

Evaluation of Cystatin C, Fibronectin and Alpha-Feto Protein as Biochemical Markers in Patients with Liver Diseases

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Abstract: *Objective:* We investigate the serum level of cystatin C as biochemical marker that can determine the severity of tissue injury resulting from chronic liver diseases. Also we investigate other two parameters - alpha-fetoprotein & fibronectin- serum levels as biomarkers of chronic liver disease progression.

Study design: This study was carried out on 66 patients (50 males & 16 female). We assessed serum levels of cystatin C, alpha-fetoprotein, albumin, ALT, AST, creatinine, urea, plasma level of fibronectin and HCV genotype.

Results: We found that (a positive & a negative) correlations between serum levels of cystatin C and plasma level of fibronectin (respectively) and progression of liver diseases. Also we found that a positive correlation between plasma level of fibronectin and both serum level of albumin and prothrombin time. We found a positive correlation between serum level of alpha-fetoprotein and liver bilharzial hepatic fibrosis. In addition we found that HCV genotype 4 was the prevalent type in Egyptian patients.

Conclusion: Serum levels of cystatin C, alpha-fetoprotein and plasma level of fibronectin can be useful markers in long-term monitoring of the progression of liver diseases.

[Mousa, M. Moustafa, El-Saeid, M. E. El-Bawab, Hamdy, M. Moustafa, Ibrahim, H. Mohamed, Magdi, M. Salama and Mohamed, H. Abd-Elzاهر **Evaluation of Cystatin C, Fibronectin and Alpha-Feto Protein as Biochemical Markers in Patients with Liver Diseases.** *J Am Sci* 2012; 8(7):770-779]. (ISSN: 1545-1003).
<http://www.americanscience.org>.

1. Introduction

Cystatins are endogenous inhibitors of lysosomal cysteine proteinases, such as cathepsin, L, H, and S (Zahran et al., 2007). Cystatin C, belonging to the type II Cystatin gene superfamily, is the most abundant extracellular inhibitor of cysteine proteinase (Merz et al., 1997). Mature cystatin C is a 13 kD, positively charged, secreted protein composed of 120-122 amino acids expressed by many cell types. It is present in large quantities in cerebrospinal fluid, seminal plasma, serum and other body fluids (Newman, 2002). One of the most prominent functions of cystatin C is related to the inactivation of the cathepsin family members of cysteine proteinase, which are involved in the cleavage of membrane and extracellular matrix proteins among others and thus is disease-related tissue remodeling. The diagnostic value and prognostic significance of cystatin C have been reported for several diseases (Koeing et al., 2005). The correlations have been found between cystatin C expression, mutations, and clinical status of patients with cerebral amyloid angiopathy, hereditary brain hemorrhage (Palsdottir et al., 2006, Levy et al., 2006), atherosclerosis, aortic aneurysms (Shi et al., 1999, Mares et al., 2003) and macular degeneration (Zurdel et al., 2002). Diseases of the liver have been an important health issue since severe hepatic diseases could lead to persistent inflammation and necrosis, and even liver cirrhosis and hepatoma. Thus indicators for the long-term monitoring on the progress of hepatitis are of great clinical importance. Liver fibrosis has been

shown to be a result from an imbalance between degradation and synthesis of extracellular matrix. Cysteine proteinases and metalloproteinases, have been found to be involved in the degradation of extracellular matrix (Chu et al., 2004). Infections caused by specific hepatitis C virus (HCV) genotypes, such as 1a and 1b, are more refractory to antiviral therapy than those caused by other genotypes (Layden et al., 2002). For this reason, HCV genotyping has become a critical component of the standard of care of HCV-infected patients (Richter, 2002)

2. Patients and Methods

Patients:

Our study was carried out on 86 individuals (patients & healthy). 66 patients (50 males & 16 females) were selected from outpatient and inpatient clinic of gastroenterology & hepatology department at Al-Azhar university hospital (Assiut branch). Their ages ranged from 31 – 65 years. They are classified as follows;

Group I: 28 patients with chronic viral hepatitis (HBV: 7 patients & HCV: 21 patients).

Group II: 27 patients with bilharzial hepatic fibrosis.

Group III: 11 patients with primary hepatocellular carcinoma.

Group IV: 20 healthy subjects as a control group

Patients were subjected to the following clinical examination and laboratory investigations: Complete history taking, full clinical examination including liver and spleen in details, routine laboratory

investigations; (urine analysis, stool analysis and complete blood picture), sigmoidoscopy and rectal snip biopsy with fresh examination for detection of *Schistosoma Mansoni* ova for group II

Abdominal ultrasonography, ultrasonography-guided liver biopsy for group III, HBV markers (HBsAg & HBeAg) for all groups to exclude HBV infection in other groups and HCV-Ab.).

Sample collection:

Blood samples were collected from all participant by vein puncture under complete aseptic conditions and divided into three parts: First part was mixed with sodium citrate (9 parts of fresh blood+ 1 part sodium citrate). Plasma was separated by centrifugation and transferred to a clean tube. Plasma was tested for fibronectin and PT.

Second part was mixed with ethylenediamine tetra acetic acid (EDTA)

(2 mg/ml blood). Plasma was separated by centrifugation and transferred to a clean tube. Plasma was tested for HCV genotyping (real time PCR). Third part was left to be clotted and serum was separated by centrifugation. Serum was divided into separate aliquots and stored at

- 70°C until used for cystatin C, AFP, ALT, AST, albumin, blood urea and creatinine, determination.

Methods:

•Measurement of serum cystatin C concentration

Cystatin in serum samples was quantified using a human cystatin C ELISA kit supplied by (BioVendor, Czech Republic) following manufacturer's instructions. Human cystatin standards were provided in the kit (200-10,000 ng/mL), and test serum samples were diluted 1:400 in the dilution buffer supplied. One hundred microliter aliquots of the diluted standards and test samples were added in duplicate to the wells of a microtiter plate coated with antihuman cystatin C antibody. Dilution buffer alone was added to a pair of duplicate wells to serve as blank. After incubation at room temperature for 30 min on an orbital shaker, the plate was washed thrice with the wash solution and 100 µL of antihuman cystatin antibody labeled with horseradish peroxidase was added to the wells.

The plate was incubated for 90 min at room temperature followed by washes as before and addition of 100 µL of substrate solution containing hydrogen peroxide and tetramethylbenzidine to the wells.

The plate was covered with aluminum foil to protect from light and incubated for 10 min to allow for color development. The reaction was stopped by the addition of 100 µL of stop solution, and the optical densities were determined by reading absorbance at 450 nm. A standard curve of concentration of cystatin versus absorbance was plotted using the four-variable function, and the test values were derived from the measured absorbance using this curve (fig.1). (Tian et al., 1997).

•HCV typing:

Serum samples were obtained from 21 patients chronically infected with HCV, genotype-specific primers were designed to detect nine genotype 1a, 1b, 1d, 2a, 2b, 3a, 3b, 4, 5a, or 6a. As there were nine different subtypes that we tried to detect, the detection primers are divided into two different types on the basis of differences in the sizes of the different bands, so that no genotype-specific bands are of similar molecular size in the same gel. All samples were analyzed twice by PCR with either mix 1 or mix 2. Mix 1 will allow for the specific detection of PCR products for HCV genotypes 1b, 2a, 2b, and 3b. Mix 2 will allow for the specific detection of HCV genotypes 1a, 3a, 4, 5a, and 6a. The detection of genotype-specific products in mix 1 and mix 2 was designed so that the differences in the sizes of PCR products could be evaluated on gels easily (fig.2). In the analysis of results, one should look for the strong specific bands seen on either gel. Faint bands are nonspecific, may be generated through weak priming. (Ohno, et al., 1997)

•Serum alpha-fetoprotein concentration (AFP)

Serum alpha-fetoprotein concentration (AFP) was measured by ELISA (Engall et al., 1980) using human AFP ELISA kit supplied by (Europa Bioproduct Ltd, United Kingdom). The serum AFP was determined from calibration curve (fig.3)

•Plasma fibronectin concentration

Plasma fibronectin concentration was measured by ELISA (Ruoslahiti et al., 1981) using human fibronectin kit supplied by (MyBiosource, USA). The plasma fibronectin was determined from calibration curve (fig.4)

•Serum albumin concentration

Serum albumin concentration was estimated colorimetrically by bromocresol green (Dumas et al., 1971) using kit supplied by (Spectrum Diagnostics, Egypt)

•Serum ALT and AST concentration

Serum ALT and AST concentration were estimated by colorimetric method (Reitman and Frankel, 1957) using kit supplied by (Spectrum Diagnostics, Egypt)

•Serum of creatinine concentration

Serum of creatinine concentration was determined by kinetic colorimetric method (Jaffe', 1986) by using kit supplied by (human

•Serum of urea concentration

Serum of urea concentration was determined by enzymatic colorimetric method (Rock, et al., 1987) using kit supplied by (Vitro-Diagnostic

•Determination of Prothrombin Time (PT) and International Normalized Ratio (INR):

Tissue thromboplastin, in the presence of calcium ions, is an activator which initiates the extrinsic pathway of coagulation. When a mixture of tissue thromboplastin and calcium ions is added to normal

anticoagulated plasma, the clotting mechanism is initiated leading to formation of a fibrin clot. If a deficiency exists within the extrinsic pathway, the time required for clot formation will be prolonged depending on the severity of the deficiency (Hull et al., 1982)

Statistical Analysis:

Statistical analysis was performed using SPSS-16 for windows. All results are expressed as mean \pm standard deviation (SD), median values and ranges. Data were analyzed by one-way analysis of variance in addition to the student–Newman post-hoc test. Coefficient correlation was evaluated using Pearson correlation. Statistical significance was established at $p < 0.05$.

3. Results:

As regard to cystatin C serum levels (mg/L) the results revealed high statistically significant difference in the serum cystatin C levels between the control, chronic hepatitis, H.bil.F and HCC groups ($p < 0.001$ for all, table 1, fig. 5). Also serum cystatin C has a positive correlation with ALT serum levels (U/I) in chronic hepatitis group ($r = 0.74$, $P < 0.01$, fig.6), hepatic bilharzial fibrosis group ($r = 0.64$, $P < 0.01$, fig.7) and in HCC group ($r = 0.8$, $P < 0.01$, fig.8). There are significant increase in serum cystatin C levels in control and three classes of different severity of hepatic disease based on serum level of ALT (Chu et al., 2004). Where class that has ($ALT \leq 35$ U/I) considered mild, that has

($35 < ALT < 105$ U/I) considered moderate and that has ($ALT \geq 105$ U/I) considered severe hepatic diseases respectively, based on three times reference value (35U/I). The results revealed high statistically significant increase in the serum cystatin C levels between the control and three classes ($P < 0.01$ for all, tabl.2,fig.9). There are a significant increase in fibronectin plasma levels ($\mu\text{g/ml}$) in chronic viral hepatitis, and hepatocellular carcinoma groups when compared with controls ($P < 0.01$ for all table 3, fig. 10). There are a significant increase in the serum alpha -feto protein (AFP) levels in chronic viral hepatitis, hepatic bilharzial fibrosis and hepatocellular carcinoma groups when compared with controls ($P < 0.01$ for both, table 4, fig.11).

AST and ALT serum levels revealed statistically very high significant increase in all groups when compared with controls ($P < 0.0001$ for all, table 5). Serum albumin levels revealed statistically high significant increase in hepatic bilharzial fibrosis and hepatocellular carcinoma groups ($P < 0.001$ for both, table 5). But in chronic viral hepatitis group there are no significant differences. Plasma prothrombin times revealed very high significant increase in hepatic bilharzial fibrosis and hepatic cellular carcinoma groups ($P < 0.0001$ for both, table 5), but insignificant differences in chronic viral hepatitis group. Percentage of each genotype of HCV. Type 4 represents most cases infected with HCV (90.4 %) while type 1 only represents (4.8 %) of cases.

Table (1): Comparison between serum cystatin C levels in control versus chronic viral hepatitis, hepatic bilharzial fibrosis and hepatocellular carcinoma groups.

	Controls	CVH	HBF	HCC
Cysatin C (mg/L) \pm S.D	0.66 \pm 0.018	0.8 \pm 0.11	0.82 \pm 0.12	0.89 \pm 0.12
P value		$P < 0.001$	$P < 0.001$	$P < 0.001$
Significance		H.S	H.S	H.S
N.	20	28	27	11

H.bil.F= hepatic bilharzial fibrosis, H.S= high significant, HCC=hepatocellular carcinoma N= number of cases.

Table (2): Comparison between cystatin C and the severity of liver diseases, patients were regrouped into three groups, mild ($ALT \leq 35$ U/L), moderate ($35 < ALT < 105$) and severe ($ALT \geq 105$), hepatic diseases respectively based on an increase more than three times reference value of ALT serum level (U/I).

	Controls	Mild H.D ($ALT \leq 35$ U/I)	Moderate H.D ($35 < ALT < 105$ U/I)	Severe H.D ($ALT > 105$ u/I)
Cysatin C (mg/L) \pm S.D	0.66 \pm 0.018	0.76 \pm 0.09	0.87 \pm 0.09	1.02 \pm 0.08
P value		$P < 0.01$	$P < 0.01$	$P < 0.01$
Significance		H.S	H.S	H.S
N.	20	30	28	10

H.D= hepatic disease, H.S= high significance, N.= number of cases.

Table (3): Comparison between plasma fibronectin levels ($\mu\text{g/ml}$) in control versus chronic viral hepatitis, hepatic bilharzial fibrosis and hepatocellular carcinoma groups.

	Controls	CVH	HBF	HCC
Fibronectin ($\mu\text{g/ml}$) \pm SD	398.2 \pm 57.06	371.8 \pm 68.6	234.4 \pm 122	322.6 \pm 80.57
P value		P<0.01	P<0.01	P<0.01
Significance		H.S	H.S	H.S
N.	20	28	27	11

H.bil.F= hepatic bilharzial fibrosis, H.S= high significant, HCC=hepatocellular carcinoma, N= number of cases.

Table (4): Comparison between serum alpha-fetoprotein levels (ng/ml) in controls versus chronic viral hepatitis, hepatic bilharzial fibrosis and hepatocellular carcinoma groups.

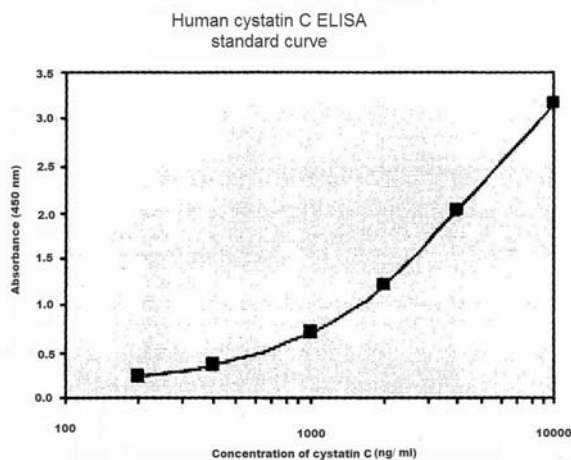
	Controls	CVH	HBF	HCC
AFP (ng/ml) \pm SD	2.23 \pm 1.05	2.47 \pm 1.7	41.7 \pm 17	174.9 \pm 92.4
P value		0.599	< 0.001	< 0.001
Significance		N.S	H.S	H.S
N.	20	28	27	11

AFP= alpha-fetoprotein, CHV=chronic viral hepatitis, HBF= hepatic bilharzial fibrosis, H.S= high significant, HCC=hepatocellular carcinoma, N= number of cases, NS= non-significant.

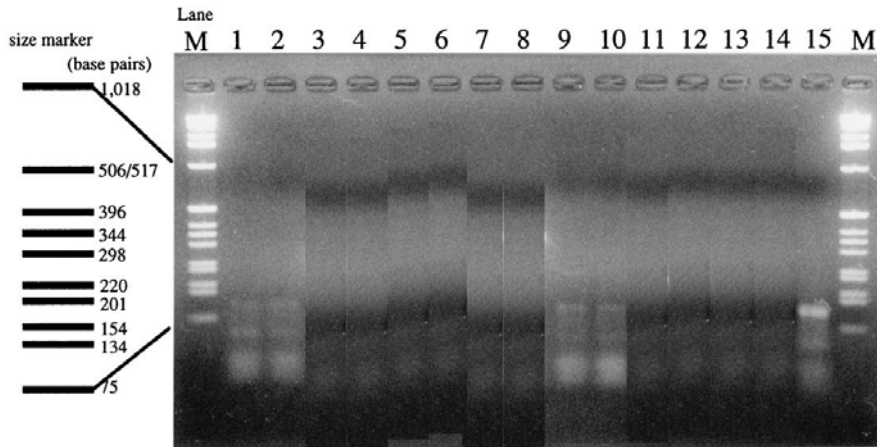
Table (5): Comparison between serum ALT, AST, creatinine, urea, albumin and prothrombin time in controls versus chronic viral hepatitis, hepatic bilharzial fibrosis and hepatocellular carcinoma groups.

parametrs	Controls	CVH	P value	HBF	P value	HCC	P value
ALT(U/I) \pm SD	14.6 \pm 3.9	50 \pm 25.9	<0.0001 VHS	37.9 \pm 21.6	<0.0001 VHS	39.4 \pm 24.1	<0.0001 VHS
AST(U/I) \pm SD	21.3 \pm 3.8	64.3 \pm 31.9	<0.0001 VHS	48.8 \pm 24.3	<0.0001 VHS	50.3 \pm 24	<0.0001 VHS
Creatinine (mg/dl) \pm SD	0.76 \pm 0.09	0.81 \pm 0.13	0.420 NS	0.79 \pm 0.1	0.420 NS	0.76 \pm 0.12	0.601 NS
Urea (mg/dl) \pm SD	15.6 \pm 1.1	16.3 \pm 1.5	0.0970 NS	14.8 \pm 2.4	0.1618 NS	14.95 \pm 0.47	0.1694 NS
Albumin (g/L) \pm SD	4.19 \pm 0.24	4.1 \pm 0.16	0.2063 NS	3 \pm 0.41	<0.001 HS	2.9 \pm 0.36	<0.001 HS
PT(sec.)	12.26 \pm 0.9	12.61 \pm 1.31	0.3021 NS	18.8 \pm 4.1	<0.0001 VHS	19 \pm 1.78	<0.0001 VHS

CHV=chronic viral hepatitis, HBF= hepatic bilharzial fibrosis, HS= high significant, HCC=hepatocellular carcinoma, N= number of cases, NS= non-significant, VHS=very high significant, ALT=alanine transferase, AST= aspartate transferase, sec= seconds.

**Fig.1:** Human cystatin C ELISA standard curve.

【Mix-1】



【Mix-2】

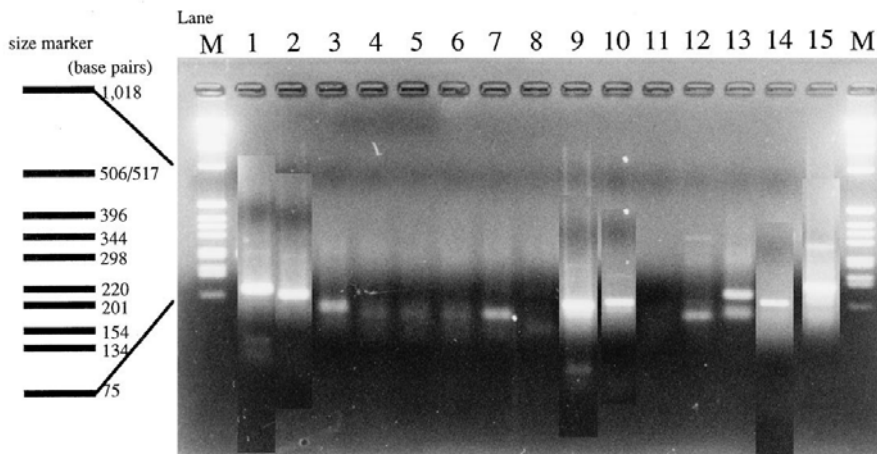


Fig.2: Typical electrophoresis patterns of PCR products from 15 cases. All samples were analyzed twice by PCR with either mix 1 or mix 2. Mix 1 will allow for the specific detection of HCV genotypes 1b, 2a, 2b, and 3b. Mix 2 will allow for the specific detection of HCV genotypes 1a, 3a, 4, 5a, and 6a. The figure shows that fifteen cases give no specific bands in mix 1 (except one at lane 15 give a specific band for 1b genotype, but fourteen cases give in mix 2 a specific bands for 4 genotype).

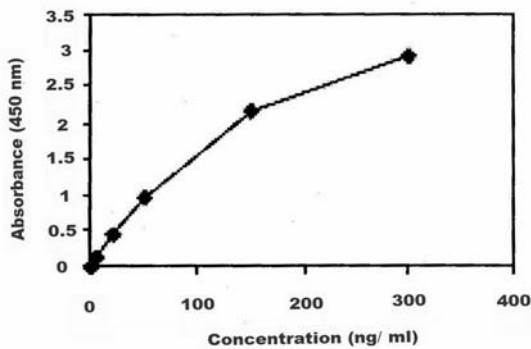


Fig.3: Alpha-fetoprotein (ng/ml) standard curve.

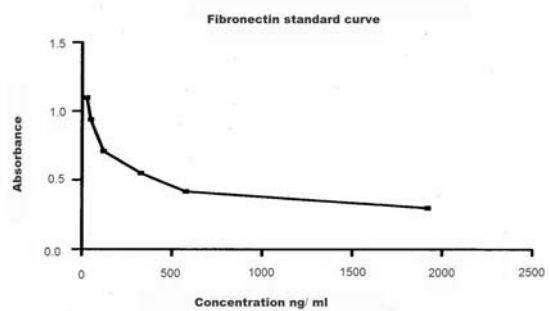


Fig.4: Fibronectin (ng/ml) standard curve.

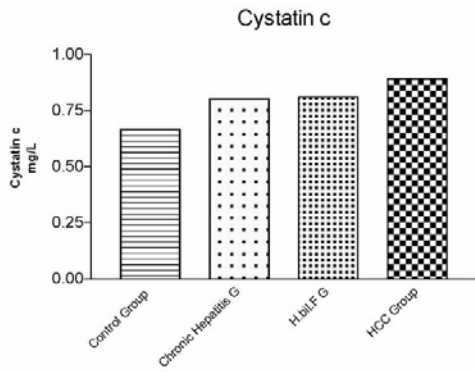


Fig.5: Comparison of cystatin c levels (mg/L) in controls and diseased groups.

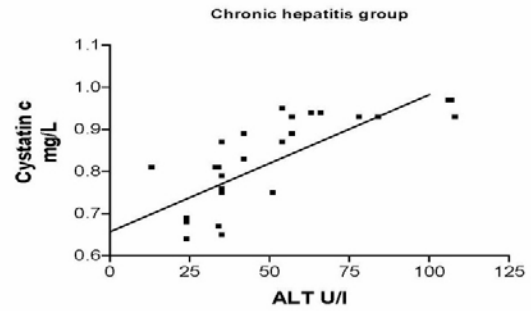


Fig.6: Positive correlation between serum cystatin C and ALT in chronic hepatitis ($r=0.74$, $P<0.001$)

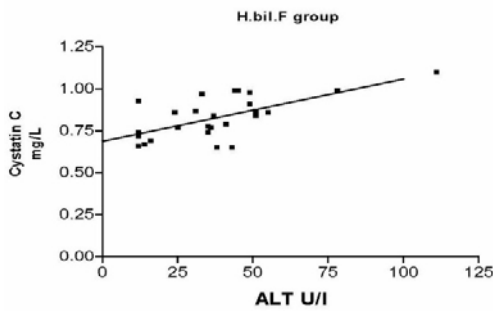


Fig.7: Positive correlation between serum cystatin C and ALT in hepatic bilharzial fibrosis ($r=0.64$, $P<0.01$)

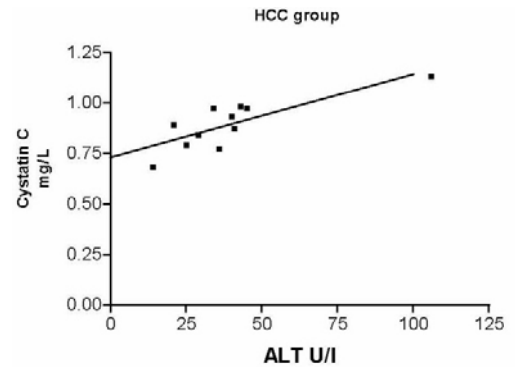


Fig.8: Positive correlation between serum cystatin C and ALT in hepatocellular carcinoma ($r=0.8$, $P<0.01$)

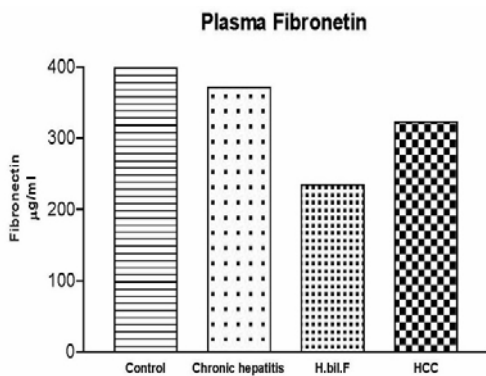


Fig.9: comparison between serum cystatin c levels in controls three different severity of hepatic disease (according to ALT serum levels)

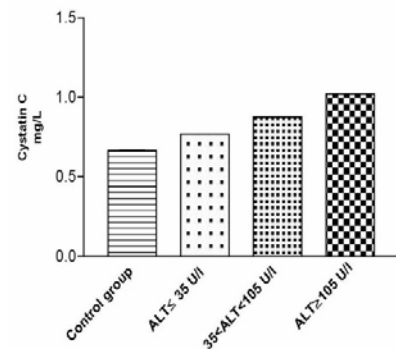


Fig.10: Comparison between plasma levels of fibronectin in controls and diseased groups

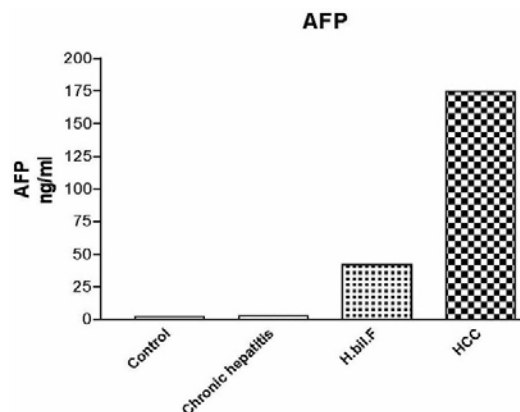


Fig.11: Comparison between serum levels of alpha-fetoprotein In controls and diseased groups

4. Discussion

A further highly relevant function of cystatin C has been recently reported, which is concentrated on the inhibitory effect on transforming growth factor- β (TGF- β)-Signal transduction in normal and malignant cells (Sokol and Schiemann, 2004). In vitro, TGF- β 1 is a potent inducer of cystatin C secretion in vascular smooth muscle cells (Shi et al., 1999). Furthermore, expression of cystatin C mRNA in astrocyte precursor cells is directly linked to the activity of TGF- β (Solem et al., 1990). TGF- β is known to be the fibrogenic master cytokine in human due to its ability to stimulate the expression and inhibition of degradation of extracellular matrix (ECM) proteins and to promote the trans-differentiation of profibrogenic hepatic stellate cells (HSCs) to ECM-producing hepatic myofibroblasts (MFBs) (Gressner et al., 2002). The phenotypic and functional trans-differentiation of HSCs is initiated in acute and chronic inflammatory liver diseases, ultimately leading to organ fibrosis and cirrhosis (Takeuchi et al., 2001). Significantly elevated serum concentration of cystatin C have been recently described in chronic liver diseases showing a strong correlation between the degree of elevation and the severity of disease (Chu et al., 2004). Based on these new findings, we tried to figure out the potential function relevance of cystatin C in various liver diseases.

Diseases of the liver have been an important health issue since severe hepatic diseases could lead to persistent inflammation and necrosis, and even liver cirrhosis and hepatoma. Thus indicators for the long-term monitoring on the progress of hepatitis are of great clinical importance. Liver fibrosis has been shown to be a result from an imbalance between degradation and synthesis of extracellular matrix. Cysteine proteinases and metalloproteinases, have been found to be involved in the degradation of extracellular matrix (Chu et al., 2004).

Since an increased activity of cystein proteinase in liver fibrosis may be accompanied by increased activity of cystatin C, a very potent inhibitor of lysosomal cysteine proteinases. So, in this study serum cystatin C concentration of 66 patients with liver diseases of various severities and 20 healthy controls were analyzed. Since renal diseases are common complications of hepatic diseases, we have eliminated the possibility of any renal disease complications by screening serum creatinine and blood urea nitrogen levels. Fourteen patients have been excluded from this study due to their abnormal kidney function tests.

In the present study, our results showed that the levels of serum cystatin C were statistically significant increased progressively with the progression of liver disease ($P < 0.001$), in chronic viral hepatitis, hepatic bilharzial fibrosis hepatic bilharzial fibrosis and hepatocellular carcinoma groups in comparison with the control group. Our results also showed that serum cystatin C concentration was significantly higher in patients with liver fibrosis and hepatocellular carcinoma than in control or chronic hepatitis

These results were in agreement with Chu et al., (2004) and Chen et al., (2005) who found that serum cystatin C concentrations significantly and progressively increased with the progression of liver disease and that is a potential marker for liver fibrosis.

ALT value is considered as a direct indicator for liver inflammation or fibrosis. To look at the correlation between cystatin C and the severity of liver diseases in our study, patients were regrouped into three groups, mild ($ALT \leq 35$ U/L), moderate ($35 < ALT < 105$) and severe ($ALT \geq 105$), hepatic diseases respectively based on a moderate increase or an increase more than three times reference value (Chu et al., 2004). We have found a positive correlation between serum cystatin C concentrations and the severity of liver diseases which determined by the increase in serum ALT ($P < 0.01$). So, serum cystatin C

was progressively increased with the progression increase of ALT. These findings were in agreement with **Chu et al., (2004)**. The previous results indicate that serum cystatin C concentration was closely related to the progression of liver diseases and chronic liver inflammation.

This study also was conducted on 28 Egyptian patients chronically infected with viral hepatitis; 75% with HCV and 25% with HBV. Chronically infected patients with HCV have been studied for HCV genotyping. HCV genotype 4 was detectable in 90.4 % of HCV patients. These reveals that the most prevalent genotype among Egyptian patients is type 4. These results were in agreement with **(Omran et al., 2009)** and **(Abdel-Hamid et al., 2007)**. They found that most prevalent type in Egyptian patients was type 4 (94.3%) and (95.9%) respectively.

Our data showed that plasma fibronectin concentrations were statistically significantly decreased in hepatic bilharzial fibrosis group ($P < 0.001$) and non-significant decrease in HCC groups. Whereas, there was non-significant difference in chronic viral hepatitis group. These finding were in agreement with **Simon et al., (1995)** , **Jitoku et al., (1996)** and **Fortunato et al., (2001)** who found significant lower values $\{ (P < 0.001)$, $(P < 0.001)$ and $(P < 0.01)$ respectively} in liver cirrhosis and non-significant lower values in chronic hepatitis group. They concluded that the determination of plasma fibronectin is not important in evaluation of degree of liver fibrosis but it is one of the liver function tests. Whereas, **Grieco et al., (1998)** found significant decrease in plasma fibronectin only in liver cirrhosis group ($P < 0.001$) while in contrast increased levels were associated with acute and chronic hepatitis group.

On the other hand, **Kandemir et al., (2004)** did not agree with our results as regards chronic hepatitis patients. In the present study we also examined the relation between plasma fibronectin and ALT levels as marker of liver inflammation and necrosis. Our data showed no correlation between plasma fibronectin concentrations and ALT levels ($P > 0.05$). These finding was in agreement with **Kandemir et al., (2004)** who found also no correlation between plasma fibronectin concentrations and ALT levels.

Furthermore, we examined the correlation between plasma fibronectin and both of serum albumin concentrations and prothrombin time (PT) as all of them are synthesized in the liver. We found positive correlation between plasma fibronectin and serum albumin ($P < 0.001$). On the other hand, we found negative correlation between plasma fibronectin and (PT) ($P < 0.001$) (i.e., positive correlation with prothrombin concentration). This finding was also reported by **Ricciardi et al., (1987)** and **Gabrielli et al., (1986)**. In contrast **Angelis et al., (1988)** found no correlation between plasma fibronectin and serum albumin in cirrhotic patients.

Other findings in this study, we found 10 cases have a fibronectin level less than 170 $\mu\text{g/ml}$, 8 of them have a past history of recurrent infection. Moreover, we found 6 patients have fibronectin level $< 140 \mu\text{g/ml}$ and all of them have severe splenomegaly. These findings may indicate that the decrease in plasma fibronectin may result from its increased consumption in the hyperactive reticulo-endothelial system. Since fibronectin acts as opsonin in reticulo-endothelial system, it is not surprising that low levels of this glycoprotein are associated with recurrent infection.

Our previous results indicate that the significant decrease in plasma fibronectin in hepatic bilharzial fibrosis patients is either due to liver function impairment and/or its increased consumption by the hyperactive reticulo-endothelial system.

Our results showed statistically significant increase of serum AFP in hepatic bilharzial fibrosis and as expected in hepatocellular carcinoma groups in comparison with the control group ($P < 0.001$). There was non-significant increase in chronic hepatitis group. So, the increase in AFP was positively related to disease progression with the highest being in hepatocellular carcinoma group ($174.9 \pm 92.4 \text{ ng/ml}$, $P < 0.001$). These results were in consistent with **Chu et al., (2004)** and **Arrieta et al., (2007)**. But **Seifi and Bafandeh, (2006)** did not agree with our results as regards chronic hepatitis group only. They found significant difference between HCV positive group in comparison with the control group ($P < 0.05$). On the other hand AFP was also increased in hepatic bilharzial fibrosis mean concentration (41.7 ng/ml). this indicates low specificity of AFP hence, there is a need for additional serum markers that will improve the detection of early hepatocellular carcinoma.

In coclusion serum cystatin C, plasma fibronectin and AFP were closely related to the progression of liver diseases and chronic liver inflammation. This suggests that the above markers may be useful to certain extent in monitoring liver function and follow up the progression of liver diseases. Besides, we need additional markers to improve early diagnosis of hepatocellular carcinoma hence improvement of prognosis.

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6/12/2012