Evaluation of Serum Fibronectin and Interleukin-10 in Egyptian Patients with Combined Viral Hepatitis C and Schistosomiasis

Mosa T. E., Khayyal A.A., Saad A and Abo-Zeid M.

1Biochemistry Department, Genetic Engineering and Biotechnology Division, National Research Center. Dokki
2Gastroenterology Center, Mansoura University, Egypt

ABSTRACT

Hepatic schistosomiasis and chronic hepatitis C virus (HCV) are the most prevalent agents causing hepatic fibrosis in humans. Fibronectin (FN) has been related to liver fibrosis and subsequent development of portal hypertension in chronic liver disease. There are no available data describing the pattern of fibronectin in combined HCV and schistosomiasis-infected patients. Thus, this study's objective was to evaluate individually and combination of the ability of routine laboratory tests and serum biomarkers of extra cellular matrix (fibronectin) to predict hepatic fibrosis and compared to serum cytokine (IL-10) in Egyptian patients which can assist the clinician in making prognostic decision. This study included four groups: Group I included 20 patients with schistosoma mansoni infection, group II included 32 patients infected with HCV, group III included 40 patients with combined chronic viral hepatitis C and schistosomiasis and group IV included 20 healthy individuals who served as a control group the following markers were assessed: Fibronectin (FN), aspartateaminotransferase (AST), alanine aminotransferase (ALT), glutamyltranspeptidase (GGT), Prothrombin time (PT), platelets, besides Interleukin10 (IL-10). Serum Fibronectin and Interleukin10 were measured in the different groups quantitatively by ELISA technique. Significantly higher serum FN and cytokine IL-10 concentrations were found in patients with combined chronic viral hepatitis C and schistosomiasis than in patients with either chronic HCV (P <0.005) or schistosomiasis (P < 0.001) alone. Also, serum FN and IL-10 were significantly higher in the patient groups than the control group (P < 0.001). The overall sensitivity, specificity, positive predictive value, negative predictive value and efficiency of serum FN and cytokine IL-10 were 92.0%, 85%, 90.2%, 84.2%, 90% and 90%, 80%, 87.8%, 80.9%, 86.6%, respectively. The combined use of fibronectin and cytokine IL-10 achieved the highest sensitivity 97.5 %. Serum tests measuring the dynamic processes of fibrogenesis and fibrolysis may reflect the severity of liver disease; fibronectin plays a role in liver fibrosis. Serum fibronectin and IL-10 can differentiate HCV or schistosomiasis infected patients with liver fibrosis from patients with non fibrosis. The combined use of FN and IL-10 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.

Key Words: Evaluation of serum, fibronectin, interleukin-10, combined viral hepatitis

Corresponding Author: Khayyal A.A.
E-mail: abeerkhaial@yahoo.com

INTRODUCTION

Hepatic fibrosis is a common response to chronic liver injury from many causes including alcohol and persistent viral and helminthes infections. Hepatic schistosomiasis is the most prevalent form of hepatic fibrosis in Egypt. The final sequel to schistosomal eggs trapped in the liver is advanced portal fibrosis with dense deposits of connective tissue in greatly expanded portal tracts. The net increase in liver connective tissue might be caused by increased biosynthesis, decreased degradation or a combination of both (Bissell and Maher, 1996 and Abou Basha, et al., 2000). Chronic HCV is a slow progressing inflammatory disease of the liver that can lead to cirrhosis and its complications. Chronic hepatitis develops in at least half of persons acutely infected with HCV. Ten to 25% of these patients develop liver cirrhosis (Bissell and Maher, 1996 and Abou Basha et al., 2000).

The best method to diagnose and quantitative hepatic fibrosis in schistosomiasis and chronic viral hepatitis patients is histological examination. Histological examination is limited because it is difficult to obtain a regular liver biopsy due to the risk of bleeding, sampling error and finally liver biopsy reflects a static picture of hepatic fibrosis (Regev et al., 2002). But, with the availability of effective treatments, such as interferon and the potential for progression to hepatocellular carcinoma (HCC) in some cases, an accurate measurement of the stage of disease is important. Assessment of hepatic...
fibrogenesis would give more important information about the ongoing process in the liver, especially at the early stage of the disease, during follow-up and under potential anti-fibrogenic therapy (Cadranel et al., 2000). Hepatic fibrogenesis results from an imbalance between enhanced connective tissue synthesis and diminished or altered matrix breakdown (Kossakowska et al., 1998). It is characterized by an excessive accumulation of Extra Cellular Matrix (ECM) components (Friedman, 1993). The ECM composition in septa and cirrhotic nodules consists mainly of collagen types I and III and other non-collagenous proteins such as laminin and fibronectin (Schuppan, 1990 and Bissell and Roll, 1990).

Fibronectin is a non-collagenous extra cellular matrix glycoprotein and one of the factors that promotes wound healing through its facilitating effects of cell adhesion or migration. Hynes (1980), in arterial walls, fibronectin can be produced by endothelial cells, smooth muscle cells and fibroblasts and exists in all layers of the walls. It has been shown to be strongly immunostained in the early stage of atherosclerosis (Statathakis et al., 1981). As a major component of the extracellular matrix, it is considered to have an important role in chronic inflammatory periodontal disease (Muro et al., 2003). Fibronectin also plays important roles in the development and pathogenesis of many disorders, including cancer. It has also been mentioned that fibronectin plays important roles in hepatic fibrogenesis (Proctor, 1987).

The mature human IL-10 is a protein with 160 amino acids and the functional IL-10 is a homodimer. IL-10 is expressed by a variety of cells, including mouse T cells (TH1, TH2 and Tr1 subsets), B cells derived from peripheral blood, tonsils or spleen, Epstein–Barr virus (EBV)-transformed B cell lines, Burkitt’s lymphoma, AIDS lymphomas, monocytes, placental trophoblasts, bronchial epithelial cells and certain tumor cells including melanomas and carcinomas of various origin (De Waal and Moore, 1998).

IL-10 plays an important role in inflammatory and immune responses. The biological activities of IL-10 include both immunosuppressive and immuno-stimulatory effects. For example, IL-10 can suppress the production of pro-inflammatory cytokines by monocytes and neutrophils and down regulates the expression of activating and co-stimulatory molecules on monocytes and dendritic cells. IL-10 also can improve the growth of B cells and mast cells and inhibit or enhance the activities of T cells depending on their activation conditions. The different polymorphisms in the IL-10 gene promoter have been demonstrated the association with both SIDS (sudden infant death syndrome) and sudden unexpected death due to infection (Mosmann, 1994 and Tsukamoto, 1998).

Human IL-10 has both anti-inflammatory and immunosuppressive properties and it down-regulates the production of pro-inflammatory cytokines by T cells. Endogenous IL-10 reduces the intrahepatic inflammatory response and curbs hepatotoxicity in several models of liver injury. It is also expressed by hepatic stellate cells and may have a role in liver matrix remodelling by reducing collagen and enhancing collagenase production. Nelson et al. reported that, though it had no antiviral activity, IL-10 could play a part in normalizing ALT serum levels, improving liver histology and reducing liver fibrosis in a large proportion of patients treated with recombinant IL-10, supporting the hypothesis that IL-10 has an anti-fibrotic effect (Nelson et al., 2000 and Jacobson and Neuman, 2001).

There are no available data describing the pattern of fibronectin and IL-10 in combined HCV and schistosoma-infected patients. Thus, the aim of this study was to evaluate individually and combination of the ability of routine laboratory tests and serum biomarkers of extra cellular matrix (fibronectin) to predict hepatic fibrosis and compared to serum cytokine (IL-10) in Egyptian patients and to determine if these parameters could assist the clinician in making prognostic decision.

PATIENTS AND METHODS

Ninety two patients (64 male (69%) and 28 female (31%); mean age = 39 ± 8.2 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 20 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 32 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 40 patients with combined chronic HCV and schistosomiasis, who were diagnosed by the above mentioned criteria. Beside the group 4 which included 20 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:
1. 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).
2. Platelets were counted on an Advia 120® Hematology System (Bayer Diagnostic Division, Tarrytown, NY, USA); the normal range was 130–400 × 1000/μL.
3. Serum IL-10 was measured with an immunosorbent assay ELISA kit (Cat. No. EL10027, ANOGEN Immunotech; Ontario, Canada). (Fiorentino et al., 1991)
4. Serum FN was measured in the different groups quantitatively by immunnoassay ELISA kit (Code No. MK 115, TAKARA) (Hynes et al., 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) (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

Patient demographics and laboratory measurements are shown in (Table 1). The mean ± SD of FN level was the highest in group 3 (137.2 ± 68.9 units/ml) compared to group 1 (78.4 ± 27.7units/ml), P < 0.001) and group 2 (79.9 ± 32.4 units/ml, P < 0.001), as shown in (Figure 1), while all groups (1, 2, 3) showed a highly significant increase in serum FN compared with the healthy controls (27.7 ± 14.3 units/ml) (P < 0.001). High serum FN levels were detected in sera in 38 patients in group 3 with a high sensitivity (92.5%) compared to 15 patients in group 1 with a sensitivity of 75% and 29 patients in group 2 with a sensitivity of 90%. In addition, this assay demonstrated a specificity of 90% where a false-positive FN result was found in 3 patients of the control group samples.

Table 1: Patient demographics and baseline characteristics.

<table>
<thead>
<tr>
<th>Group 1 (n = 20)</th>
<th>Group 2 (n = 32)</th>
<th>Group 3 (n = 40)</th>
<th>Group 4 (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex:</strong></td>
<td><strong>Age (years)</strong></td>
<td><strong>ALT (up to 32 IU/L)</strong></td>
<td><strong>AST (up to 37 IU/L)</strong></td>
</tr>
<tr>
<td>Male</td>
<td>15 (75%)</td>
<td>23.7±4.6</td>
<td>37.6±8.8</td>
</tr>
<tr>
<td>Female</td>
<td>5 (25%)</td>
<td>52±28.2*</td>
<td>41.0±9.2*</td>
</tr>
<tr>
<td><strong>ALT (up to 32 IU/L)</strong></td>
<td>70.7±4.6*</td>
<td>70.7±34.6*</td>
<td>44.6±6.8*</td>
</tr>
<tr>
<td><strong>AST (up to 37 IU/L)</strong></td>
<td>31.4±12.8</td>
<td>31.4±12.8</td>
<td>31.4±12.8</td>
</tr>
<tr>
<td><strong>GGT (up to 50 IU/L)</strong></td>
<td>170.9±23.2</td>
<td>170.9±23.2</td>
<td>170.9±23.2</td>
</tr>
<tr>
<td><strong>Platelets counts (130–400×1000/μL)</strong></td>
<td>142.7±32.6*</td>
<td>142.7±32.6*</td>
<td>142.7±32.6*</td>
</tr>
<tr>
<td><strong>Prothrombine INR (70-110%):</strong></td>
<td>76.4±4.4</td>
<td>76.4±4.4</td>
<td>76.4±4.4</td>
</tr>
<tr>
<td><strong>Fibronectin (up to 45 ng/ml):</strong></td>
<td>78.4±27.7*</td>
<td>78.4±27.7*</td>
<td>78.4±27.7*</td>
</tr>
<tr>
<td><strong>IL-10 (up to 30 ng/ml):</strong></td>
<td>68.5±18.9*</td>
<td>68.5±18.9*</td>
<td>68.5±18.9*</td>
</tr>
</tbody>
</table>

Data expressed as mean ± standard deviation.
Group1: Schistosomiasis group.
Group2: HCV-infected group.
Group3: Combined group.
Group4: Control group.
* = significance P<0.05 compared to control group.

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

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.

The mean ± SD of IL-10 level was the highest level in group 3 (92.9 ± 23.3 units/ml) compared to group 1 (68.5 ± 18.9 units/ml, P < 0.001) and group 2 (52.0 ± 14.6 units/ml, P = 0.005), as shown in (Figure 2), while all groups (1, 2, 3) showed highly significant increase in serum IL-10 compared with the healthy controls (28.2 ± 8.2 units/ml) (P< 0.001). High serum IL-10 levels were detected in serum in 36 patients in group 3 with a high sensitivity (90%) compared to 14 in group 1 with a sensitivity of 70% and 28 in group 2 with a sensitivity of 87.5%. In addition, this assay demonstrated a specificity of 80% where a false-positive IL-10 result was found in 4 of the control group samples.

**Table 2:** Overall sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of FN and IL-10 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>FN</td>
<td>92.5</td>
<td>85.0</td>
<td>90.2</td>
<td>84.2</td>
<td>90.0</td>
</tr>
<tr>
<td>IL-10</td>
<td>90.0</td>
<td>80.0</td>
<td>87.8</td>
<td>80.9</td>
<td>86.6</td>
</tr>
<tr>
<td>FN + IL-10</td>
<td>97.5</td>
<td>95.0</td>
<td>97.5</td>
<td>95.0</td>
<td>96.7</td>
</tr>
</tbody>
</table>

**Figure 2:** Mean ± SD of serum IL-10 levels in all tested group using ELISA technique.

The overall sensitivity, specificity, positive predictive value, negative predictive value and efficiency of serum FN and cytokine IL-10 were 92.0%, 85%, 90.2%, 84.2%, 90% and 90%, 80%, 87.9%, 80.9%, 86.6%, respectively. The combined use of fibronectin and cytokine IL-10 achieved the highest sensitivity 97.5%.

The results showed that the cut off for FN was 36 U/L, at sensitivity 92.5% and specificity 85%, while the sensitivity and specificity for IL-10 were 90% and 80%, respectively. The combined use of FN (92.5%) as well as IL-10 (90%) achieved the highest sensitivity (97.5%) 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 fibronectin and IL-10 for discriminating patients with liver fibrosis from those with no fibrosis livers and its p value were 0.91 and 0.81 (P<0.001), respectively as shown in (Figure 3). Also indicating that these tests in discriminating the high and low level of fibronectin and interleukin with very good validity.

**DISCUSSION**

Fibronectin plays crucial roles in various cellular functions, including cell adhesion, migration, proliferation and differentiation (Hynes, 1980). According to previous reports, plasma fibronectin is mainly produced by hepatocytes and cellular fibronectin in liver is produced, at least in part, by endothelial cells, fat-storing cells and to a lesser extent, by hepatocytes. It has also been mentioned that fibronectin plays important roles in hepatic fibrogenesis (Matsui et al., 1997). There is a need for accurate biochemical markers to aid in early diagnosis of liver fibrosis. Several connective tissue substances have been studied as noninvasive markers for liver cirrhosis, e.g., hyaluronic acid, type III procollagen N-terminal peptide, collagen type I, II, IV, laminin and fibronectin and have been proposed to represent hepatic fibrosis, focusing particularly on the diagnosis of hepatic cirrhosis (Fortunato et al., 2001).

The diagnostic accuracy of serum FN as an index of liver fibrosis was studied by several investigators in patients with schistosomiasis (Burchard et al., 1998 and Strickland, 2006) and in patients with chronic viral hepatitis C (Attallah et al., 2006, El Masry et al., 2000 and Lebensztejn, 2001). To the best of our knowledge, this is the first studies of...
pattern of fibronectin in combined viral hepatitis C and schistosomiasis patients in a high prevalence setting of these agents in Egypt. Several immunodiagnostic assays based on anti-FN monoclonal antibody for detection of serum FN in liver fibrotic patients have been described (Rockey, 2005). These assays cannot be easily applied in screening programs where they need a long time for completion; moreover, special and highly expensive equipment is used. Therefore, there is a strong demand for a rapid, simple and reliable test for detection of FN.

The current study analyzed the FN concentrations in the sera of 92 patients with liver fibrosis due to schistosomiasis (n = 20), HCV (n = 32) and combined schistosoma and HCV infections (n = 40). The results showed that serum FN was significantly increased in all liver fibrotic patients when compared to the control group. In addition, serum FN concentrations were increased in the sera of patients had combined schistosomiasis and viral hepatitis patients when compared to schistosomiasis or viral hepatitis C patients only. The significant increase in FN in our results might be due to intense stimulation of fibrinogenesis especially in the cases with the dual infections (schistosoma and HCV). These results are comparable to those obtained previously (Kardorff et al., 1999 and Cai et al., 2003), where a significantly increased level of serum FN in chronic hepatitis or schistosomiasis cases was determined. Burchard et al. reported that there was a lack of correlation between liver morphology in ultrasound and connective tissue metabolites in serum of patients recently exposed to S. mansoni infection (Burchard et al., 1998).

Also, high serum FN levels were detected in sera in combined group with a high sensitivity (92.5%) compared to schistosomiasis group with a sensitivity of 75% and HCV-infected group with a sensitivity of 90%. In addition, this assay demonstrated a specificity of 80%. The explanation is due to many cytokines secreted by T helper cell (Th) type 1 and 2 (Th1 and Th2) cells are involved in the immune response to HCV infection and progression of HCV-related liver disease. Th1 cells release TNFα, INF-γ and IL-2, causing inflammation and necrosis and Th2 release IL-4 and IL-10, which modulate hepatic injury by suppressing the Th1 response and counteracting the fibrogenic effects of TNFα, INF γ and IL-2 (Jacobson and Neuman, 2001). The higher levels of IL-10 present in those individuals are associated with a higher risk of an inefficient clearance of the HCV and the development of chronic HCV infection patients (Islam et al., 2005).

In our study, the increased of AST due to the progression of liver fibrosis, this may reduce the clearance of AST leading to increased serum AST levels (Kamimoto et al., 1985.) In addition, advancing liver disease may be associated with mitochondrial injury, resulting in preferential release of AST, which is present in mitochondria as well as in the cytoplasm of hepatocytes (Okuda et al., 2002). Platelet count decreases, with increasing liver fibrosis, due to worsening of portal hypertension with consequent increased platelet sequestration and destruction in an enlarging spleen (Aster, 1966).

Noninvasive methods to measure severity of liver injury are clinically important in Egypt where advanced liver disease from HCV is common and access to liver biopsy is limited (Strickland, 2006 and Waked et al., 1995). In addition, reliability of the biopsy to detect and measure hepatic pathology is not ideal (Afshal and Nunes and 2004, Halfon et al. (2007) and Fortunato et al (2001) results were in-agreement with the results of Natalia et al. (2006) and Cacciarelli (1996), they found that IL-10 is a Th2 cytokine which down regulates the Th1 effector mechanisms and the elevated serum levels of this cytokine have been observed to occur in patients with chronic HCV infection (Paladino et al., 2006 and Cacciarelli et al., 1996). In line with our finding, the Japanese study by Hamada et al (2003) suggests that high production of IL-10 may cause inhibition of liver fibrosis progression and also, agrees with Tsai et al (1997) who observed increase IL-10 level of patients with acute infection who developed a chronic disease (Hamada et al., 2003 and Tsai et al., 1997).

The results of the current study analyzed the IL-10 concentrations in the sera of 92 patients with liver fibrosis due to schistosomiasis, HCV and combined schistosoma and HCV infections. The results showed that serum IL-10 was significantly increased in all liver fibrotic patients when compared to the control group (P < 0.001). In addition, serum IL-10 concentrations were increased in the sera of schistosomiasis and viral hepatitis patients as compared with schistosomiasis or viral hepatitis C patients only. These results were in-agreement with the results of Natalia et al. (2006) and Cacciarelli (1996), they found that IL-10 is a Th2 cytokine which down regulates the Th1 effector mechanisms and the elevated serum levels of this cytokine have been observed to occur in patients with chronic HCV infection (Paladino et al., 2006 and Cacciarelli et al., 1996). In line with our finding, the Japanese study by Hamada et al (2003) suggests that high production of IL-10 may cause inhibition of liver fibrosis progression and also, agrees with Tsai et al (1997) who observed increase IL-10 level of patients with acute infection who developed a chronic disease (Hamada et al., 2003 and Tsai et al., 1997).
et al., 2006). For instance, in a multicenter study validating biochemical markers for predicting liver fibrosis in patients with chronic HCV and schistosomiasis, the authors proposed discordance caused by interpretation of the biopsy (4%) as frequent as in the Fibrotest (5%) being evaluated. Many of the reports evaluating biomarkers for detecting hepatic fibrosis have used scoring systems encompassing combinations of results from several blood tests and demographic data (Poynard et al., 2004. and Imbert Bismut et al., 2001).

Most of the indexes proposed in these studies would not be practical in Egypt and other developing countries because of cost and unavailability of some tests. For this reason we evaluated a few blood tests routinely performed on patients with chronic HCV and schistosomiasis in Egypt in addition to two commercially available tests for measuring hepatic fibrosis. Predictability of hepatic injury was significantly improved when we evaluated combinations of results from two or more tests.

In the present work, the combined use of FN and IL-10 achieve the highest sensitivity to detect liver fibrosis due to combined infections. The overall sensitivity, specificity, positive predictive value, negative predictive value and efficiency for estimation of serum FN and cytokine IL-10 were 92.0%, 85%, 90.2%, 84.2%, 90% and 90%, 80%, 87.8%, 80.9%, 86.6%, respectively.

The combined use of FN (92.5%) and IL-10 (90%) achieved the highest sensitivity (97.5%) 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 fibronectin and IL-10 for discriminating patients with liver fibrosis from those with no fibrosis livers and its p value were 0.91 and 0.81 (P<0.001), respectively, also indicating that these tests in discriminating the high and low level of fibronectin and interleukin with very good validity.

CONCLUSION

Serum tests measuring the dynamic processes of fibrogenesis and fibrolysis may reflect the severity of liver disease; fibronectin plays a role in liver fibrosis. Serum fibronectin and IL-10 can differentiate HCV or schistosomiasis infected patients with liver fibrosis from patients with non fibrosis. The combined use of FN and IL-10 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.

REFERENCES


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