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RESEARCH ARTICLE

SERUM FETUIN-A LEVEL IN PATIENTS WITH CHRONIC LIVER DISEASE AND HEPATOCELLULAR CARCINOMA

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Abstract

Introduction:

Assessment of the levels of glycoproteins as fetuin-A which provides insights into the pathogenic mechanisms of various liver diseases and improved capability to identify disease-specific profiles of proteins leads to accurate prediction of disease classes and development of novel treatments.

Aim and Methods:

The aim of this study was to evaluate the levels of fetuin-A and its relationship with the severity of cirrhosis and hepatocellular carcinoma.

This study included three groups. Group A (n= 30) are patients with liver cirrhosis, Child–Pugh class A (n=10), class B (n=10) and C (n=10). Group B (n=10) are patients with hepatocellular carcinoma. Group C (n=10) are healthy subjects matched for age and sex as a control. All subjects were subjected to : thorough history, clinical examination, routine laboratory investigations including liver function tests, renal function tests, urine analysis, complete blood counts, lipid profile, fasting and postprandial blood glucose, hepatitis Bs Ag, hepatitis B core Ab, HCV Ab, alpha-fetoprotein, abdominal ultrasound, abdominal triphasic computed tomography, serum fetuin-A. In addition, cardiac assessment using resting electrocardiography and echocardiography done.

Results:

Serum levels of fetuin-A showed statistically significant difference among patients with liver cirrhosis; marked decrease in the mean levels with the increased severity of the disease. Fetuin-A (ug/ml) significantly decreased in class C (mean 346.07±10.9) than class B (mean 411.1±17.04) than class a (mean 444.1±40.5). Fetuin-A (ug/ml) was significantly lower in HCC patients (mean 307.2±31.2) in comparison with liver cirrhosis patients (mean 400.4±48.5) than in controls (mean 551.3±42.3). There was significant negative correlation between serum fetuin A with the scores of the Child–Pugh ($r= -0.82$, $p<0.001$). There were significant negative correlations of serum fetuin-A-levels with bilirubin ($r = -0.68$, $p <0.001$), INR ($r = -0.56$, $p <0.001$), the degree of ascites ($r = -0.80$, $p <0.001$) and the grade of HE ($r = -0.33$, $p <0.001$) while there was high significant positive correlation of serum fetuin-A-levels with the level of albumin ($r = 0.61$, $p <0.001$).

Conclusion:

Fetuin-A was significantly lower in patients with liver cirrhosis and hepatocellular carcinoma than in healthy controls with lower levels in patients with hepatocellular carcinoma than liver cirrhosis. In patients with cirrhosis, marked decrease in the mean levels with the increased severity of the disease noticed.

INTRODUCTION

Fetuin-A is a major plasma glycoprotein that is mainly made in the liver. Fetuin-A is around 450–600 μg/ml in healthy persons. Fetuin is more abundant in fetal blood, hence the name "fetuin" (from Latin, fetus) **(1).**

Fetuin-A is one of the negative acute phase proteins (APP) (i.e. decreased in inflammation and malignancy) as prealbumin, albumin and transferrin **(2).**

There are few reports on fetuin-A levels in patients with liver diseases, and if these levels reflect the acute phase reaction or the parenchymal cell damage of the liver & if these levels can predict the fatal outcome of these patients or can predict the response to interferon in chronic hepatitis **(3).**

The rate of synthesis of fetuin by hepatocytes is reduced by cytokines and partial hepatectomy.

Circulating fetuin-A levels were found elevated in patients with non-alcoholic fatty liver disease (NAFLD) as the hepatic transcription of fetuin-A is co-regulated with genes regulating glucose and fat metabolism (chromosome 3q27).On the other hand, metformin induced a dose-dependent decrease in fetuin A secretion in vitro **(4).**

Aim of work:

Assessment of the relation of serum fetuin-A in patients with liver cirrhosis with various grades of severity of Child–Pugh classification and hepatocellular carcinoma.

SUBJECTS AND METHODS:

This work carried out in the Internal Medicine & Microbiology and Immunology Departments, Faculty of Medicine, Zagazig University, during the year 2014.

Subjects:

There were two groups of patients, liver cirrhotic & HCC patients. The first group included 30 patients with liver cirrhosis. The three Child–Pugh classification was represented (10 patients in each class). They were 19 males & 11 females with ages ranging from 47-59 years with a mean age ±SD 52.5±6.5 years. This group included nine HCV patients; three HBV & 18 patients have both HCV + HBV. The second group of patients included ten patients of hepatocellular carcinoma. They were eight males & two females with ages ranging from 52-55 years with a mean age ±SD 53.5±1.6 years. All cases of hepatocellular carcinoma were Child Pugh class C.

The study also included ten healthy subjects matched with age and sex with patients in our study as a control. They were five males & five females with ages ranging from 50-53 years with a mean age ±SD 51.3±1.7 years.

The three classes of liver cirrhotic patients according to Child–Pugh scores were:

- **Class A:** this class included 10 patients with CP score 5.8 ± 0.42. They were six males & four females, with ages ranging from 52-58 years with a mean ±SD 55.1±3.6 years. This class included three HCV patients, 1 HBV & six have mixed infection. The clinical & laboratory characteristics of this class were as the following: (bilirubin → 0.89 ± 0.35, albumin → 3.4 ± 0.38 & INR → 1.3 ± 0.25). The patients of this class had neither HE nor ascites except two patients with mild ascites.
- **Class B:** this class included 10 patients with CP score 8.1 ± 0.37. They were six males & four females with ages ranging from 47-59 years with a mean ±SD 53.8±6.4 years. This class included four HCV patients, 2 HBV & four have both HCV + HBV. The clinical and laboratory characteristics of this class were as the following: (bilirubin → 0.94 ± 0.55, albumin → 2.7 ± 0.37 & INR → 1.2 ± 0.16). Nine patients of this class have mild ascites & seven with grade I HE.
- **Class C:** this class included 10 patients with CP score 12.4 ± 1.3. They were seven males & three females, with ages ranging from 51-59 years with a mean ±SD 55.2±4.6 years. This class included two HCV patients & eight have both HCV + HBV. The clinical & laboratory characteristics of this class were as the following: (bilirubin → 3.9 ± 1.7, albumin → 2.04 ± 0.51 & INR → 1.6 ± 0.26). Ten patients of this class have tense ascites, one patient with grade II HE, 3 with grade III & 6 with grade IV.

Exclusion criteria: Patients who had received antiviral therapy within 6 months or lipid-lowering drugs for 6 weeks before the study, subjects with medical history of renal dialysis, diabetes mellitus, cardiovascular diseases or ischemic injury.

Methods

All subjects were subjected to:

a) Thorough history taking and clinical examination.

b) Routine laboratory investigations including liver function tests, renal function tests, urine analysis, complete blood picture, lipid profile, fasting and 2 hours postprandial blood glucose. Patients assessed for the presence of CVD using resting electrocardiography and echocardiography.

c) Research Investigations

1. Viral markers including hepatitis BsAg and HCV Ab by ELISA.
2. Alpha-fetoprotein tested .(5) ; using **Abbott AYSYM** automated immunoassay analyzer using a cut-off level of 200ng/ml **(6).**
3. Radiological investigations:
   - Abdominal ultrasound.
   - Abdominal triphasic computed tomography.

Diagnosis of cirrhosis and HCC in our study based on the radiological features using ultrasound & triphasic computed tomography (arterial phase enhancement followed by washout in venous and delayed phase).

4. Measurement of serum fetuin-A of the collected blood samples by ELISA: After blood sampling, the samples clotted at room temperature, and centrifuged at 3500 rpm for 15 min to collect serum samples. The sera to be tested were stored at −80°C until analysis and supernatant sera diluted to a ratio of 1: 10000. Fetuin-A was measured in diluted serum solutions by means of a human enzyme-linked immune-sorbent assay (ELISA) kit **(BioVendor Company)**.Fetuin-A ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human Fetuin-A protein in serum.

Test Principle

- Surface of wells in micro titration plate coated with polyclonal anti-human Fetuin-A specific antibody. Standards, Quality Controls (QC) and diluted samples are pipette into the wells.
- Any human Fetuin-A present captured by immobilized antibody and unbound protein washed away after the first incubation period. Then a horseradish peroxidase (HRP) conjugated polyclonal anti-human Fetuin-A antibody is added to the wells and incubated.
- After another washing step, when unbound antibody-HRP conjugate removed, a substrate solution (H_2O_2 and TMB) added to the wells.
- The enzymatic reaction yields a blue product that turns yellow when acidic stop solution added.
- The intensity of the color, measured spectro-photochemically at 450 nm, is directly proportional to the amount of the human Fetuin-A bound in the initial step.

Statistical analysis:

The statistical analysis of data was done by using excel program and SPSS program statistical packages for social science version 16. The description of the data done inform of mean (+/-) SD for quantitative data. Student t test used to test the significance between two means.

Correlation between variables was done using correlation coefficient (r) which detects if the change in one variable was accompanied by a corresponding change in the other variable or not.

Data analyzed by ANOVA, level of < 0.001 considered significant.

Spearman rank correlation coefficient was calculated to access correlation between fetuin-A and numerical parameters except for ordinal data Kendall tau rank correlation coefficient was calculated.

RESULTS

There were no significant differences between the three classes of liver cirrhotic patients as regard age, sex &AFP. There was a statistically significant difference as regard CP score, bilirubin, albumin, INR, presence & severity of ascites and the grade of encephalopathy among classes of liver cirrhotic patients (table1).

There was a statistically significant difference among the three classes of liver cirrhotic patients as regard fetuin-A, with marked decrease of the mean levels with the severity of the disease. Fetuin-A was significantly decreased in class B &C of CP compared to class A, with significant decrease in class C compared to class B. the mean level of serum fetuin-A was significantly higher in control group versus cirrhotic group. As regard fetuin-A, it was significantly lower in HCC patients versus the liver cirrhotic patients (table 1).

The AFP of HCC patients was below the cut-off level in 70% of the studied HCC patients and it was equal or above the cutoff level in only 30% of patients (table 2).

Our study showed significant negative correlation between serum fetuin A with the scores of the three classes of CP ($r=-0.82, p<0.001$).There were high significant negative correlations of serum fetuin-A-levels with bilirubin ($r=-0.68$, $p<0.001$),INR($r=-0.56$, $p<0.001$), the degree of ascites ($r = -0.80$, $p <0.001$) &the grade of HE ($r = -0.33$, $p <0.001$) , while there were high significant positive correlation of serum fetuin-A-levels with albumin ($r = 0.61$, $p <0.001$) (table3).

Table 1: Demographic and laboratory characteristics of the studied groups.

	Healthy controls	Chronic liver disease			HCC	F	P
		Class A	**Class B**	**Class C**			
Age	*51.3±1.7*	*55.1±3.6*	*53.8±6.4*	*55.2 ±4.6*	*53.5±1.6*	*0.23*	*NS*
Gender							
Male	*5*	*6*	*6*	*7*	*8*	*0.28*	*NS*
Female	*5*	*4*	*4*	*3*	*2*		
CP class		*5.8±0.42*	*8.1±0.37*	*12.4±1.3*	*12.6±1.4*	*121.6*	*<0.001*
Ascites							
No		*8*	*1*	*0*	*0*	*44.8*	*<0.001*
Mild		*2*	*9*	*0*	*2*		
Tense		*0*	*0*	*10*	*8*		
Grade of HE							
No		*10*	*3*	*0*	*0*	*46.1*	*<0.001*
I		*0*	*7*	*0*	*1*		
II		*0*	*0*	*1*	*1*		
III		*0*	*0*	*3*	*2*		
IV		*0*	*0*	*6*	*6*		
Albumin		*3.4±0.38*	*2.7±0.37*	*2.04±0.51*	*2.01±05*	*25.2*	*<0.001*

	Healthy controls	Chronic liver disease			HCC	F	P
		Class A	**Class B**	**Class C**			
Bilirubin		*0.89±0.35*	*0.94±0.55*	*3.9±1.7*	*3.8±1.90*	*24.7*	*<0.001*
INR		*1.3±0.25*	*1.2±0.16*	*1.6±0.26*	*1.61±0.29*	*9.2*	*<0.001*
AFP		*10±1.7*	*11±1.90*	*11.2±1.8*	*11.3±0.1.81*	*1.2*	*NS*
Fetuin-A	*551.3±42.3*	*444.1±40.5*	*411.±17.04*	*347.07±10.9*	*307.2±31.20*	*88.5*	*<0.001*
	551.3±42.3	*400.4±48.5*				*T-=8.7*	*<0.001*

Data presented in table (1) in the form of mean±SD. Albumin in gm/dl, bilirubin in mg /dl, AFP in ng/ ml, fetuin in ug/ ml. NS= non-significant

Table 2: Alpha fetoprotein (AFP) in the studied HCC patients.

AFP level	**Number of patients**	**mean±SD**
≥200	*3*	*470.3± 97*
<200	*7*	*72.1± 41.2*

Table 3 Correlation between fetuin A and CP score, albumin, bilirubin, degree of ascites and the grade of HE.

	r	P
Child Pugh score	*-0.82*	*<0.001*
Albumin	*0.61*	*<0.001*
Bilirubin	*-0.68*	*<0.001*
INR	*-0.56*	*<0.001*
Degree of ascites	*-0.80*	*<0.001*
Grade of HE	*-0.33*	*<0.001*

Discussion

Fetuin-A is predominantly expressed in the liver, and to a lesser degree in the placenta and the tongue. Placental expression is only relevant during pregnancy and the tongue is not an organ with endocrine activity. Therefore, the liver is the only organ regulating circulating fetuin-A levels **(7).**

Fetuin-A is around 450–600µg/ml in healthy persons. Because its serum level down regulated in infection, inflammation and malignancy, fetuin-A is known as a negative acute phase protein (APP) **(1).** The results of this study detected that the mean value of fetuin-A in liver cirrhosis was significantly lower than a healthy control cases. The results in our study agrees with **Verma-Gandhu et al., (8)** study, which demonstrated low serum fetuin-A levels in patients with biopsy proven hepatic fibrosis & cirrhosis related to HCV. They found that the incubation of fetuin-A with hepatic stellate cells samples (the major cell involved in hepatic cirrhosis) leads to significantly inhibited collagen synthesis in HSCs samples, as fetuin-A antagonist transforming growth factor beta (TGF-beta) & reduced platelet derived growth factor (PDGF) by 70% **(8).**

The results also were in agreement with **Ma et al., (9)** who demonstrated that low serum fetuin-A levels in patients with chronic hepatitis B virus(HBV) and it was significantly up regulated in HBe Ag seroconversion patients during IFN therapy **(9).**

Our results also agree with **Dai et al., (10)** study on chronic HBV cirrhotic patients, who showed that serum fetuin-A level was low in these patients **(10).**

The results of this study were also consistent with another study that done by **Cheung et al., (11)** on HCV cirrhotic patients, in whom they found low serum fetuin-A levels in these patients **(11).**

The results of our study found that the mean value of fetuin-A was significantly lower in HCC patients versus the liver cirrhotic patients. This finding is in agreement with those reported by **Kalabay et al., (3)** who demonstrated low serum fetuin-A levels in patients with various liver diseases &alterations were most marked in patients with alcoholic liver cirrhosis and hepatocellular carcinoma **(3).**

That is also consistent with **Baskies et al., (2)**. in a study of serum levels of acute-phase proteins in patients with solid malignancies either confined to the primary site or with regional spread only, they found low fetuin-A levels and the low levels of fetuin-A were correlated with tumor extent and quantitative delayed hypersensitivity **(2).** The results also are in agreement with Li et al. who stated that, fetuin-A expression in human HCC are reduced by proinflammatory cytokines as TNF, IL-1, IL-6 and IFN-ɣ. IFN-ɣ at concentration as low as 10-50 ng/ml, reduced fetuin-A expression by as much as 50-70 % **(12).**

Alpha fetoprotein (AFP), which is one of the tumor markers used for diagnosis of HCC, was below the cutoff level in 70% of the studied HCC patients and it was equal or above the cutoff level in only 30% of patients in our study. That is consistent with **Torbenson et al., (13)** who stated that AFP was limited in its diagnostic utility because 35%-50% of patients with HCC do not show high levels of AFP **(13).**

The results also in agreement with **Soresi et al., (14)** who stated that the positive predictive value of AFP is significantly lower in detecting HCC patients with viral etiology **(14).** In our study, we observed various changes in expression of fetuin-A in line with cirrhosis progression (Child-Pugh classes).

We found that there were high significant negative correlations of serum fetuin-A levels with the scores of the 3 classes of CP, INR, bilirubin, the degree of ascites and the grade of HE, indicating that with progression of cirrhosis and hepatocyte dysfunction, fetuin-A levels were decreased and the CP scores were increased & progression of grades of ascites, HE, and jaundice were occurred.

These findings were going hand in hand with results reported by **Kalabay et al., (15) (**They found that the sensitivity, specificity and predictive values of serum fetuin-A concentration exceed those of the CP & found significant negative correlations of fetuin-A with the CP scores, INR, bilirubin, the degree of ascites &the grade of HE **(15).** The results also were in agreement with the finding of the study of **Cheung et al., (11)** that done to find a useful fibrosis marker in HCV patient's sera of different fibrosis degrees (METAVIR F0-F4). They detected that fetuin-A exhibited a significant decreasing trend towards cirrhosis (F4) and significantly decreased in mild fibrosis (F1) in contrast to no fibrosis (F0). Fetuin-A significantly decreased in F4 opposite F0 and F1 (negative correlation with the liver pathological stage). So assay of fetuin-A were successful in predicting stages of fibrosis and cirrhosis.

Our results also agree with **Dai et al., (10)** study on chronic HBV cirrhotic patients, who showed that serum fetuin-A level was significantly higher in the surviving patients compared with those who succumbed to their illness and there was a negative correlation of serum fetuin-A with total serum bilirubin concentration. These findings indicate that the serum fetuin-A level directly linked to the severity of liver damage and might represent poor prognosis.

In our results, there were high significant positive correlations of serum

Fetuin –A levels with albumin. The positive correlation of serum fetuin-A levels with albumin can be interpreted in both ways of decreased levels of fetuin-A in liver cirrhosis as albumin is a negative acute phase reactant but is also widely considered an independent indicator of the reserve of hepatic protein synthesis. That is consistent with **Kalabay et al., (15).**

Consequently, the results of the present study are consistent with other studies, which suggest that serum fetuin-A level is decreasing in liver cirrhosis and HCC.

Alteration is most marked in patients with HCC. In addition, the results show the significant negative correlations of fetuin-A with the CP scores.

Conclusion

Fetuin-A was significantly lower in patients with liver cirrhosis and hepatocellular cancer. Alterations were most marked in patients with hepatocellular cancer than liver cirrhosis. There were high significant negative correlations of serum fetuin-A levels with the scores of the 3 classes of CP. This study showed also the correlation of fetuin–A level with the clinical and laboratory measures of liver cirrhosis as the following: high significant negative correlations of serum fetuin-A levels with INR, bilirubin, ascites and HE. On the other side, there were high significant positive correlations of serum fetuin-A levels with albumin.

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