Assessment of Liver Fibrosis in HCV Infection in Egyptian Patients

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Abstract: One trial to replace liver biopsy with a simple blood test(s) (whose levels can reflect the severity of liver disease) is the aim of this work. The present investigation was carried out on 72 cases (62 fibrotics and 10 hepatocellular carcinomas; HCCs) who referred to the Early Cancer Detection Unit belonging to the Faculty of Medicine, Zagazig University, Egypt for liver biopsy assessment. Their sera were tested for liver enzymes (alanine aminotrasferase [ALT], aspartate aminotransferase [AST] and AST/ALT ratio), HCV viraemia and type, matrix metalloproteinase-9 (MMP-9) and alpha fetoprotein (AFP). The relationships between the values of these serum tests and the stages of liver fibrosis or the presence of HCC were studied in this work. The results indicated that, the serum ALT level at 60 U/L was indicative of significant fibrosis in 81%. Serum AST level at 130 U/L was indicative of significant fibrosis in 88%. However, the transaminases levels can't differentiate, at any level between cancerous and non-cancerous lesions. The transaminases ratio (AST/ALT) at a cut off value 1.0 reflected significant fibrosis in 93% of patients but can't differentiate between cancerous and non-cancerous lesions. Similarly, the serum level of MMP-9 was diagnostic at a level of 160 mg/dl or less for severe fibrosis in 87% of patients but not for HCC. On the other hand, the level of AFP at 1000 ng/ml or more was diagnostic for cancerous lesions in 90% of patients but cannot differentiate at any level between mild and significant fibrosis. Unfortunately, the HCV level of viraemia and type did not affect the severity of liver disease. The age of patient at the biopsy was found to correlate positively with liver disease. The significant fibrosis was found in 81% of patients aged 45 years or more. While, at a cut off value of 55 years, age at the biopsy was diagnostic for HCCs in 88% of patients with a specificity of 71%. On the other hand, the sex of the patient had no effect on severity of liver disease. In conclusion, there is no single blood test whose value can predict the severity of liver diseases in HCV infection with 100% accuracy, but the use of the above significant serum parameters together with age of the patient can help to exclude the need for liver biopsy in many patients, at least those with contraindications for liver biopsy or those refuting this investigation.
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Key words: HCV infection, Egyptian patients, Liver fibrosis, Histopathology, Serum markers.

1. Introduction:

Hepatitis C virus has been encountered worldwide with WHO estimates of 170 million infected patients **(Booth *et al.*, 2001)**. In the United States and Europe, HCV infection has been detected in 1% to 2% of the general population and fewer than 1% of volunteer blood donors **(Alter, 1997)**. In most developed countries, HCV infection is associated with percutaneous blood exposures, such as blood transfusion and injection drug use **(Yoshiba *et al.*, 1996)**. In contrast, a high prevalence of anti-HCV has been found among apparently healthy Egyptian population, such as military recruits (22%-33%) **(Farghaly & Barakat, 1993)**, expatriate workers in the Gulf Region (31%) **(Mohamed *et al.*, 1996)**, and blood donors (28%) **(Arthur *et al.*, 1997)**. Blood transfusion and illicit drug use, as risk factors are rare in Egypt. However, Schistosomiasis infection and the parenteral therapy of this infection have been proposed as risk factors for both hepatitis B virus (HBV) and HCV infection in Egypt **(Frank *et al.*, 2000 and El-Sadawy *et al.*, 2004)**. It is believed that, up to 85% of infected individuals will develop chronic HCV infection. One of the main problems with chronic HCV is that, it generally leads to hepatic fibrosis, cirrhosis and ultimately hepatocellular carcinoma **(El-Serag, 2002)**.

In patients infected with HCV 20% to 30% will progress to cirrhosis in over two to three decades and the development of histologic cirrhosis is silent in most patients **(Wiley *et al.*, 1998)**. The risk of developing cirrhosis appears to be related to the degree of inflammation and fibrosis present on liver biopsy at any given time, and varies from less than 2% risk per year in those with mild disease to over 10% in patients with severe inflammation **(Yano *et al.*, 1996)**. Hepatocellular carcinoma is a significant complication of HCV infection, although it rarely occurs in the absence of cirrhosis **(El-Serag, 2002)**. The degrees of hepatic fibrosis and cirrhosis are undoubtedly associated with patient prognosis and survival. Hepatic fibrosis may occur at varying rates.

However, male sex, higher alcohol use, older age at the time of infection, duration of infection and higher necroinflammatory score at the initial liver biopsy are probable predictors of increasing fibrosis **(Cacciola *et al.*, 1999)**. It is, therefore, important to evaluate the degree of hepatic histopathological changes when diagnosing and treating HCV-infected patients. The evaluation of these changes is difficult without liver biopsy **(Scheuer *et al.*, 1992)**. Nevertheless, the technique of taking liver biopsy have many contraindications and limitations **(Booth *et al.*, 2001).**

The present study is a trial to identify simple tests that might reflect the severity of hepatic fibrosis and the occurrence of liver cancer.

2. Patients and Methods

In the present study, all available cases (82) with liver disease referred to the Early Cancer Detection Unit belonging to the Faculty of Medicine, Zagazig University, Egypt, between April 2001 to April 2004 for liver biopsy assessment were included. Preserved serum samples of these patients were used for the following manifestations:

Anti-HCV antibody testing was done for all serum samples applying the micro particle enzyme immunoassay (MEIA) using the I_{mx} automated system (Abbott Diagnostics, USA) and following the manufacturer's instructions. Negative cases for anti-HCV and positive cases for HbsAg were excluded from this study. Positive samples for anti-HCV and negative for HBsAg were subjected to reverse transcription polymerase chain reaction (RT-PCR) testing to differentiate samples containing HCV-RNA from those with eradicated HCV viraemia. Negative RT-PCR samples were excluded.

To all HCV-infected patients, as confirmed by RT-PCR, the following tests were done:

I- Paraffin bocks of the RT-PCR positive cases were cut and mounted for histopathological, and histochemical assessment:

1- For histopathological studies, the paraffin sections were routinely stained with haematoxylin and eosin according to **Culling (1974)** and examined microscopically by an experienced pathologist blinded to the biochemical results to evaluate the fibrosis stage of each sample using the METAVIR system **(Metavir, 1994)**.

2- The distribution of collagen and reticulin was demonstrated by staining the paraffin sections with Masson's trichrome stain **(Luna, 1972)** and with Gordon and Sweets reticulin stain **(Gordon and Sweets, 1936)**.

II- The serum samples were used for the following assays:

1- Quantitation of the level of viraemia using the Rel Time Detection polymerase chain reaction (RTD-PCR):

A single-tube RT-PCR was optimized for the quantitation of the 5` NCR (non-coding region) of HCV by using the Taqman technology (Roche Molecular Diagnostics), which exploits the 5`-3` nucleolytic activity of AmpliTaq DNA polymerase first described by **Holland et al** (**1991**). The use of a sequence detector (ABI Prism 5700; Applied Biosystems, Foster City, California) allowed measurement of the amplified product in direct proportion to the increase in fluorescence emission continuously during the PCR amplification. The amplification plot was examined early in the reaction at a point that represents the logarithmic phase of product accumulation. The point representing the detection threshold of the increase in the fluorescent signal associated with the exponential growth of the PCR product for the sequence detector is defined as the cycle threshold (C_T). C_T values are predictive of the quantity of the input target **(Heid *et al.*, 1996)**; that is, when the conditions of the PCR are the same, the larger the starting concentration of a template, the lower the C_T.

The standard curve was created automatically by the ABI Prism 5700 detection system (Foster City, CA, USA) by plotting the C_T against each standard dilution of known concentration.

2-HCV serotyping:

The viral serotypes were determined by using the commercial HCV serotyping 1- **6 assay** (Murex diagnostics corporation, UK), and the tests were performed according to the manufacture's instructions.

3- **Liver function tests**, included alanin aminotransferase (ALT), aspartate aminotransferase (AST) and AST/ALT ratio. These biochemical studies were carried out using an automated system (Dimension, DuPont Medical Products Wilmington, Delaware, USA) according to the manufacturer's instructions.

4- **Matrix metaloproteinase-9 (MMP9):** this assay employs the quantitative sandwich immunoassay technique using the ELISA quantikin Kit (R & D, UK) according to the manufacturer's instructions.

5- **Alpha fetoprotein (AFP):** The LIA-mat AFP is a two-site immunoluminometric assay (sandwich principle) using two highly specific monoclonal antibodies. Antibody-coated polystyrene tubes serve as the solid phase. The tracer antibody and the coated antibody react simultaneously with the AFP present in patient samples or standards. Unbound material is removed by a washing step. The AFP values were measured in ng/ml.

Statistical analysis:

All results were expressed in means ± SD. Differences in means of the studied parameters between stages of fibrosis and/or cancerous and noncancerous lesions were tested using one-way analysis of variance (ANOVA). The influence of gender and viral genotype on stage of fibrosis and/or cancerous and noncancerous lesions was tested using a Pearson's chi-square test. The data were entered, checked and analyzed using the SPSS software program (SPSS Inc, hicago,IL). *P* values less than 0.05 were considered to be significant.

The clinical usefulness of the studied serum markers of fibrosis and/or hepatocellular carcinoma (HCC) were assessed, according to **Galen & Gambino (1977)**, by the determination of: sensitivity, specificity, positive and negative predictive values and ROC (receiver operating characteristic) curve.

III- Tabulation, photographing and interpretation of the results were done.

3. Results

Only 72 cases (out of 82), who met the proposed criteria of the study, were used in this work (62 fibrotic cases and 10 cancerous ones). These cases were the subject of the following studies.

I- Histopathological results:

The distribution of fibrosis stages in the studied cases (62 fibrotics) is shown in table (1). From this table, it is clear that, all cases have at least a fibrosis stage 1 or more, none was found in stage 0.

Stage 1 fibrosis (Plate I, A) comprised 7 (11.3%) cases. The Masson's trichrome and reticulin stained sections from this stage showed minimal amounts of collagen and reticulin fibers distributed within the portal tract and pericellular in the hepatic lobules (Plate I, B and C).

Most cases 28 (45.2%) were found in stage 2 fibrosis. At this stage, in addition to portal tract enlargement, there was septa formation between portal areas (Plate II, A). Examination of the trichrome and reticulin stained sections revealed a relative increase in collagen deposition within the portal tracts and pericellular in the hepatic lobules (Plate II, B), with a slight decrease in reticulin staining (Plate II, C).

Table (1): The distribution and frequencies of histopathological changes seen in hepatic tissue sections of hepatitis C virus infected patients.

Pathological changes observed in liver biopsy	No. of cases	%
1- Fibrosis Stages (METAVIR):	**62/72**	**86.1%**
a- Portal tract expansion (stage 1)	7/62	11.3%
b-Portal tract enlargement with rare septa formation (stage 2).	28/62	45.2%
c-Numerous septa formation (stage 3)	16/62	25.8%
d- Cirrhosis (stage 4)	11/62	17.7%
2- Hepatocellular Carcinoma	**10/72**	**13.9%**

Sixteen (25.8%) cases had stage 3 fibrosis. At this stage, there was fibrosotic bridging between portal areas (Plate III, A) and between portal areas and central veins. Masson's trichrome and reticulin stained sections of this stage revealed increased collagen and reduced reticulin fibers within portal tracts and pericellular in hepatic lobules (Plate III, B and C).

In cirrhotics (Stage 4 fibrosis), all pathological changes of stage 3 were present besides pseudolobule formation (Plate IV, A). Eleven cases (17.7%) were present in this stage. Masson's trichrome stained sections revealed thick collagen fibrous bands (Plate IV, B) connecting the portal tracts together and with central veins. These connections were confirmed by reticulin stain, which revealed bridging necrosis and progressive fibrosis (Plate IV, C).

Hepatocellular carcinoma was observed in only 10 cases out of the 72 studied ones (Plate V, A). This figure revealed that, this hepatocellular carcinoma is composed of liver cords which are much wider than the normal liver plate which is two cells thick. There was no discernable normal lobular architecture, though vascular structures were present. Also, the collagenous fibers were increased (Plate V, B) and reticulin fibers were obviously decreased (Plate V, C).

II- Non-Histological Study:

The non-histological study included the historical and the immunochemical features of the studied cases. These features are listed in table 2.

Historical features:

1- Age at biopsy:

The mean age of the studied population was 46.4 ± 10.7 years. It was 43.9 (± 9.2) (range 26-64, median 45 ± 1.17) in fibrotic cases. As a preliminary test, the ages of this population were classified into two groups; group 1 (< 40 years old) and group 2 (>= 40 years old) and the correspondant mean fibrosis stage was calculated. The mean fibrosis stage in the first group was 1.7 while that in the second group was 3.3 (*P= 0.001*) (table, 2). So, one can expect a positive correlation between age at liver biopsy and stage of fibrosis. Also, the distribution of the studied cases was higher in the second group than in the first one

(41 versus 21), indicating an increase in HCV infection in the latter group.

Plate (I)

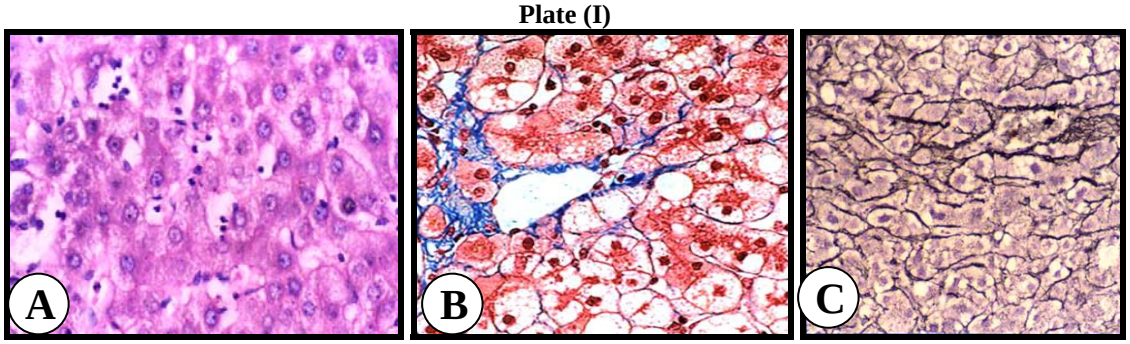

Plate (I): Liver sections from a female patient aged 28 years and infected with HCV showing mild fibrosis: A- Haematoxylin and Eosin stain (x 250), B- Masson's Trichrome stain (x 450) and C- Reticulin stain (x 250).

Plate (II)

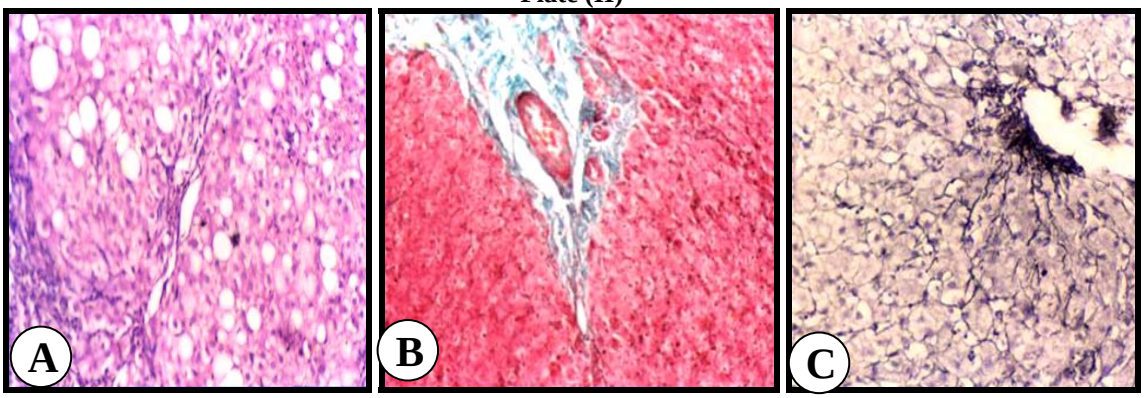

Plate (II): Liver sections from HCV-infected male patient aged 35 years showing short fibrous septa formation (METAVIR stage 2): A- Haematoxylin and Eosin stain (x 250), B- Masson's Trichrome stain (x 250) and C- Reticulin stain (x 250).

Plate (III)

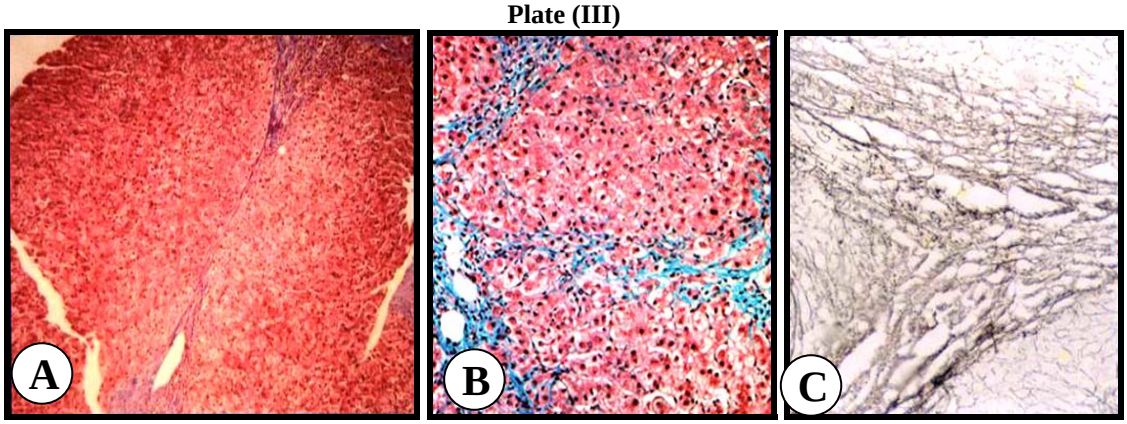

Plate (III): Liver sections showing bridging fibrosis (METAVIR stage 3) from HCV-infected male patient aged 46 years: A- Haematoxylin and Eosin stain (x 250), B- Masson's Trichrome stain (x 250) and C- Reticulin stain (x 250).

Plate (IV)

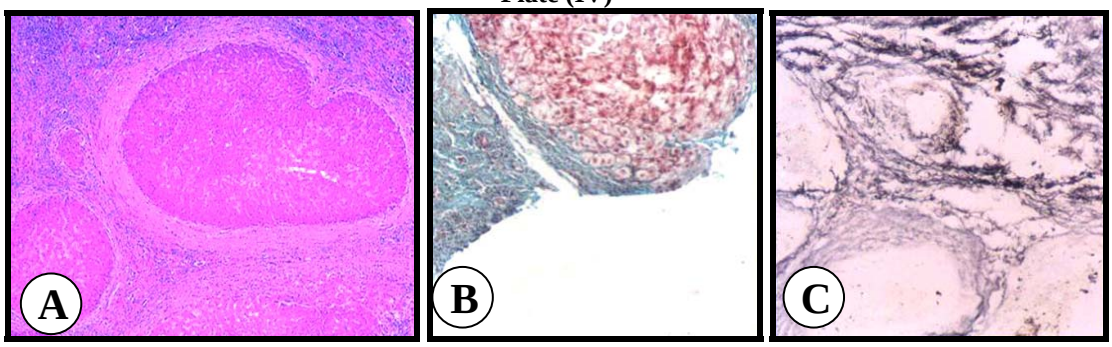

Plate (IV): Liver sections from a male patient aged 52 years and infected with HCV revealing cirrhotic changes (METAVIR stage 4): A- Haematoxylin and Eosin stain (x 150), B- Masson's Trichrome stain (x 150) and C- Reticulin stain (x 150).

Plate (V)

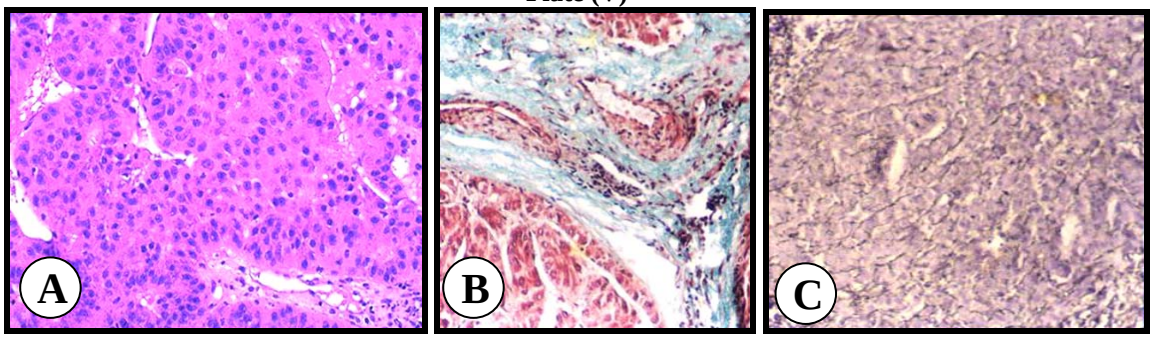

Plate (V): Liver sections from a 63 years old male patient infected with HCV and showing hepatocellular carcinoma: A- Haematoxylin and Eosin stain (x 250), B- Masson's Trichrome stain (x 250) and C- Reticulin stain (x 250).

Table (2): Historical and immunochemical characteristics of fibrotic cases in HCV infected patients.

Character	Number	%	Mean Fibrosis stage (METAVIR)	*P* value
Age (years):				
< 40	21	33.9	1.7	*P* = 0.001
≥ 40	41	66.1	3.3	
Gender:				
Male	52	83.9	2.5	*P* = 0.84
Female	10	16.1	2.3	
ALT (U/L):				
< 40	11	17.7	1.7	*P* = 0.049
≥ 40	51	82.3	2.7	
AST (U/L):				
< 40	6	9.7	1.4	*P* = 0.053
≥ 40	56	90.3	2.7	
AST/ALT:				
< 1.0	35	56.5	1.8	P = 0.001
≥ 1.0	27	43.5	3.3	
Viraemia (copy/ml)				
< 8800	31	50	2.4	*P* = 0.967
≥ 8800	31	50	2.5	
Type:				
1	5	8.1	3.4	*P* = 0.026
4	53	85.5	2.5	
6	4	6.4	2.0	
MMP-9 (mg/dl):				
≤ 160	27	43.6	3.3	*P* < 0.009
> 160	35	56.4	1.8	
AFP (ng/ml):				
< 400	49	79	2.3	*P* = 0.155
≥ 400	13	21	3.4	

Figure (1) is a diagrammatic representaion of fibrosis stages and their correspondant mean ages at biopsy and shows a parallel relationship between age at biopsy and stage of fibrosis. Using a cutoff value of 45 years, age at biopsy was found to have 81% sensitivity, 63 % specificity, 83% positive predictive value and 58% negative predictive value when differentiating between severe and non-severe fibrosis (Table 3). Age at liver biopsy was found to have a fair discriminating power in differentiating severe and non-severe fibrosis (area under the ROC curve= 0.721) (Chart, 1).

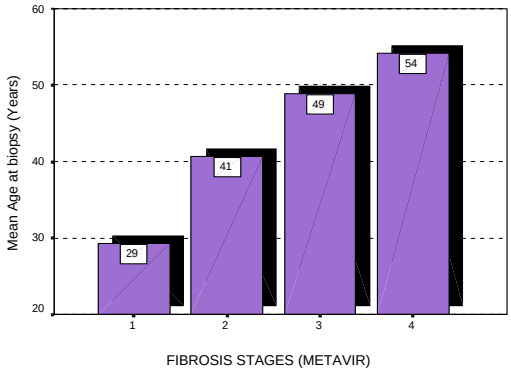

Figure (1): Diagrammatic representation showing the relationship between age at biopsy and stages of liver fibrosis in HCV infected patients.

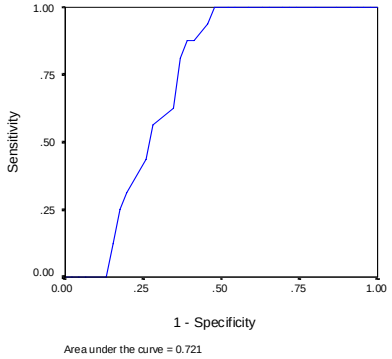

Chart (1): The ROC curve of age at biopsy when used to diagnose significant fibrosis showing a fair discriminating power (Area under the curve = 721).

In HCC cases, the mean age at biopsy was 62 ± 4.2 (median 63 ± 3.1, range 56- 67). When using a cutoff value of 55 years for age to diagnose hepatocellular carcinoma, age at biopsy was found to have 88% sensitivity, 71% specificity, 77% positive predictive value and 68% negative predictive value (Table, 4). The ROC curve of age at biopsy in HCC cases revealed a good discreminating power (area under the curve = 0.78) for differentiating cancerous from non-cancerous lesions (Chart, 2).

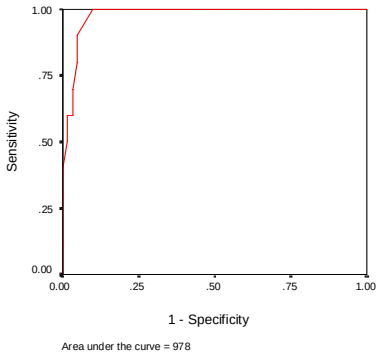

Chart (2): The ROC curve of age at biopsy when used to diagnose hepatocellular carcinoma showing an improvement in its discriminating power (area under the curve = 0.78).

2- Gender:

The population of this study has a high frequency of male sex over females (52 males versus 10 females in fibrotic cases and 9 versus 1 in HCCs) (Table 2). In other words, males have a higher prevalence of HCV infection than females. The mean fibrosis stage of the two sex groups did not differ significantly (2.5 in males versus 2.3 in females, *P*= 0.84, Fig., 2). Therefore, the sex of the patient has no effect on fibrosis progression.

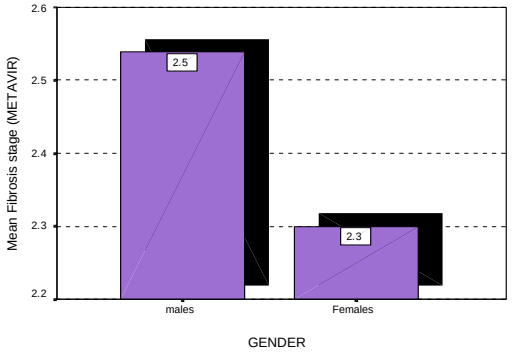

Figure (2): Diagrammatic representation showing no significant difference between males and females in the mean fibrosis stage in HCV-infected patients.

Immunochemical features:

Serum alanine aminotransferase (ALT):

The mean values of serum ALT levels in the studied cases was 82.2 ± 53.3 U/L. In fibrotics it was 70.3 ± 32.4 U/L (range 15-144, median 66 ± 4.1). When the latter cases were divided into two groups according to the serum level of ALT; group (1) with normal levels and group (2) with elevated levels

(Tabe, 2) and the mean fibrosis stage of each group was calculated, a statistical difference in mean fibrosis stages (1.7 versus 2.7) was found between both groups ($P < 0.049$). Figure (3) shows the relationship between mean values of serum ALT levels and the stages of liver fibrosis. In severe fibrotics the mean ALT value was 81.3 ± 14.3, while in mild fibrosis it was 27.4 ± 10.0 U/L. Using a cutoff value of 60 U/L, serum ALT was found to differentiate between severe and non-severe fibrosis by 81% sensitivity, 57% specificity and 80% positive predective value (Table, 3). When it was used for diagnosing severe fibrosis, the level of ALT was found to have a fair discreminating power as the area under the ROC curve = 0.695 (Chart, 3).

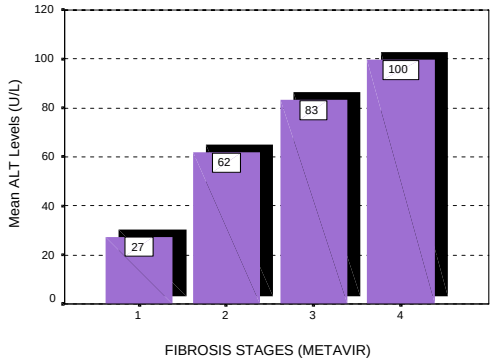

Figure (3): Diagrammatic representation showing an increament in the mean ALT serum levels with progression of liver fibrosis in HCV infection.

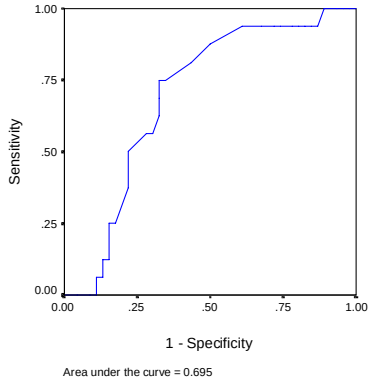

Chart (3): Receiver operating characteristic curve of serum ALT levels for diagnosing significant fibrosis showing a fair diagnostic power (area under the curve = 0.695).

In HCC cases, the mean serum ALT level was 115.7 ± 91.5 U/L (median 112 ± 28.9, range 66 -336), while it was 70.3 ± 32.4 U/L (range 15-144, median 66 ± 4.1) in non-cancerous cases. Nontheless, the serum ALT level was shown not to have any diagnostic ability when differentiating cancerous from non-cancerous lesions (area under the ROC curve = 0.310, Chart, 4).

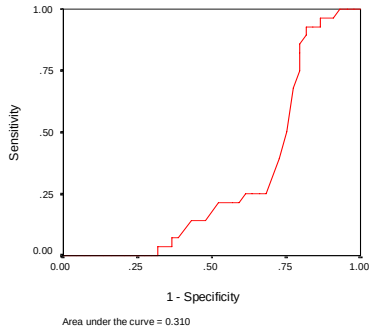

Chart (4): Receiver operating characteristic curve of serum ALT level when used to diagnose hepatocellular carcinoma in HCV-infected patients revealing a bad discriminating ability (area under the curve = 0.310).

Aspartate aminotransferase (AST):

The mean AST level in the studied cases was 149.2 ± 107.7 U/L. It was 125.6 ±74.5 U/L (range 20-322, median 101.5 ± 9.5) in fibrotic cases. When the latter cases were divided into two groups (AST normal group and AST elevated group), the mean fibrosis score of the second group was found to be significantly higher than that of the normal group (2.7 versus 1.4, *P= 0.053*, Tablee, 2). The activities of AST and their relationships with the histologic characters are shown in Fig. (4).

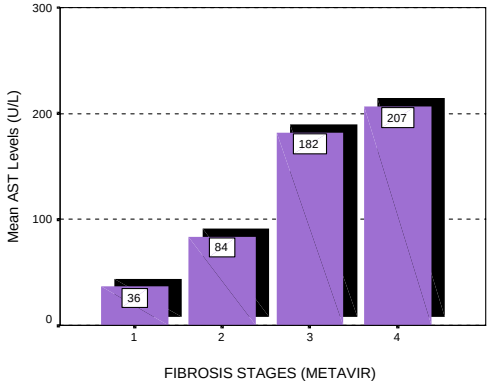

Fig. (4): The relationship between serum AST levels and stages of liver fibrosis in HCV-infection was shown to be parallel.

A cutoff value of 130 U/L for AST concentration was found to have 88% sensitivity, 72% specificity, 84% positive predective value and 71% negative predective value when it was used to differentiate between severe and non-severe fibrosis. The area under the ROC curve when diagnosing severe fibrosis was 0.815 indicating a good discreminating power (Chart, 5).

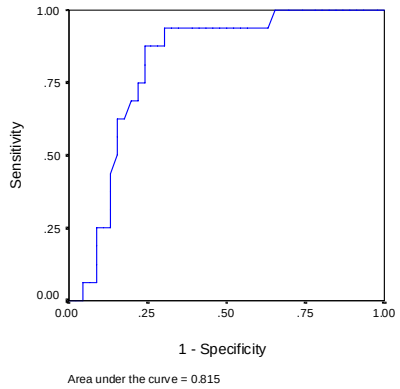

Chart (5):ROC Curve of AST when it was used to diagnose significant fibrosis revealing a good discriminating power (AUC = 0.815).

On the other hand, the mean value of serum AST level in the 10 cancerous cases was 295.7 ± 161.9 U/L (median 232 ± 51.2, range 142 - 621), while in non-cancerous cases it was 125.6 ±74.5 U/L (range 20-322, median 101.5 ± 9.5), indicating a positive relationship. However, the serum level of AST appeared not to have any diagnostic power when diagnosing hepatocellular carcinoma, because the area under the ROC curve was 0.194 (Chart, 6).

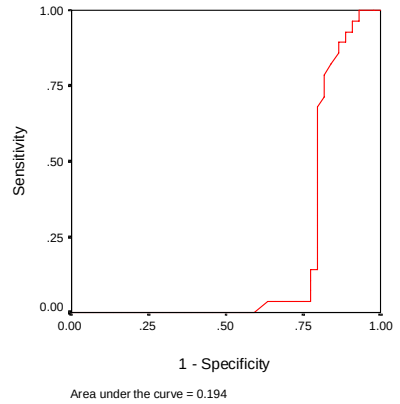

Chart (6):ROC curve of AST level to differentiate between cancerous and non-cancerous lesions in HCV infection revealing a bad diagnostic power (AUC = 0.194).

AST/ALT ratio:

The mean AST/ALT ratio in the studied cases was 1.08 ± 0.35 and it was 1.06 ± 0.37 (range 0.33-2.2, median 0.89 ± 0.05) in fibrotic cases. The fibrotic cases were divided into two groups according to the value of AST/ALT ratio (group 1 < 1, group 2 ≥ 1). The mean fibrosis scores of the two groups were significantly differerent (1.8 versus 3.3, p<0.01).

The mean AST/ALT ratio in severe fibrotics (n: 55) was higher than that in non-severe fibrotics (n: 7) (1.2 ± 0.06 versus 0.7 ± 0.09; P< 0.001). A ratio ≥ 1 had 77% specificity and 89 % positive predictive value in distinguishing severe from non-severe fibrotics, with a 93% sensitivity and 80.7% negative predictive value. The ratio correlated positively with the stage of fibrosis (Fig., 5).

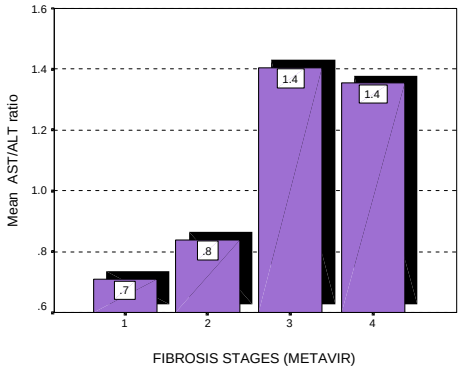

Figure (5): Diagrammatic representation of mean transaminases ratio in the different hepatic fibrosis stages due to HCV infection.

The ROC curve of the transaminases ratio showed a good diagnostic power (AUC = 0.872) when it was used to differentiate between fibrosis stage 1 and higher stages (Chart, 7).

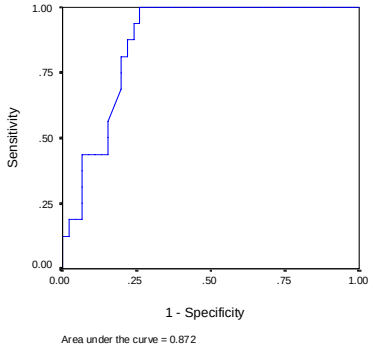

Chart (7): ROC curve of transaminases ratio revealing a good diagnostic power (area under the curve = 0.872) when differentiating severe from non-severe fibrosis.

In HCC cases, the mean AST/ALT ratio was 1.17 ± 0.102 (range 1.02 – 1.32, median 1.2 ± 0.03). While in noncancerous cases it was 1.06 ± 0.37 (range 0.33 – 2.17). Therefore, there was no statistical difference (P = 0.78) between cancerous and non-cancerous cases in terms of AST/ALT ratio. The ROC curve of AST/ALT (Chart 8) revealed a bad diagnostic power for differentiating between cancerous and non-cancerous lesions (AUC: 0.293).

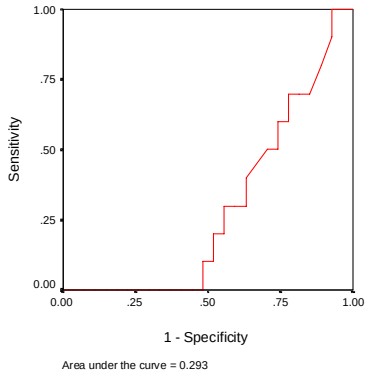

Chart (8): ROC curve of AST/ALT ratio showing a bad discriminating power (area under the curve = 0.293) when diagnosing HCC.

Serum Matrix Metaloproteinase 9 (MMP9):

The mean level of serum MMP9 in the studied population was 217.1 ± 121.7 mg/dl (range 77-654, median 193.5 ± 15.5). When the studied cases were divided into two groups (group 1 with MMP-9 ≥ 169 and group 2 with reduced levels) and the correspondent mean values of fibrosis stages were calculated. The mean fibrosis stage of the first group (≥ 169) was found to be 1.88, while that of the group with reduced levels was 3.25 (*P< 0.009*). The mean serum levels of MMP9 measured in the different histological changes are shown in figure (6). From this figure the mean values of serum MMP9 concentrations decrease, with the severity of the liver fibrosis. In other words, there is a negative correlation between the stage of liver fibrosis and the level of serum MMP9 (*P< 0.0001*).

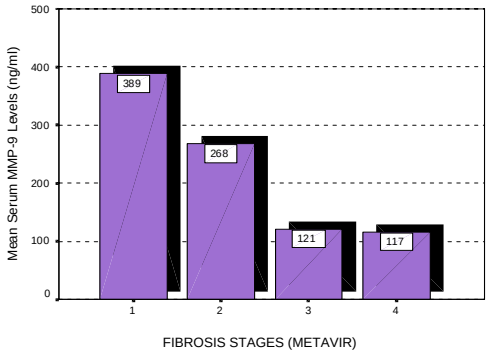

Fig. (6): An inverse relationship between stages of liver fibrosis and the level of serum MMP-9 in HCV infected patients.

Thirty five patients (56.4%) had normal levels of serum MMP9 while 27 patients (43.6%) had reduced levels (Table 2). Moreover, the number of patients having reduced levels increases with fibrosis stage (1 versus 6 in stage 1; 2 versus 26 in stage 2; 14 versus 2 in stage 3 and 11 versus 0 in stage 4).

The test has, at a cutoff value of 160 mg/dl, a 87 % sensitivity and 72% specificity in differentiating severe fibrotics.

The discreminating power of this serum test in diagnosing severe from non-severe fibrosis was good (area under the ROC curve = 0.846, Chart, 9).

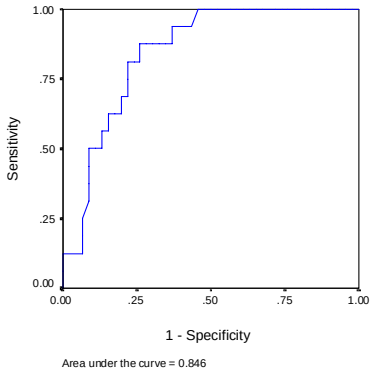

Chart (9):A good discriminating power of serum MMP-9 level when diagnosing severe fibrosis showed by the large area (0.846) under the ROC curve.

The mean level of MMP9 in cancerous cases was 91.2 ± 46.5 mg/dl while in non-cancerous cases it was 119.4 ± 32.7 mg/dl indicating no statistically significant difference (*P* = 0.82) between both groups. Hence, the serum MMP-9 is useful in diagnosing severe fibrosis but not HCC. Moreovere, the area under the ROC curve is 0.196 indicating a bad discriminating power (Chart, 10).

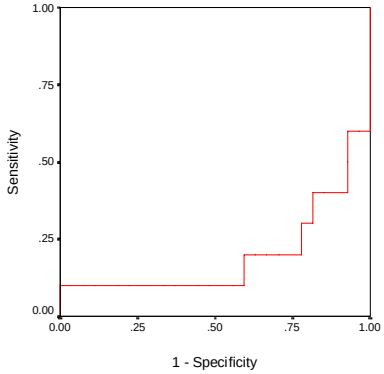

Chart (10): A bad diagnostic power (AUC = 0.196) of serum MMP-9 level when differentiating cancerous from non-cancerous lesions seen in liver sections of HCV infected patients.

HCV-Viraemia:

Qualitatively, HCV infection has been proved by using RT-PCR technique. According to this technique, 72 cases out of 78 were positive (92.3%), indicating that, HCV infection is a major etiological factor for liver disease in this population.

The level of viraemia in the sera of the 72 RT-PCR positive cases was measured by the real-time PCR using ABI prism 5700. Figure (7) shows the amplification curve of some samples. The amount of circulating HCV-RNA in the sera of the studied cases ranged from 140 to 92000000 copies/ml (mean 1836235 ± 10893436.2, median 8750 ± 1283804). In fibrotic cases, the mean level of viraemia is 231657 ± 1172639.4 (median 8800 ± 1489412, range 140 – 92000000).

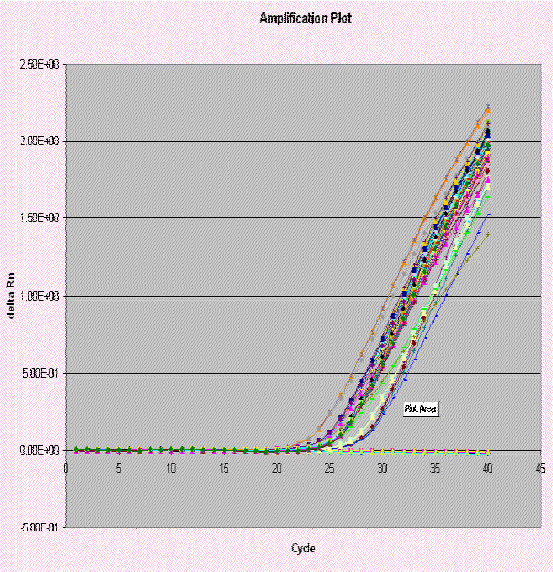

Fig. (7): The amplification curves of some samples using the real time-PCR technique for HCV-RNA quantification.

When the studied cases were divided into two groups according to the level of viraemia (< 8800 and ≥ 8800), the mean fibrosis stage of the first group did not differ significantly from that of the second group (2.25 versus 2.28, *P= 0.967*). Moreover, figure (8) represents the mean values of HCV viraemia calculated in the four fibrosis stages and shows no correlation between stage of fibrosis and level of HCV viraemia. In addition, the area under the ROC curve revealed a bad diagnostic power (Chart, 11, area under the curve = 0.382). Therefore, these data demonstrate that, the amount of HCV-RNA did not correlate with liver histology.

In HCC cases the mean level of viraemia was 191560 ± 231633 while in non-cancerous cases the mean level of viraemia was 231657 ± 1172639.4 (median 8800 ± 1489412, range 140 – 92000000), indicating no significant difference (*P* = 0.334) although the mean level of viraemia is reduced in HCC cases. Also, the ROC curve revealed a bad diagnostic power (Chart 12).

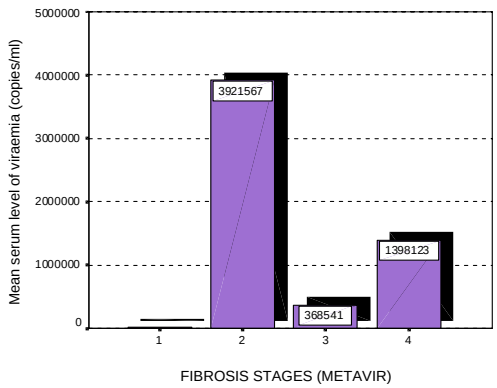

Fig. (8): A fluctuating level of HCV viraemia in the different fibrosis stages indicating no relationship.

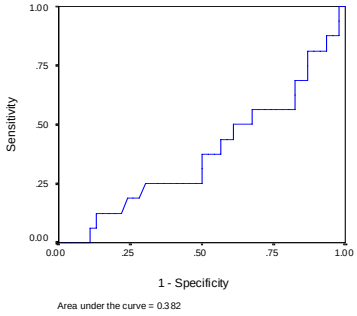

Chart (11): The receiver operating charactaristic curve of serum level of viraemia in fibrotic cases showing a bad discriminating power when differentiating between severe and non-severe fibrosis (AUC = 0.382).

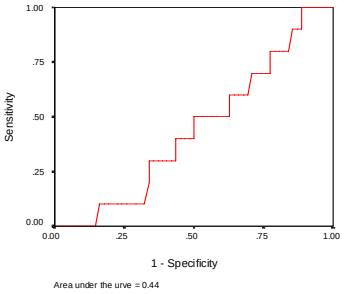

Chart (12):A bad diagnostic power of serum level of HCV viraemia in discriminating between severe and non-severe fibrotics and between cancerous and noncancerous lesions in HCV infected patients.

HCV serotype:

The frequency of the presence of HCV serotypes in the present population was as follows: In fibrotics, the HCV serotypes were represented by only types 1 (5 cases), 4 (53 cases) and 6 (4 cases).

The mean fibrosis stage in patients with HCV type 1 was 2.0, type 4 was 1.9 and type 6 was 1.8; Fig. 9). Thus, the fibrosis stage did not differ significantly by the type of HCV. Therefore, the type of HCV appears to have no effect on fibrosis staging. In HCC cases only types 4 (8/10) and type 1(2/10) were present, while type 6 was absent from HCC cases.

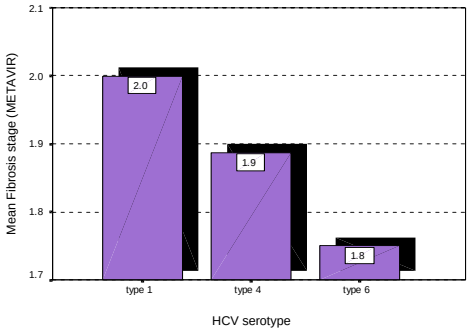

Fig. (9): A diagrammatic representation of mean fibrosis stages in the HCV –infected patients with the different serotypes showing no significant difference**.**

Serum AFP:

The mean AFP level in the studied population (72 cases) was 226.7 ± 416.5 ng/ml. In fibrotic cases, the mean serum AFP level was 83.5 ± 186.9 ng/ml (range 3-888, median 10.5 ± 23.7). When the levels of serum AFP were divided into two groups; normal and elevated, and the mean fibrosis scores of the two groups were calculated, a slight statistical difference was found in the fibrosis score between the two groups ($P< 0.05$). The distribution of the level of AFP in the different fibrosis stages was shown in figure (10). The mean AFP levels in mild fibrosis was 7.7 ± 2.9, while that in significant fibrosis was 93.2 ± 196.5 ng/ml. Therefore, there is a significant difference in the mean level of serum AFP between mild and significant fibrosis ($P = 0.023$). However, when the diagnostic value of serum AFP level in differentiationg mild from significant fibrosis was studied, serum AFP was found to have a bad discreminating power (area under the ROC curve= 0.349, Chart, 13).

In HCC cases, the mean serum level of AFP was 1004.5 ± 207.1 ng/ml (median 1081.5 ± 65.5, range 888 - 1750), while that in fibrotics was 83.5 ± 186.9 ng/ml (range 3-888, median 10.5 ± 23.7), indicating a positive relationship. At a cut off value 1000 ng/ml serum AFP level was found to have 90% sensitivity, 77% specificity, 93% positive predictive value and 71% negative predictive value when differentiating cancerous from non-cancerous hepatic lesions. Moreover, the area under the ROC curve (Chart 14) showed an excellent diagnostic power of serum AFP level for HCC (AUC = 949).

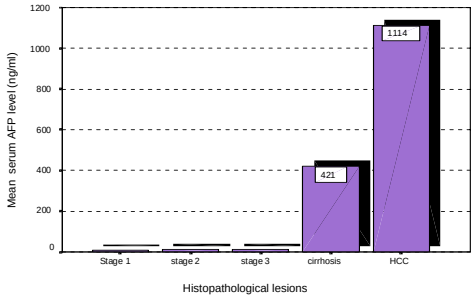

Fig. (10): Mean levels of serum AFP in HCV infected patients showing no significant correlation with the severity of liver fibrosis in spite of the increase in AFP concentration in cirrhotics. On the other hand there was a significant difference between cancerous and non-cancerous lesions in the AFP level.

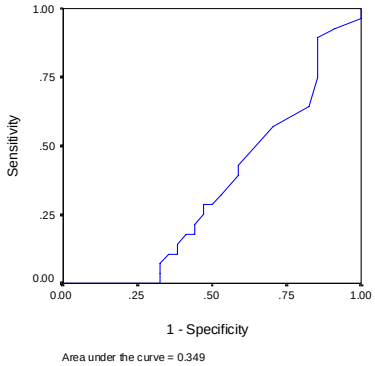

Chart (13): The ROC curve of serum AFP level when it was used to diagnose significant fibrosis showing a bad diagnostic power (AUC = 0.349).

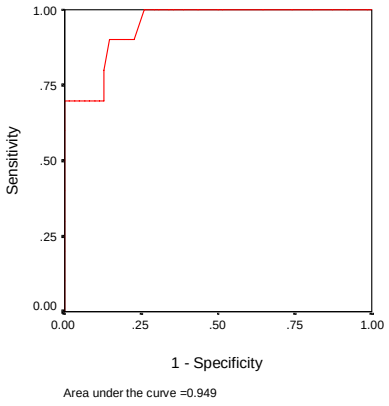

Chart (14): An excellent diagnostic power of the ROC curve of serum AFP level in diagnosing HCC in HCV infection (AUC = 0.949).

Table (3): Operating characteristics of indices of clinically significant fibrosis.

Indices	Cut-off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Area under ROC curve
Age at biopsy	45	81	63	83	58	0.721
ALT	60	81	57	80	56	0.695
AST	130	88	72	84	71	0.815
AST/ALT	1.0	93	77	89	80.7	0.872
MMP-9	160	87	72	85.7	81.2	0.846

Table (4): Operating characteristics of indices of hepatocellular carcinoma.

indices	Cut-off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Area under ROC curve
Age at biopsy	55	88	71	77	68	0.978
AFP	1000	90	57	93	71	0.947

4. Discussion

In the present investigation, the histopathological results indicated that, most patients were in stage 2 (28, 45.2%), while stage 1 was represented by few cases (7, 11.3%). In other words, most of the included patients (55, 88.7%) had significant fibrosis (i.e., METAVIR stages more than 1). These results indicated, therefore that, HCV infection has a rapid course of disease progression in the studied population. Similar results were reported in a similar Egyptian population by **Mangoud *et al.* (2004a)**. These authors attributed the progressive nature of the disease to the concomitant infection with other viruses like HBV. However, in this study patients with HBV concomitant infection were excluded. Hence, METAVIR stage 1 fibrosis was absent from the study of **Mangoud *et al.* (2004b)** (and METAVIR stage 2 replaced stage 1 in this study instead) due to the rapid course of disease progression caused by other concomitant infections.

In the present study, 10 cases (out of 82, 13.9%) were diagnosed as hepatocellular carcinoma. Several studies have shown that, HCV is positively related to HCC **(Kalamani *et al.*, 1991 and Simonetti *et al.*, 1992)**. Therefore, HCV infected individuals are regarded as a high-risk group for HCC. Several lines of evidence indicate a strong causal association between HCV and HCC. HCV-RNA can be found in the serum, liver, and tumor tissues of patients with HCC, but unlike HBV it does not integrate into host genome. Moreover, the age-standardized death rates owing to HCC are significantly correlated with the seroprevalence of HCV in the general population **(Deuffic & Poynard, 1999)**. Hepatitis C virus infection increases the risk for HCC probably by promoting fibrosis and cirrhosis; virtually all HCV-related HCC cases occur among patients with cirrhosis. With the exception of areas in the world where hepatitis B is endemic, it is uncommon to find HCC in the absence of cirrhosis **(Fattovich, 1998)**.

The choice of METAVIR fibrosis score for this analysis deserves comment, because there is no perfect fibrosis scoring system. METAVIR scores, which had been shown to be very reproducible for fibrosis evaluation (at least by the pathologists who created it; The METAVIR cooperative group**)** as well as more linear (i.e., no missing numbers) than the Knodell fibrosis score. Subsequently, more sensitive scores with greater numbers of categories have been published, but none as yet has become the standard for these types of studies. For ongoing and future studies, other scoring systems will be used and compared with previous methods, Knodell and METAVIR scores, to determine whether any of these is clearly superior. One of the most recent and more population specific score was that created by **Mangoud *et al.* (2004b)** through an Egyptian population screening study. However, the new concept stated by these authors needs further validations.

A percutaneous liver biopsy can be useful in patients with chronic hepatitis C virus (HCV) infection by providing information regarding the stage of fibrosis and grade of inflammation **(Garcia & Keeffe, 2001)**. However, patients with chronic hepatitis C are not always eager to have a liver biopsy. They frequently have anticipatory anxiety, which would be expected of a procedure that is associated with pain in 35% of patients, severe complications in 0.3% and death in 0.03% **(Piccinino *et al.*, 1986 and Cadranel *et al.*, 2000)**. Liver biopsy also adds significant direct costs (equipment, observation time, and time of a skilled clinician and pathologist) and indirect costs (time away from work and home) to the management of patients with chronic hepatitis C. Finally, a cost-effectiveness analysis suggested that, the best strategy in the management of chronic HCV infection is to offer therapy to all patients and not perform liver biopsies **(Wong *et al.*, 1998)**.

The use of biopsy to confirm HCC remains controversial for the following reasons; it can be difficult to distinguish large cirrhotic nodules from well-differentiated HCC or low-grade dysplastic nodules from HCC in either needle or wedge biopsies; liver biopsy carries a small risk of tumor spread along

the needle track; finally, fine-needle aspirates provide cells without some of the architectural abnormalities that are important in making a diagnosis **(El-Serag, 2002)**.

Other researchers have sought other markers that could signal liver damage without the need for biopsy. Therefore, liver biopsy would be less important where other clinical or laboratory tests available that could reliably predict the grade of inflammatory injury or stage of fibrosis. However, fibrosis is most important when considering the natural history of hepatitis C because fibrosis, not inflammation *per se*, leads to the sequelae of liver disease **(poynard *et al.*, 1997)**. Therefore, this study is a trial to find a non-histological marker(s) of fibrosis in the hepatitis C infected patients. The non-histological study included the historical and the immunochemical features of the studied cases. An alternative approach for the noninvasive prediction of fibrosis is the use of historical features, including advanced age at infection, male sex and alcohol consumption, which are all known to accelerate fibrosis progression **(Poynard *et al.*, 2001 and Myers *et al.*, 2001)**. This is a frequently used approach in the clinical setting. This approach has the advantage that, it is without cost and is relatively simple **(Alter, 1997)**, but it has yet to be validated. Furthermore, it cannot be used in patients who have received antiviral treatment because of the anti-fibrotic effects of current therapies **(Poynard *et al.*, 2000)**.

Considering the relationship between the age of the patient and the prevalence of HCV infection, anti-HCV positivity was more prevalent in older ages than in younger ones [51(70.8%) cases in patients with 40 years old or more versus 21 (29.2%) cases with younger ages]. The increase of anti-HCV prevalence with age has been reported in other studies in Egypt. **Darwish *et al.* (1992)** studying 90 volunteer Egyptian blood donors, reported that, anti-HCV among those 20 to 30 years old was 6% as compared with 37.5% among those older than 30 years, which is substantially lower than the estimates published in the study of **Abdel-Aziz *et al.* (2000)**. A study of 270 residents of another Egyptian village in the Nile Delta had a similar increase in anti-HCV prevalence after the age of 25 years from 12.7% to 36.7% **(Abdel-Wahab *et al.*, 1994)**. The rise in anti-HCV positivity with age in these studies could either be the result of continuous exposure, a cohort effect, or a combination of both. In Egypt, there is evidence for both continuous and cohort effects, reflecting both historical and continuing patterns of infection, with potentially different risk factors **(Frank *et al.*, 2000)**.

In the present study, liver fibrosis from portal tract enlargement (stage 1) to cirrhosis (stage 4) was almost linear according to age at biopsy (means; 29 in F1, 41 in F2, 49 in F3 and 54 in F4). Similar results were reported in a similar Egyptian population by **El-Shorbagy *et al.* (2004)**. In addition, **Poynard *et al.* (1997)** found that, the rate of fibrosis progression was low in individuals younger than 20 years, intermediate in those aged 21-40 years, increased in those aged 41-50 years, and highest in those older than 50 years. Although these investigators did not know why age is a risk factor, it may be that the host defense mechanisms against HCV is weaker in older people. The analysis of the impact of the historical features revealed that, older age at biopsy was an independent predictor of more advanced fibrosis. In discordance with other reports, older age at biopsy was not independently associated with significant fibrosis **(Poynard *et al.*, 2001)**. The reasons for this discrepancy remain unclear but may relate to the small size of the current study [62 versus > 2200 in the studies of **Poynard *et al.* (2001)**].

In the present study, patients with HCC were older (62 ± 4.2 years) than patients without HCC (43.9 ± 9.2 years). These results are in agreement with those reported in another study from Taiwan by **Peng *et al.* (1999)**. HCC is rarely seen during the first 4 decades of life except in populations in which HBV infection is hyperendemic. The incidence of HCC increases progressively with older age, reaching a peak between the ages of 70 and 75 years **(El-Serag, 2001)**. However, the increase in HCC cannot be explained solely by the effect of aging in the general population **(El-Serag, 2002)**. In the present study, a direct relationship exists between the HCC risk and the age at liver biopsy. In line with this observation is the study of **Tradati *et al.* (1998)**. Why patients of older ages are at higher risk of HCC is unknown. However, an increased exposure to environmental factors is responsible for cirrhosis or liver cancer or an increased vulnerability of the older liver to genotoxic agents are possible explanations.

The present study revealed that, the HCV infection was more prevalent in males than in females (61 versus 11). Similar observations were reported by other investigators (**El-Khoby *et al.*, 2000 and Frank *et al.*, 2000)**. The association between male sex and HCV positivity reflected the increased risk of exposure to the infection in males than in females. For example, males, who were frequently have schistosomiasis than females **(El-Khoby *et al.*, 2000)** and those more than 30 years of age who had risk of exposure to parenteral antischistosomal therapy (PAT) **(Frank *et al.*, 2000)**. The gender difference in anti-HCV prevalence being present in adults more than 30 years of age **(Frank *et al.*, 2000)** is further support for the present study. However, a history of therapy for schistosomiasis increased the risk of HCV infection by three times **(Frank *et al.*, 2000)**. The latter authors were unable to

explain the association between male sex and HCV prevalence. But, they suggested that, it may be due to the increased risk factors of HCV infection in males than in females like shaving at barber-shop, smoking goza in groups and PAT.

In the present investigation, although male sex was associated with HCV infection, yet it does not affect fibrosis. The difference in fibrosis stages between males and females was not significant (2.5 in males versus 2.3 in females, P= 0.84).

According to **Kew (2000),** serum aminotransferase concentrations are an indicator not of hepatocellular dysfunction but of hepatocellular damage. In the present study, the mean levels of AST were increased by the stage of liver fibrosis (36 U/L in F1, 84 U/L in F2, 182 U/L in F3 and 207 U/L in F4). A further increase in the mean level of serum AST concentration (125.6 U/L) was seen in the HCC cases. Although, ALT is considered to be more specific for liver disease than AST (because AST is found in more types of cells e.g., heart, intestine and muscle)**,** a notable increase in AST was shown in patients with viral hepatitis by **Gordon *et al.* (2000)** who found that, AST had a stronger correlation with liver histology, in particular hepatic fibrosis. More recently, **El-Shorbagy *et al.* (2004)** reported a parallel relationship between serum level of AST activity and the degree of liver disease in an Egyptian population infected with HCV.

Kaplan (1993) reported the commonly held view that, AST concentrations exceeding 300-400 U/L usually indicate acute hepatocellular disease such as hepatitis or drug toxicity, and points out that, similar values may sometimes occur with extrahepatic obstruction but are uncommon. He also declared that, the greatest elevations (> 1000 U/L) are seen in the early phase of viral hepatitis, in toxic injury, and with circulatory collapse, but the last of these is not considered a common cause. In an important British study **(Ellis *et al.,* 1978)** on serum enzyme concentrations in diseases of the liver and biliary tree involving over 1100 patients, circulatory collapse did not feature at all. **McIntyre & Rosalkis (1991)** stated that, AST concentrations greater than 20 times normal are strongly suggestive of viral or drug hepatitis but it was noted that, similar concentrations may sometimes be seen in shock and heart failure, usually with a very abrupt rise. The authors also remark on the occasional striking elevation of AST in extrahepatic biliary obstruction which may mimic hepatitis.

In the present study, the serum AST level at a cutoff value 130 U/L was found to have an 88% sensitivity and 72% specificity when it is used to differentiate between mild and significant fibrosis. While in HCC cases the AST concentration was found not to have any diagnostic value, in spite of its increase in HCCs when compared with non-cancerous cases. Raised AST concentrations, generally below 400 U/L, are also seen in intrahepatic and extrahepatic cholestasis, in cirrhosis, and in primary and secondary liver tumors **(Ellis *et al.,* 1978 and Reichling & Kaplan, 1988)**.

In the present investigation, the mean serum ALT level was increased by the progression of liver fibrosis. In agreement with this finding, the study reported by **El-Shorbagy *et al.* (2004)**. A number of studies have shown that, ALT does not necessarily predict liver disease. A patient can have moderate or advanced disease and have a normal ALT (**Mathurin *et al.,* 1998; Marcellin, 1999; Ghany *et al.,* 2000** and **Marcellin *et al.,* 2001**). According to **Mathiesen *et al.* (1999)** there is little information on the spectrum of pathological liver changes which can be found in patients with hypertransaminasaemia of unknown etiology. This is mainly because most histological studies in large series of patients were performed before the discovery of hepatitis C **(Berasain *et al.,* 2000).**

In the present study, 11 cases have normal ALT values in spite of having some degree of fibrotic changes. However, the mean fibrosis stage in ALT normal group was significantly lower than that in ALT elevated one (1.7 versus 2.7, P = 0.049). Controversies exist concerning histological findings in patients with normal ALT levels. Some authors have shown that, in this group of patients, liver histology is virtually normal **(Brillanti *et al.,* 1993)**. In contrast, other authors have shown that HCV, viraemia in persons with normal ALT is consistently associated with liver damage **(Alberti *et al.,* 1992 and Stanley *et al.,* 1996)**. These studies do not focus on fibrosis, which is clinically more important than the activity grade **(Poynard *et al.,* 2001)**. It can't be explained why patients remain asymptomatic (with normal ALT) despite having considerable amounts of HCV-RNA in the serum. However, **Okanoue *et al.,* (1996)** speculated that, asymptomatic patients may have had chronic hepatitis prior to entry and/or had developed transient elevation of serum transaminases in the past.

Mathurin *et al.* (1998) studied the rate of fibrosis progression in patients with persistently normal and elevated ALT values. These investigators found that, lower ALT values correlated with lower histologic activity scores, and that these differences were not related to viral load or genotypes. Moreover, the rate of fibrosis progression remained lower among these patients. Another study found that, HCV patients with persistently normal ALT levels not only had lower inflammatory and fibrosis scores than patients with abnormal ALT values, but that levels of viremia were also lower in this subset **(Jamal *et al.,* 1999)**. In

addition, in a study from Italy, none of 37 patients had a worsening of hepatic fibrosis on follow-up liver biopsy 5 years later **(Persico *et al.*, 2000)**. In a study from France, there was no significant changes in the Ishak fibrosis scores among 24 patients who had a second liver biopsy 3 to 5 years later **(Martinot-Peignoux *et al.*, 2001)**. On the other hand, in both of these studies, a proportion of patients (approximately 5% per year) developed abnormal ALT levels during follow-up. Thus, patients may have a change in disease activity over time and the lack of fibrosis progression during one period may not predict future lack of progression of disease.

Although the elevation of serum ALT and AST concentrations above the range of normal values is the most frequent feature of acute or chronic hepatitis, serum aminotransferase activity elevation is not specific (57% for ALT and 72% for AST), because it is seen in numerous liver disorders of various etiologies **(Hoofngle, 2002)**. It is also poorly sensitive (81% for ALT and 88% for AST), since ALT and AST can remain within the normal range for long periods of time in patients with chronic HCV infection, in spite of progressive liver disease **(Seeff, 2002)**.

In spite of lacking optimal sensitivity and specificity of serum transaminases levels in assessing disease progression, the present study supports the clinical usefulness of the enzymes as an alternative to liver biopsy, at least in those patients refuting this technique. However, the validity of this approach and the level above which the ALT elevations are predictive of more rapid progression require further delineation.

In the present study, mean levels of serum ALT and AST were higher in HCC groups than in the group without HCC (ALT: 155.7 versus 70.3; AST: 295.7 versus 125.6). This finding is in agreement with the report by **Sato *et al.* (1996)**. They compared cirrhotic patients with HBV or HCV infection and clearly demonstrated that, persistent elevation of ALT is an important factor for the development of HCV-related HCC. The persistently high levels of ALT was found by **Tarao *et al.* (2000)** to be also associated with more rapid recurrence of hepatocellular carcinoma in hepatoctomized patients with HCV-associated liver cirrhosis or hepatocellular carcinoma than those with persistently normal ALT levels. The authors found that, HCC was recurred within 3 years in 70.6% of patients with high (> 80 IU/ml) ALT levels, while it recurred in only 18.8% of low ALT group within the same period ($P<0.05$).

Overall fibrosis stages an AST/ALT ratio had a significant positive relationship. Thus, the present study agrees with the previous findings of **Correia *et al.* (1981); Williams & Hoofnagle (1988); Sheth *et al.* (1998) and Giannini *et al.* (1999)**. Moreover, this study confirms the usefulness of the AST/ALT ratio as a means for separating patients with mild fibrosis from those with severe fibrosis and cirrhosis as stated by **Myers *et al.* (2002)**. The mean fibrosis score in patients with AST/ALT ratio ≥ 1 was significantly higher than that of patients with <1 ratio (3.3 versus 1.8, $P< 0.001$).

Noninvasive determination of cirrhosis using the ratio of serum AST/ALT has also been previously examined **(Williams & Hoofnagle, 1988 and Sheth *et al.*, 1998)**. Typically, this ratio is > 2.0 in alcoholic liver disease and < 1.0 in viral hepatitis. **Williams and Hoofnagle (1988)** reported on the accuracy of the ratio in a small group of patients with non-A, non-B hepatitis. They found that, when the AST/ALT was > or = 1.0, the likelihood ratio for the presence of cirrhosis was 2.0. The AST to ALT ratio appeared to be more predictive in a separate population of patients with documented hepatitis B.

Considering the diagnostic power of the transaminases ratio for separating mild from significant fibrosis, the ratio at a cutoff value 1.0 was found in the present study to have a 93% sensitivity and 77% specificity. **Sheth *et al.* (1998)** found the AST/ALT ratio to be highly predictive of cirrhosis in their patients with hepatitis C. These authors found that, the AST/ALT ratio had 100% specificity, 100% positive predictive value, and 81% negative predictive value. The operating characteristics of this serum test were reduced in this study because of the dilution effect caused by the lower stages of fibrosis. **Reedy *et al.* (1998)** did not find the AST/ALT ratio to be clinically useful in a small population of patients with hepatitis C.

The mechanism responsible for raised AST/ALT ratio in liver diseases is not fully understood. However, **Fleisher & Wakim (1963)** demonstrated that, AST is electively taken up by the liver, while **Kanimoto *et al.* (1985)** stated that, plasma clearance of AST is predominantly carried out by sinusoidal liver cells. **Giannini *et al.* (1999)** suggested that, an increase in AST/ALT ratio in progressive degrees of disease depends on diminished liver cell uptake of AST due to impaired functional liver blood flow. Although the mechanisms of the AST/ALT ratio modifications in advanced disease are not yet clear, its documented correlation with progressive liver function impairment might suggest its use in clinical practice. The usefulness of evaluating the serum AST/ALT ratio has been highlighted in previous studies examining patients with liver diseases of different etiology (**Correia *et al.*, 1981; Gitlin, 1982 and Williams & Hoofnagle, 1988**). Therefore, the ratio of AST to ALT in serum may help in the diagnosis of some liver diseases. In most patients with acute liver injury, the ratio is 1 or

less, whereas in alcoholic hepatitis it is generally about 2 (**Cohen & Kaplan, 1979**). Moreover, **Sheth *et al.* (1998)** demonstrated that, AST/ALT ratio alone can be used as a diagnostic tool for identifying the appearance of cirrhosis in chronic hepatitis patients infected by hepatitis C virus. More specific tests of hepatocellular damage will undoubtedly become available in due course, but in the meantime, measurement of serum aminotransferase concentrations best fulfills this purpose. According to **Pontisso *et al.* (1999),** hepatitis C virus infection was assured in this study both qualitatively by using RT-PCR and quantitatively by using the real time PCR technique. Chronic infection with hepatitis C virus is characterized by persistent viraemia.

The amount of circulating HCV-RNA in the serum samples of the studied cases ranged from 140 to 9200 000 copies/ml. No correlation between viral load and degree of liver injury was found in the present study. Controversial reports have been published on this point; in some studies high titer viraemia was correlated with advanced stage of liver disease **(Gretch *et al.*, 1995 and Hagiwara *et al.*, 1993)**, while others found no correlation with either histology or aminotransferase activities **(Chayama *et al.*, 1993)**. In addition, in most studies in which the intrahepatic HCV RNA level was evaluated, no correlation with liver injury was shown **(Sakamoto *et al.*, 1994; Coelho-Little *et al.*, 1995 and McGuiness *et al.*, 1996)**. The present analysis can't determine why the amount of HCV-RNA was not correlated with liver histology. However, it should be noted that, the characteristics of the population studied are important variables for the interpretation of the results, since significant differences were only observed when extremely different clinical settings were considered, for example, asymptomatic HCV carriers versus end stage liver disease (**Gretch *et al.*, 1995 and Hagiwara *et al.*, 1993)**.

In the present investigation, the study population included patients with disease severity ranging from mild chronic hepatitis to liver cirrhosis; there was only a few cases with minimal features of hepatitis. It is possible that, the wide range in viral load (140-9 x 10^6) detected in individual patients does not allow identification of any difference, unless extreme situations are considered. On the other hand, the putative mechanism of liver injury is not yet fully clarified, while the contribution of a direct cytopathic effect of HCV to liver damage is still controversial. Several lines of evidence, including the existence of chronic HCV infections without clinically overt disease (**Brillanti *et al.*, 1993**) and the detection of diffuse viral antigens in the liver of immunosuppressed transplant patients (**Krawczynski *et al.*, 1992**) indicate that, immune mediated mechanisms, already described for hepatitis B virus infection (**Thomas *et al.*, 1988**) are likely to play an important role in the pathogenesis of hepatitis C. However, at variance with hepatitis B, where impairment of the virus specific T cell response has been observed (**Barnaba & Balsano, 1992**) in patients with hepatitis C. A valid T cell response to HCV proteins has been detected both in the liver (**Koziel *et al., 1992)*** and in the peripheral blood lymphocytes (**Ferrari *et al.*, 1994**). Whether the level of viral load is the result of immune surveillance or whether it acts as an independent variable awaits the development of reliable cell culture systems.

Most cross-sectional studies have reported the absence of correlation between serum HCV RNA levels and the activity or grade of liver disease **(Zeuzem *et al.*, 1996)**. Interestingly, patients with chronic HCV infection who have normal serum alanine aminotransferase (ALT) levels and nearly normal liver histology may have high serum HCV RNA levels **(Martinot-Peignoux *et al.*, 1994)**. However, serum HCV RNA levels are an indirect reflection of intrahepatic HCV replication, and the correlation between fibrosis and intrahepatic levels of HCV RNA has not been adequately investigated and should be included in prospective studies **(Gervais *et al.*, 2001)**. **Brillanti *et al.* (1993)** recorded that, HCV infection may persist for several years without biochemical or histological evidence of liver disease. Fluctuating ALT levels characterize chronic hepatitis C but the authors found the activities to be consistently normal during a 5 year follow-up period. Also, they did not found any histological changes at liver biopsy after 18-24 months of clinical observation. The investigators could not explain this absence of liver disease. But they proposed three hypotheses; these are, the patient may have been infected by non-virulent HCV strain **(Weiner *et al.*, 1991)**; the pathogenesis of HCV-induced liver disease is mediated by the host immune response, and the patients may have been tolerant to HCV infection **(Brillanti *et al.*, 1992)**. These findings demonstrate that, active HCV infection may persist for a long time in the absence of HCV-induced liver disease and thus support the existence of healthy carriers of HCV. Moreover **Navas *et al.* (1993)** detected plus and minus HCV-RNA in peripheral blood mononuclear cells and in liver biopsy specimens in HCV-infected patients with persistently normal ALT levels. These results suggested that, in some symptom-free HCV carriers, HCV may replicate in the liver without inducing histological lesions. Furthermore, HCV-RNA may replicate in the peripheral blood mononuclear cells of symptomless patients. The normality of the liver of these patients is probably intrinsically related to one type of HCV strain or a lack of recognition of HCV by the immunological system **(Navas *et al.*, 1993)**.

In the present study, only three types of HCV were detected in the studied cases (type 1 in 7, type 4 in 61 and type 6 in 4 cases). Therefore, type 4 is the most prevalent one in this population. A similar finding was observed by **Mangoud *et al.* (2004b)** in a similar Egyptian population. However, the type of HCV was found not to affect the stage of liver fibrosis (mean fibrosis stage is 1.3 in type 1, 1.3 in type 4 and 1.0 in type 6). The difference in mean fibrosis stage in cases infected with different HCV types was statistically not significant (P = 0.064), indicating no effect of HCV type on fibrosis progression.

The influence of viral genotype in the pathogenesis of the liver disease is not completely resolved. In several early studies, HCV genotype 1 (particularly 1b) was found to be associated with a more severe liver disease, including a higher frequency of cirrhosis and hepatocellular carcinoma **(Silini *et al.*, 1996)**. However, many of these studies did not control for important confounding factors, such as age, source, and duration of infection. Genotype 1b is more common among older than younger patients and has been commonly linked to spread by blood transfusion. In studies with adjustment for these variables, the association between genotype 1b and a more severe liver disease has not been found **(Ghany *et al.*, 2000 and Zeuzem *et al.*, 1996)**. Interestingly, the distribution of genotypes is not different in patients with chronic hepatitis C and normal serum ALT levels, as compared with those with increased serum levels **(Marcellin, 1999)**. Further studies are needed to better determination of the possible role of genotype in the outcome of HCV-related liver disease. On the other hand, several studies have shown a modest association between a high quasispecies heterogeneity and more severe liver injury in chronic hepatitis C **(Pawlotsky *et al.*, 1998)**. In one study, quasispecies heterogeneity was less in patients with normal ALT levels compared with those with elevated levels **(Asselah *et al.*, 2002)**. Quasispecies heterogeneity can also be confounded by features of gender, age, and duration of disease. Further studies are needed on the significance of quasispecies heterogeneity in the natural history of hepatitis C and its association with hepatic fibrosis.

In the present study, HCV type 4 is also more prevalent in HCC cases (6/10) than type 1 (3/10) and type 6 (1/10). In cross-sectional studies, HCV genotype 1 (1b in particular) is the most prevalent genotype worldwide and also is the most common genotype found among patients with HCC. However, all HCV genotypes have been described in HCV-related HCC. There was conflicting data as to whether genotype 1 is a risk factor for cirrhosis or HCC independent of older age **(Bruno *et al.*, 1997 and Lopez-Labrador *et al.*, 1997)**. It has been suggested that, the higher prevalence of these genotypes reported in some studies represents a cohort effect in which older persons (those at greatest risk for cirrhosis and HCC) were infected at a time when genotype 1 was most prevalent **(Lopez-Labrador *et al.*, 1997)**. Lastly, there is no evidence that either viral load or viral quasispecies are important in determining the risk for HCV progression to cirrhosis or HCC **(El-Serag, 2002)**.

In the present study, the levels of matrix metalloproteinase 9 was inversely correlated with the stage of liver fibrosis. Similar results were recently reported by **Mangoud *et al.* (2004b)**. Moreover, **El-Shorbagy *et al.* (2004)** reported a negative relationship between serum MMP-9 level and the severity of liver disease. The present study revealed a high sensitivity and specificity (87% and 72%, respectively) of this serum test in differentiating mild fibrosis from severe fibrosis that may reduce the need for liver biopsy in many patients especially when it was combined with transaminases ratio. Moreover, the serum MMP9 test has a fairly high positive and negative predictive values (86% and 82%, respectively) which supports the usefulness of its use in the clinical settings as a serum fibrosis marker.

Circulating levels of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) have been shown by several investigators to correlate with the development of cirrhosis **(Murawaki *et al.*, 1999; Muzzillo *et al.*, 1993 and Tsutsumi *et al.*, 1996)**. The results of the present study showed that, circulating concentrations of serum MMP9 change in the course of chronic hepatitis C. The changes in the concentrations of MMPs and TIMPs have been described by other investigators. **Lichtinghagen *et al.* (2000)** reported a slight decrease in MMP2 and MMP9 in patients with CAH but the decrease was almost doubled in patients with cirrhosis, while TIMP2 was increased in hepatitis and cirrhotic patients. In contrast to the present findings, plasma MMP9 concentrations have been reported to be within the reference intervals in patients with chronic liver diseases and even cirrhosis, but increased in patients with HCC **(Hayasaka *et al.*, 1996)**. The difference between the results of the present study and those reported by the later authors can possibly be explained by the fact that, the Japanese results were obtained in EDTA plasma, whereas serum samples were assayed in this study. **Jung *et al.* (1998)** found that, the Fuji-MMP9 antibody, which is used in the commercial assays used in this study and by the Japanese group, gives measurable MMP9 levels in EDTA plasma that may be up to 20-folds higher than that in serum.

Benyon & Arthur (2001) reported that, inactive metalloproteinases can be either activated through proteolytic cleavage, or inhibited by binding to specific inhibitors known as TIMPs (tissue inhibitors of metalloproteinases). So, progressive fibrosis will be associated with marked increases in TIMPs leading to a net decrease in protease activity of the MMPs and therefore promoting matrix accumulation by slowing down collagen breakdown **(Iredale, 1997)**. This assumption was assured by the findings of **Arthur (1997)** who reported an increase in serum and liver TIMP-1 in cirrhosis and that adds another support to the findings of the present study. The observations of **Arthur (1997)** are in agreement with those of **Murawaki *et al.* (1999)** and **Tsutsumi *et al.* (1996)**. Studies in animal models and human liver fibrosis indicated that, interstitial collagenolytic activity decreases in liver extracts in advanced fibrosis **(Okazaki & Maruyama, 1974; Takahashi *et al.*, 1980 and Maruyama *et al.*, 1982)** which would promote net collagen deposition. There is increasing evidence that, collagenase inhibition may arise from increased expression in fibrotic liver of endogenous MMP inhibitors (TIMPs). Expression of both TIMP-1 and –2 is increased in human and rat model fibrotic liver **(Rojkind *et al.*, 1979 and Han *et al.*, 1980),** and in human liver the degree of TIMP-1 expression correlates with extent of fibrosis **(Benyon *et al.*, 1996)** assessed by hydroxyproline content. In rat models of liver fibrosis, TIMP-1 is expressed early in fibrogenesis before apparent collagen deposition **(Iredale *et al.*, 1996)**. The resulting increase in TIMP:MMP ratio in liver may promote fibrosis by protecting deposited ECM from degeneration by MMPs.

In the present study, there was no statistical difference in the mean AFP serum level between mild and significant fibrosis (7.7 versus 16.7 ng/ml, P = 0.75). However, the receiver operating characteristic curve revealed a very good diagnostic power of serum AFP for HCC diagnosis (area under the curve = 0.949). When the predictive power of serum AFP was assessed in a prospective fashion, it was found to be higher in population-based versus clinic-based studies, as a consequence of the many false-positive results in patients with cirrhosis **(Sato *et al.*, 1993 and Colombo *et al.*, 1991)**. The clinical significance of an elevated AFP level in chronic hepatitis C remains to be identified. By using the most commonly reported cutoff value of a positive test result for hepatocellular carcinoma (AFP level > 1000 ng/ml) the test sensitivity was 90%, specificity 57%, positive predictive value 87% and negative predictive value was 59%. Data for AFP at higher cutoff values suggest that AFP, although not sensitive, can be highly specific for hepatocellular carcinoma. Therefore, a low AFP level (< 1000 ng/ml) would not be informative enough to stop further search for hepatocellular carcinomas but an AFP level higher than 1000 ng/ml would strongly suggest that, cancer is present, allowing for earlier counseling of patient **(Gupta *et al.*, 2003)**.

Although the National Cancer Institute recommended against screening for hepatocellular carcinoma, many physicians still screen high-risk populations with various strategies, including serum AFP, ultrasonography and computed tomography **(El-Serag, 2002)**. The use of AFP to detect these tumors has been widely debated **(Lin & Liaw, 2001; Sherman, 2001 and Johnson, 2002)**. Many conclude that, AFP is not a useful diagnostic test **(Sherman, 2001 and Tong *et al.*, 2001)**, but AFP continues to be commonly used. Patients with cirrhosis have a higher risk for cancer **(Tsukuma *et al.*, 1990)** but commonly have elevated levels of AFP thought to be unrelated to hepatocellular carcinoma **(Taketa, 1990)**, leading to an unknown effect on sensitivity and specificity. Some studies have shown that, AFP has different test characteristics in patients with hepatitis B virus than in those with HCV **(Tsai *et al.*, 1994 and Gupta *et al.*, 2003)**, but the sensitivities and specificities in the study of **Cedrone *et al.* (2000)** were within the range of values reported by other studies which included only patients with HCV.

With the recent development of improved resectional and ablative therapies, cirrhotic patients are now eligible for the treatment of HCC. The potential for cure of HCC depends on early detection **(Gogel *et al.*, 2000)**. Therefore, screening of high-risk population will be necessary to achieve early diagnosis. Most HCC screening protocols use ultrasound and serum AFP, although the use of AFP as a screening test is complicated by frequent false-negative and false-positive results. **Torzilli *et al.* (1999)** reported a sensitivity of 68% and specificity of 20% using AFP to diagnose early (less than 3 cm) HCC. In another study from China, serum AFP levels were elevated only in 60% to 70% of patients with small HCC lesions, and fewer than 20% of the elevated levels of AFP were due to HCC **(Lok & Lai, 1989)**. HCCs in symptomatic patients are generally large and demonstrate extrahepatic spread, thus rendering them unresectable **(Kassianides & Kew, 1987)**. Median survival in patients with clinically apparent HCC is less than 6 months, and 2 year survival is virtually nonexistent **(Gogel *et al.*, 2000)**. Early detection offers the best potential for curative intervention. Therefore, in contrast to certain other cancers, screening for liver cancer has become, at least among hepatologists, an accepted part of the management of patients with end-stage liver disease **(Collier & Sherman, 1998)**. A number of screening and surveillance programs have

been reported **(Lok & Lai, 1989 and Sherman *et al.*, 1995)**. However, the results range from very optimistic to downright pessimistic.

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