). The results showed that there was significantly increased in serum LAMININ in all patients with hepatic fibrosis when compared to the healthy group. Also, in the sera of patients had combined schistosomiasis and viral hepatitis patients, serum LAMININ concentrations were increased when compared to viral hepatitis C or schistosomiasis patients only. In our results, the significant increase in LAMININ might be due to highly stimulation of fibrinogenesis especially in the dual infections of hepatitis C virus and schistosoma (Cai et al., 2003; Kardorff et al., 1999).

In sera in combined group, high serum LAMININ levels were detected with a high sensitivity (84.5%) compared to schistosomiasis group 80.8% sensitivity and HCV-infected group 90% sensitivity. Also, this assay demonstrated a specificity of 90%, so serum LAMININ can be used as screening marker of hepatic fibrosis. These results were in-agreement with the results of dos Santos et al., (2005) and Fortunato et al., (2001), they found that the serum laminin level increased in fibrosis patients with HCV infection and also agrees with Junge et al., (1988).

The results analyzed the IL-6 levels in the 111 patients’ sera with hepatic fibrosis due to Hepatitis C Virus, schistosomiasis and combined infection (schistosoma and HCV). The results showed that serum IL-6 was significantly increased in all hepatic patients when compared to the healthy group (P < 0.001). Also, serum IL-6 levels were increased in the sera of combined group as compared with viral hepatitis C patients or schistosomiasis only. These results were in-agreement with the results of Natalia et al (2006) and Cacciarelli (1996), they found that IL-6 is elevated in patients with chronic hepatitis infection. In line with our finding, the Japanese study by Hamada et al., (2003) suggests that high production of IL-6 may cause inhibition of hepatic fibrosis progression and also, agrees with Tsai et al., (1997) who observed increase IL-6 level of patients with chronic disease.

Also, high serum IL-6 concentrations were detected in sera in combined group with a 88.8% high sensitivity compared to schistosomiasis group (69.2% sensitivity) and HCV-infected group (85% sensitivity). The assay demonstrated a specificity of 81%. The explanation is due to many cytokines secreted by T helper cell (Th) (type 1 and 2 cells) in the immune response to Hepatitis C Virus infection. Th2 release IL-4 and IL-6, which modulate hepatic injury by suppressing the Th1 response and counteracting the fibrogenic effects of TNFα, INF γ and IL-2 (Brown and Neuman, 2001; Islam et al., 2005).

In our study, the increased of AST is accompanied the progression of liver fibrosis [40], where, advancing hepatic diseases may be associated with mitochondrial injury, leading to release of AST (Jun et al., 2016; Okuda et al., 2002).

Advanced hepatic diseases from Hepatitis C are common in Egypt, liver biopsy is the golden standard to detect, diagnose and measure the hepatic pathology, but, it is not ideal (Bourliere et al., 2006, Denzer and Lüth, 2009).

Non-invasive (non-progressive) methods are clinically important to measure severity of liver injury (Behairy et al., 2016). Many studies evaluating biomarkers for diagnostic liver fibrosis have used scoring systems including combinations of results ( as Fibro-test ) from
several blood tests and demographic data (Myers et al., 2003; Poynard et al., 2009).

Most of the biomarkers and indexes mentioned in the studies wouldn’t be practical in developing countries as Egypt due to its cost and unavailability of some tests. So, we evaluated two biomarkers for measuring hepatic fibrosis, Laminin and IL-6. In addition to tests routinely performed on patients with chronic Hepatitis C and schistosomiasis. Predictability of hepatic injury was significantly increased by evaluating combinations of biomarkers results from two or more tests.

In the present work, the combined use of LAMININ and IL-6 achieve the highest sensitivity to detect liver fibrosis due to combined infections. The sensitivity, specificity, positive predictive value, negative predictive value and efficiency for estimation of serum LAMININ and cytokine IL-6 were 92.5%, 86%, 90.8%, 85.1%, 90% and 89.8%, 81%, 88%, 82.2%, 85.9% respectively.

The combined use of LAMININ (92.5%) and IL-6 (89.8%) achieved the highest sensitivity (96.8%) for detecting the hepatic fibrosis, making them useful tools for screening patients with combined HCV and schistosomiasis. The area under the receiver operating characteristic (ROC) curve of laminin and IL-6 for discriminating hepatic fibrotic patients from those with no fibrotic livers, its p-value were 0.91 and 0.81 (P<0.001) respectively, also indicating that these tests in discriminating the high and low laminin level and interleukin with very good validity. Finally, the measurements of serum laminin levels may become an essential biomarker parameter of patients for the indication of fibrosis.

CONCLUSION

In conclusion, Serum laminin and IL-6 can differentiate Hepatitis C or schistosomiasis infected patients with hepatic fibrosis from patients with non fibrosis. The combined use of LAMININ and IL-6 achieved the highest sensitivity to detect liver fibrosis due to combined infections so these biomarkers could assist the clinician in diagnosis, also they could have a clinical value in making prognostic and therapeutic decisions for patients with combined HCV and schistosomiasis.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

TEM & HAE designed and also wrote the manuscript. ASE and KH performed chemical analysis and data analysis. AMA designed experiments and reviewed the manuscript. MAZ collected the samples. All authors read and approved the final version.

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