Evaluation of correlation between serum immunoglobulin levels and extent of hepatic fibrosis in patients with chronic B hepatitis

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Abstract: Background: Hypergammaglobulinaemia is a common finding in patients with chronic liver diseases. The mechanism is thought to involve reduced kupffer cell clearance of antigens delivered, resulting in stimulate activity and proliferation of stellate cells and at least fibrogenesis. The aim of this study was to evaluate the correlation between serum immunoglobulin levels and possibility of use this noninvasive markers to determination of extent of hepatic fibrosis.Methods and materials: In this sectional study 50 chronic B hepatitis patients with positive virological markers and 50 people (normal control) selected from Tabriz Emam hospital during 2006-2009 and their serum IgG, IgM, IgA, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), Total protein, total bilirubin and albumin in both groups with immunoturbidometric assay and comparison with liver biopsies specimen that scoring with modified Knodell and statistical analysis was performed. Results: Results of study show significant prediction between serum total IgG ($P<0.00001$) and immunoglubolins($P<0.0001$) levels with extent of liver fibrosis , but similar relation with another serum markers specially IgA and IgM not found. Conclusion: Total imunoglobulins and IgG serum levels had significant independent prediction of necro inflammatory and extent of liver fibrosis bit other markers did not had this relation. Also we can use total immunoglobulins and IgG serum levels as a predictor of liver fibrosis and a noninvasive method to replacement of invasive liver biopsy method.
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1. Introduction

Liver fibrosis is considered to be the result of the wound healing responses to chronic liver injury caused by chronic viral hepatitis, alcoholic hepatitis, non-alcoholic steatohepatitis, hemochromatosis, or autoimmune hepatitis, which result in hepatocyte damage and abnormal proliferation (Lee et al, 2010), (Khedmat et al, 2007), (Okpalugo et al,2008).

The development of hepatic fibrosis in patients with liver disease is associated with cirrhosis and an increased risk of liver cancer. Assessing the degree of hepatic fibrosis and is therefore one of the most important factors in treatment planning (Hotta et al, 2007)

Hepatitis B virus (HBV) infection is the most common cause of acute and chronic liver disease worldwide, eventually progressing from fibrosis to cirrhosis and/ or hepatocellular carcinoma. An estimated 400 million people worldwide are carriers of HBV infection. Compensated chronic hepatitis progresses to cirrhosis in 12–20% of patients, and compensated cirrhosis progresses to hepatic decompensation and hepatocellular carcinoma with in 5 years in 20–25% and 6–16% of patients respectively (Fattovich et al, 1991), (Ikeda et al, 1998), (Befeler et al, 2000), (Lok et al, 2001), (Liaw et al, 1988), (Yu et al, 1997). Approximately 250 000 deaths occur each year as a consequence of fulminant hepatic failure, cirrhosis and hepatocellular carcinoma (Befeler et al, 2000), (Schmilovitz-Weiss et al, 2006).

Liver biopsy is the gold-standard procedure, for determining the severity of necro-inflammatory activity and fibrosis, features potentially useful for predicting treatment response (Shiffman et al,2003) and prognosis in Hepatitis C virus (HCV) (Shiffman et al, 2003). Repeated biopsies are performed in patients with recurrent HCV to estimate disease progression, to exclude other causes of elevated serum liver enzyme levels and to evaluate antiviral treatment response . but it is costly, invasive, and has inherent risks (morbidity 3%, mortality 0.03%) (Piccinino et al, 1986). In addition, sampling errors and and intraobserver variations may lead to under staging of cirrhosis, particularly macronodular cirrhosis (Maharaj et al, 1986). Accordingly, alternative simple, accurate, and noninvasive tests are

needed to assess disease activity and fibrosis stage (Poynard et al, 2002) , (Kim et al,2007).

Although several serum-based markers have shown promise for the detection of advanced fibrosis (Callewaert et al,2004), these tests, so far, are not commonly measured, and are extremely costly, and their sensitivities for milder stages of fibrosis (<2) are poor.Liver function tests are essential parts of assessing liver damage, but have poor correlation with histology (Hayes et al,1990).

Hypergammaglobulinaemia is a common finding in patients with cirrhosis of various etiologies (Triger et al,1973), (Triger et al,1972), (Prytz et al, 1977), (Husby et al, 1977). The mechanism is thought to involve reduced Kupffer cell clearance of antigens delivered by the portal venous system, resulting in increased exposure to the systemic circulation and antibody producing sites (Triger et al,1972), (Prytz et al,1977). Consequently, elevated serum immunoglobulin levels have been thought to be a result rather than cause of cirrhosis (Triger et al, 1973), (Triger et al,1972), (Prytz et al,1977), (Husby et al, 1977), (Thomas et al, 1973), (Watt et al,2004).

We carried out a comparative analysis of 50 patients with chronic liver diseases by comparing their serum biochemical markers with histopathological findings in liver biopsy, of patients with chronic liver diseases and the grading and staging of liver tissues, and to provide clues and basis for the noninvasive diagnosis of liver fibrosis.

2. Material and Methods

Patients

In this research we had two groups: group 1: as control group, they had no elevation in liver enzymes and had negative viral markers, their serum sampled and stored at -70C. In the group2, patients with chronic hepatitis B included. Patients with any other cause of chronic liver disease, rheumatic diseases, patients that may have had other types of fibrosis due to renal or pulmonary diseases, nonalcoholic steatohepatitis (NASH), auto immune hepatitis, drug poisoning and patients with malignancies were excluded. Liver biopsies were performed under ultrasonographic guidance.

Histological assessment of liver damage

Fifty patients underwent a liver biopsy for assessing the presence and severity of liver disease. The biopsy fragments were fixed in a 10% formalin solution for 12 hours and embedded in paraffin. Sections were stained with hematoxylin-eosin, Masson's trichrome and reticulin stain (Parsian et al,2010).

The original histology activity index proposed by Knodell (Knodell et al,1981) was used for grading inflammation/necrosis and for staging fibrosis. By this scoring system inflammation/necrosis score ranges from 0 to 18 (0-10 periportal ± bridging necrosis, 0-4 intralobular degeneration and focal necrosis, 0-4 portal inflammation), while fibrosis stage includes only four stages: 0 (no fibrosis), 1 (fibrous portal expansion), 3 (bridging fibrosis), 4 (cirrhosis). Steatosis was scored semi-quantitatively as: 0, no steatosis, 1, steatosis ≤ 33% of hepatocytes, 2, steatosis in 34%-66% of hepatocytes, 3, > 66% of hepatocytes.

Biochemistry:

In all serum samples levels of Ig M, Ig G and Ig A were measured using auto analyzer (Abott Alycon). Also serum biochemical factors including ALP,SGOT,SGPT,albumin, total protein and total bilirubin were measured by commercial kits (Bashir et al,2009).

According to manufacture instructions, the normal range for IgG was 700-1600 mg/dl, for IgM was 40-230 mg/dl and for IgA was 70-400 mg/dl.

Statistics:

The SPSS 16 software was used for analsing the results and Student T test, Logistic linear and one way analysis of variance were used for comparing the groups and $P<0.05$ used as significant level for comparing the groups.

3. Results

These results were obtained considering markers and lab tests after taking biopsies from patients and their serum reserves that were gained for routine lab tests before biopsies and also from normal people's serum reserves.

Personal and biochemical specifications of the patients are mentioned in the table 1. The patients group mean age was 47.78 ± 11.84 and the control group mean age was 43.76 ± 9.29. In the patients group 26 were female and 24 were male and in control group, 32 were female and 18 were male. The results showed that the levels of total immunoglobulin, IgG, IgM, IgA, total bilirubin, albumin and total protein were higher in hepatitis B patients comparing to control group significantly ($P < 0.0001$). The ALP level was higher in patients group comparing to control group significantly ($P < 0.005$), but there was no difference in SGPT and SGOT levels between two groups.

Among patients, 24 patients were mild, 20 were moderate and 6 were severe. Analysis of serum IgG level in comparison between the mild group with the moderate group and between the mild group with the severe one and between the moderate groups with the

severe one had significant difference (P < 0.0001) and has a high predictive value.

Table 1.Serum biochemical profile of patients group and control group.

Control group	Patients group	
43.76 ± 9.29	48.91±8.65	Age
32(64)	26(52)	Gender(female)
971.78 ± 201.41	2375.42±1046.43	IgG (mg/dl)
98.46±38.78	215.92±93.72	IgM(mg/dl)
5.66 ± 1.86	311.80±70.84	IgA(mg/dl)
8.13±0.92	5.66±1.86	Total Protein(mg/dl)
24.24 ± 19.61	24.02±21.01	SGOT(I.U/L)
18.90±13.08	21.44±18.03	SGPT(I.U/L)
126.76 ± 51.76	186.32±155.99	ALP(I.U/L)
0.54±0.39	2.41±1.76	Total bilirubin(mg/dl)
4.38 ± 0.51	3.07±0.78	Albumin(mg/dl)
1256.44 ± 214.25	2903.14 ± 1071.69	Total immunoglobulin(mg/dl)

But other immunoglobulins did not show significant differences between different groups with different intensities. There is significant difference among mild, moderate and severe groups only considering total immunoglobulin and IgG levels that showed this significant difference (P < 0.0001) but other markers do not have this predictive value (Table 2).

Table 2. Serum biochemical profile of patients with different grade of liver tissue fibrosis.

Severe	Moderate	Mild	
4848.01 ±505.24.	3182.05 ± 772.81	2184.51 ± 552.01	Total immunoglobulin(I.U/L)
4264.83 ± 60.55	2644.1 ±749.19	1679.16 ± 523.87	IgG(mg/dl)
287.83 ±209.10	217.95 ± 77.26	196.25 ±50.21	IgM(mg/dl)
295.33 ±73.89	320 ±68.63	309.08±74.01	IgA(mg/dl)
6 ±1.77	5.43 ±1.95	5.77 ±1.86	Total protein(mg/dl)
20.51 ± 8.51	18.1 ± 9.48	29.83 ±9.84	SGOT(I.U/L)
13.83 ±6.58	14.25 ±6.17	29.33 ± 6.26	SGPT(I.U/L)
193.1 ±101.81	156.05 ±103.86	209.88 ±155.63	ALP(I.U/L)
2.07 ± 1.04	2.76 ± 1.73	2.21 ± 2.09	Total Bilirubin(mg/dl)
3.06 ±0.87	3.21 ± 0.7	2.96 ± 0.85	Albumin(mg/dl)

About the relation of different markers with each other Pierson correlation test was used.

Analysis of serum IgG level amounts has significant and direct relation with IgM (P < 0.0001). Also it has significant and direct relation with IgA (P < 0.0001).

IgG has a significant but reversed relation (P < 0.0001) with albumin and has the same relation (P < 0.0001) with total protein and Ig M, also analysis of serum level amounts showed that with Ig A there is a very significant (P < 0.0001) and direct relation. Also IgM has a significant and reversed relation with total protein (P < 0.0001) and albumin (P < 0.0001) but there isn't any significant relation (P > 0.05) with SGOT, SGPT, ALP and total bilirubin. Analysis of IgA serum level amounts showed that IgA has a reversed and very significant relation with total protein (P < 0.0001) and albumin (P < 0.0001) and a reversed and insignificant relation with SGOT P > 0.05 and also a direct insignificant relation with SGPT and ALP and a direct significant relation (P < 0.05) with total bilirubin.

Serum total protein level amounts also has a direct significant relation with albumin (P < 0.0001) and a reversed significant relation with total bilirubin (P < 0.05) and a reversed insignificant relation with SGPT and ALP and P > 0.05 and a direct insignificant relation with SGOT and ALP.

SGOT didn't have any significant relation with any of the markers except IgG that has a reversed insignificant relation and with others, this relation was direct and insignificant (P > 0.05). Analysis of serum SGPT level amounts also showed that like SGOT it didn't have significant relation with analyzed markers and like that it had a reversed insignificant relation just with IgG. The present study showed that ALP also doesn't have any significant relation with any of the markers (p > 0.05) and with SGPT and total bilirubin and albumin, this relation was reversed and insignificant . Also total bilirubin had a direct significant relation with IgA and IgG and a reversed significant relation with total protein and albumin (P < 0.005).

Albumin also according to the mentioned data had a reversed significant relation with IgG, IgM, IgA and total bilirubin and a direct significant relation with total protein. Also it showed a reversed insignificant relation with other markers.

Analysis of linear regression showed that the level of IgG with P < 0.0001 has a significant relation with the stage but IgM with P > 0.05 doesn't have a significant relation with the stage. Also IgA with P = 0.21 doesn't have a significant relation with the stage. Also linear regression showed that IgG level with P < 0.0001 has a significant relation with the grade and IgM and IgA with P > 0.05 don't have these significant relations with the grade. ANOVA statistical analysis shows that total immunoglobulin level has a significant relation (P < 0.0001) between the groups.

Also POST HOC tests analysis by the Tukey method showed that between the mild group with the moderate group (P < 0.0001) and the mild group and the severe one (P < 0.0001), there is a very significant relation. Also between the moderate group and the severe one this significant relation was detected. About the relation of total immunoglobulin with the other markers, the obtained results showed that total immunoglobulin had a reversed significant relation with total protein (P < 0.0001) and a reversed

insignificant relation with P > 0/05 and a relative r about - 0/66 with SGOT and SGPT.

Also it had a reversed significant relation with albumin (P < 0.0001) and a direct significant relation with total bilirubin (P > 0.05) but it didn't have any significant relation with ALP.

In the study of relation of immunoglobulin levels with the severity of liver fibrosis, statistical analysis with linear regression showed that only total immunoglobulin level (P < 0.0001) and IgG (P < 0.0001) are predictive of disease severity.

4. Discussions

In this study, we evaluated the relation between serum immunoglobulin levels and fibrosis stages in hepatitis B patients and the possibility of substitution of a noninvasive method that can be repeated (which can have markers)with the invasive method of liver needle biopsy. In this study 48 percent of the patients according to the severity of liver fibrosis were in the mild group, 40 percent were in the moderate group and 12 percent were in the severe group.

In liver cirrhosis, liver injury is accompanied by a characteristic increase in serum levels of IgA, IgG and IgM, the origin of which remains to be elucidated fully. In Recent results from two animal studies suggest that immunoglobulins may play an important role in the pathogenesis of hepatic fibrosis (Shen et al,2001) ,(Yokoyama et al,1995). In a study by Yokoyama et al. Yokoyama et al,1995). ethanol alone was reported to have limited fibrogenic properties when administered to adult guinea pigs. However, when administered in conjunction with IgA immunoglobulins, extensive hepatic fibrosis occurred.

Our esults suggested a very significant relation between liver fibrosis staging with IgGand IgA level , but no significant relation could be found with serum IgM level. The severity of fibrosis (grade) also had a very significant relation with IgG level (P = 0.001) but there wasn't any significant relation with serum IgM and IgA levels.

Watt et al (Watt et al,2004), indicated that Ig M levels didn't correlate with fibrosis grade , our in vitro data indicate that rat hepatic stellate cells possess Fc receptors (which bind to IgA and IgG but not IgM immunoglobulins) . Whether complement receptors (required for IgM immunoglobulin binding) also exist in this cell population has yet to be determined. However,their results showed that there is a significant correlation between fibrosis and serum Ig A levels. In the present study Ig level and total immunoglobulin showed a more significant and direct relation with liver fibrosis staging (P < 0.0001) and other markers levels did not have any significant relation.

Schmilovitz-Weiss H et al (Schmilovitz-Weiss et al, 2006) showed that there is a strong association between serum levels of globulin and IgG and extent of hepatic fibrosis in patients with chronic HBV infection. These simple laboratory measurements can serve as noninvasive markers for disease progression. Also Pradat et al (Pradat et al,2002) in a study by the title of the predictive value of serum level of liver enzymes in histologic findings of patients suffering from C hepatitis came in to conclusion that there is an apparent relation between IgG and IgA and total immunoglobulin with fibrosis severity. Also SGOT showed a significant relation with liver fibrosis severity. This study showed that this significant relation also exists with inflammation severity (grade). But in this present study this relation did not exist about liver enzymes, neither in staging nor in grading.

Wai et al (Wai et al, 2003) also in a study for the reason of studying the power of noninvasive indicators in predicting fibrosis severity and cirrhosis in patients with C hepatitis showed that serum SGOT level is considered a useful predictive factor in C hepatitis. But in the present study serum SGOT and SGPT amounts did not show a significant value with liver fibrosis.

HUI et al (Hui et al,2005) also showed that the markers like bilirubin and albumin have useful predictive value in determining liver fibrosis severity in patients with B hepatitis.

Also he mentioned that albumin can be a prognostic factor in these patients survival.

But in the present study this significant relation was not detected with bilirubin and albumin. Schmilovitz-Weiss et al (Schmilovitz-Weiss et al,2007), reported that, there is a strong association between serum globulin and immunoglobulins levels and extent of hepatic fibrosis in patients with recurrent HCV infection after liver transplantation. Serum globulin and certain immunoglobulins level can serve as a non-invasive marker following antiviral treatment.

At present, the diagnosis of liver fibrosis still depends on pathological examination of liver biopsy. Since the procedure is invasive, its application and extensive use in clinical practice are still limited. So great attention has been paid to search for and clinical study of a non-invasive diagnostic parameter for liver fibrosis (Thabut et al,2003), It would not only speed up the study of basic medical theory about liver disease, but also be of value (Botta et al, 2003). Our study indicated that there is significant relation between Ig G level and fibrosis grade in hepatitis B patients.

Authors' Contribution

Rasoul Estakhri and Mohammad Hossein Somi designed and supervised the study, Mehdi Abdolreza Taban Sadeghi studied pathologic findings, Babak Hajipour collected the data and wrote the paper. Faeghe Tajallayi contributed to the analyzing and interpreting the data. All authors read and approved the final revision.

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