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Serum Mannose - Binding Lectin in Egyptian Patients with Chronic Hepatitis C

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Approximately 60 – 85% of HCV infections cannot eradicate the virus and progress to chronic hepatitis, this may be explained by failure in host immune responses or by the ability of HCV to defeat host defense mechanisms. Mannose-binding lectin (MBL) may play an immunomodulatory role. This work intended to evaluate the relationship of MBL concentration to chronic HCV infection. Two groups, a patient group (n=50) with chronic hepatitis C, and a control group, (n=35) apparently healthy non-infected subjects were studied. MBL was measured by ELISA in both patients and controls. MBL was higher in chronic HCV patients (1159.86 ± 710.29) than in controls (329.7 ± 68.0) (p < 0.001). There was a negative correlation with the level of vireamia, and a positive correlation with the alpha-fetoprotein level. We conclude that MBL may play a role in modulating host immunity, persistence of HCV infection and the disease progression.

Hepatitis C is a contagious liver disease caused by the HCV. The virus is endemic throughout the world and it currently infects an estimated 175 million people worldwide. The WHO estimated that about 3% of the world population has HCV and that there are about 4 million carriers in Europe alone. Anti-HCV antibody is a sign of previous and current infection and does not distinguish between acute and chronic infections [1].

HCV can cause both acute and chronic hepatitis infection. Acute Hepatitis C is a short-term illness that develops early after exposure to the HCV within the first 6 months and it is usually a sub-clinical disease. Approximately 60 – 85 % of patients develop "chronic," or lifelong, infection. Most of these patients can show serious liver complications, such as liver damage, cirrhosis, liver failure, or liver cancer. The incubation period for acute HCV infection ranges from 14 to 180 days with an average of 6 to 7 weeks [2].

Mannose binding lectin (MBL) is an important constituent of the human innate immune system, considered as an acute-phase reactant, released into the circulation by the liver. MBL is a circulating C-type lectin that can bind through multiple carbohydrate recognition domain (CRD) to polysaccharide glycans expressed on the surface of a wide range of microorganisms, and play a role in activation of macrophages and the complement system cascade. It was also reported that serum MBL plays a regularity role in release of pro-inflammatory cytokines such as TNF-α, IL-6 and IL-1β from phagocytes in response to microbial infection [3].

The role of MBL in HCV pathogenesis has received a lot of attention in the last few years. The clear implication that MBL plays a major role in elimination of the virus raises the possibility that MBL replacement therapy may be beneficial for hepatitis C carriers with low levels of MBL. MBL may play an immunomodulatory role during treatment with IFN, as MBL regulates the release of different cytokines from immune cells in response to infection [4].

The aim of this work was to evaluate the relationship of MBL concentration to chronic HCV infection.

Subjects and Methods

This is a cross sectional study conducted at the Departments of Clinical Pathology and Tropical Medicine, Sohag University Hospital, Egypt. The study included 50 chronic HCV patients who were recruited from outpatient clinics of the Tropical Medicine Department, Sohag university hospital and 35 apparently normal persons as a control group. The study was approved by the Ethics Committee of Sohag faculty of medicine, Sohag University and written informed consents were obtained from all participants.

All Subjects were subjected to history taking including age, sex, occupation, residence, exposure to risk factors like previous operations or blood transfusion (for HCV). Complete clinical examination including manifestations of hepatitis and liver cell failure such as jaundice, hepatomegaly, splenomegaly, ascitis, and lower limb oedema was performed. Patients were subjected to abdominal ultrasonography (U/S) to document the presence of hepatic enlargement, focal lesion(s) and cirrhosis.

Laboratory investigations

10 ml of venous blood were withdrawn from each subject under aseptic conditions and by using sterile disposable gloves.

- The following investigations were performed:

1. Liver function tests including Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline phosphatase (ALP), total bilirubin (TB) and Albumin were determined by Cobas c311 Chemistry Analyzer System (Roche Diagnostics, GmbH, Mannheim, Germany).

2. Complete Blood Count (CBC): CBC was done by CEII – DYN 3700 (Abbott Laboratories, Diagnostic Division, IL, USA).

3. Alpha-fetoprotein (AFP) and serological testing for both anti-HCV and HBsAg were evaluated by Architect i1000SR system (Abbott Laboratories, Diagnostics Division, Abbott Park, IL).

4. Quantitative Real time PCR for the detection of HCV RNA and viral load:

A- Extraction (Isolation) of RNA from serum: HCV viral RNA was extracted form serum samples by the use of fully automated QIAcube instrument and using specific kits; QIAgen columns (QIAamp Viral RNA Mini kit plus QIAmp Viral RNA Mini Accessory SET, cat. No. 1048147 QIagen Inc) according to the manufacturer' instructions.

B- Real time quantitative PCR: HCV viral load was quantified by using specific TaqMAn® probe-based technology and 7500 Fast Real Time-PCR system (Applied Biosystems, CA, USA), according to the manufacturer' instructions. The reaction mixture was used in a total volume of 25 µ including 10 µ of the sample extract and 15 µ of the Master Mix which prepared by: 6 µ Hep. C virus Master A and 9 µ Hep. C virus Master B. The real-time cycler conditions were performed according to the following conditions: Incubation at 50° C for 30 min then at 95° C for 15 min. to activate theTaq enzyme followed by 50 amplification cycles. Each cycle consists of denaturation at 95°C for 30 sec, primer annealing at 50 °C for 60 sec and primer extension at 72 °C for 30 s.

5. Detection of Mannose Binding Lectin (MBL) in serum:

Blood samples were withdrawn from the patients and controls for detection of serum level of MBL. The blood samples were allowed to clot for 10 – 20 minutes at room temperature and then were centrifuged for 20 minutes at 1000×g. The supernatants were collected and were divided into aliquots and stored at -20˚C.

MBL was measured in serum samples using an enzyme-linked immunosorbent assay (ELISA) kit (Glory Science Co., Ltd, 2400 Veterans Blvd. Suite 16 – 101, Del Rio, TX 78840, USA, CATALOG #: 11430), according to the manufacturer' instructions. A standard curve was prepared by serial dilution of standards supplied with the assay. Standards and diluted serum samples were added to a 96-well plate coated with purified human MBL antibody. The plate was incubated for 30 min. at 37 °C. Following complete washing, combined MBL which with HRP labeled antibody, become antibody-antigen-enzyme-antibody complex. The detection antibody was incubated for 30 min. at 37 °C, and after complete washing, tetramethylbenzidine (TMB) substrate was added. TMB substrate became blue colour as HRP enzyme-catalyzed. The enzyme reaction was stopped

by the addition of a stop solution. The absorbance was measured at 450 nm using the Stat fax 2600 microplate reader (Awareness Technologies, USA). The human MBL concentration of samples, was determined by comparing the O.D. of the samples to a standard curve.

Statistical Methods

Data were analyzed using SPSS (Statistical Package for Science and Society). Descriptive statistics are presented as means ± standard deviations (SD) for the quantitative variables. Independent sample t-test was used for comparing normally distributed quantitative variables between groups. Correlation analysis was used when appropriate P value lower than 0.05 were considered statistically significant for all tests.

Results

Our study included 50 patients with positive HCV (group I), their age ranged from 21 to 59 years (39 males, 11 females, with mean±SD age value: 45.92±9.64). The control group (group II) included 35 healthy subjects, their age ranged from 22 and 55 years (25 males, 10 females, with mean age value: 43.17 ± SD 8.72).

Serum MBL concentrations were significantly higher in the chronic HCV (group I) (1159.86 ± 710.29) than in the control (group II) (329.7 ± 68.0) (P<0.001).

There was a significant negative correlation between MBL concentrations in HCV patients (group I) and HCV viral load (r= - 0.999; P <0.01) (Figure 1).

There was a significant positive correlation between MBL concentrations in HCV patients (group I) and alpha-fetoprotein (r= - 0.996; P<0.01) (Figure 2).

There was a significant difference in AFP level between group I as compared with group II (P< 0.001) (Table 1).

Regarding liver function tests, a significant difference among the two studied groups was observed, (table II). Serum ALT and serum AST levels showed significant elevation in chronic HCV group (group I) as compared to the control group (group II) (P<0.001), while serum total protein and serum albumin level showed significant decrease in chronic HCV group (group I) as compared to control group (group II) (P<0.001).

Comparison of laboratory parameters of CBC for the studied groups is shown in table II. The mean HB concentration and platelet count decreased in chronic HCV group (group I) as compared to controls (group II) (P < 0.05) and (P < 0.001), respectively. However, there was no significant difference in WBCs among the two groups.

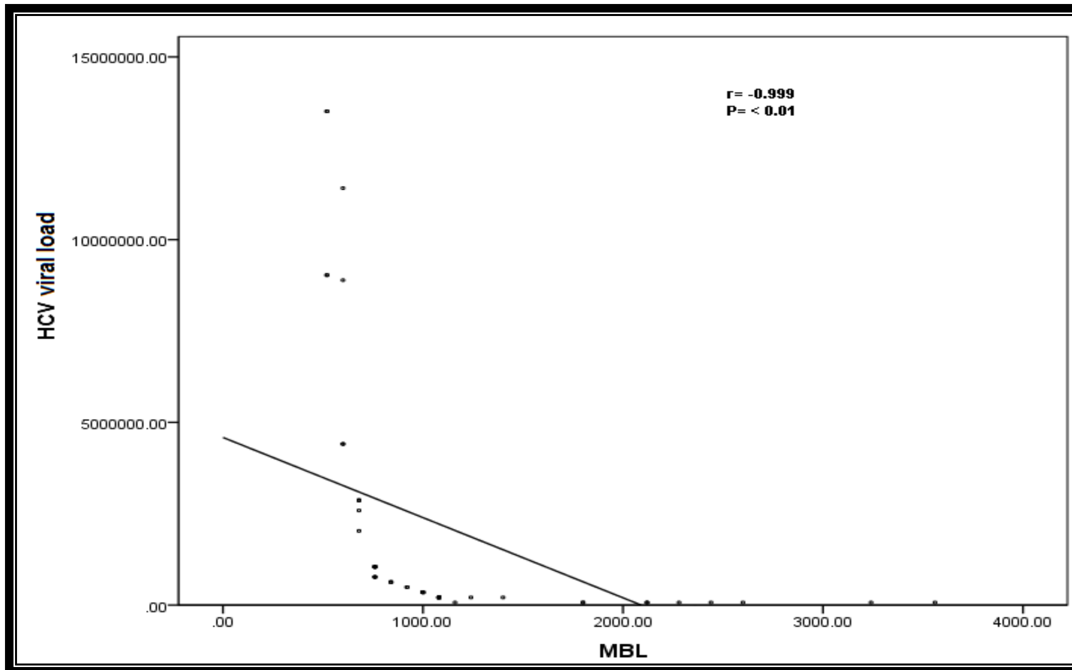

Figure 1. Correlation between MBL concentrations and HCV viral load in HCV infected patients. (r = - 0.999, $P < 0.01$; Spearman Correlation Test).

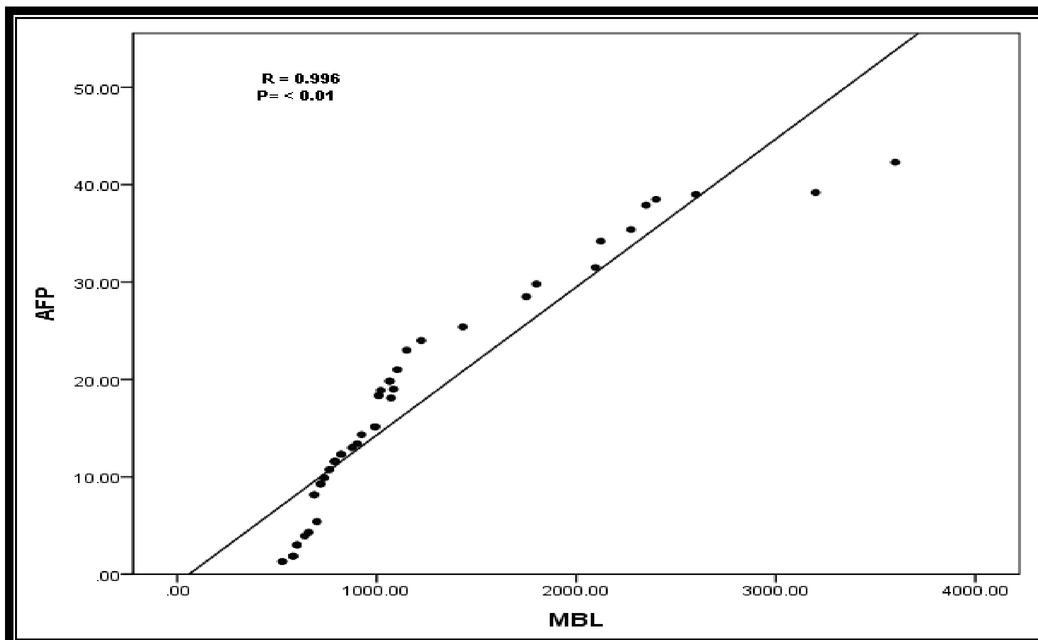

Figure 2. Correlation between MBL concentrations and AFP in HCV infected patients. (r = 0.996, $P < 0.01$; Spearman Correlation Test).

Table 1. Comparison of clinical data of group I versus group II.

	Group I (Chronic HCV patients) Mean ± SD	Group II (Control) Mean ± SD	*P* value
1-Age (years)	45.92 ± 9.64	43.17 ± 8.72	
2-Body weight (kg)	74.18 ± 8.23	75.00 ± 8.26	
3-liver function tests:			
a- ALP (IU/L)	90.22 ± 50.56	78.17 ± 17.84	NS
b- AST (IU/L)	69.42 ± 20.19	16.06 ± 5.7	< 0.001
c- ALT (IU/L)	59.3 ± 45.57	20.51 ± 5.59	< 0.001
d- Albumin (g/dl)	2.18 ± 0.24	5.19 ± 0.32	< 0.001
e- T. Protein (g/dl)	5.65 ± 0.39	7.62 ± 0.95	< 0.001
4- CBC:			
a- Hb (gm/dl)	12.84 ± 2.06	13.77 ± 0.92	<0.05
b- Platelets/ mm^3	229.62 ± 87.03	296.62 ± 48.13	<0.001
c- WBCs/ mm^3	6.46 ± 3	6.06 ± 1.96	NS
5- AFP (ng/ml)	16.72 ± 11.55	1.56 ± 1.08	< 0.001
6- HCV viral load (IU/ml)	2042945.08 ± 3551960.73	---	---
7-MBL (µg/l)	1159.86 ± 710.29	329.7 ± 68.0	< 0.001

P > 0.05 is not significant (NS)

Discussion

The present study evaluated the serum concentration of MBL concentration in Egyptian patients with chronic Hepatitis C. We found that serum MBL concentration was significantly increased in chronic hepatitis C patients than healthy control group ($P < 0.05$). These results are compatible with other studies, Kilpatrick *et al.* [5] explained these observations as another reflection of MBL as an acute phase reactant and is consistent with the observed increase in MBL in hepatitis C patients compared to controls in the study of Esmat *et al.* [4].

The results of our study did not agree with some previous researches [6, 7] who found that the overall circulating levels of MBL showed no significant difference between HCV patients and controls. Also, it did not agree with Yuen *et al.* [8] who found that MBL levels in hepatitis C patients with and without symptomatic cirrhosis were significantly lower than those in the controls, he explained the decrease in MBL levels in patients with chronic hepatitis C infection in his study that, unlike in acute infection, chronic viral infection suppresses protein production non specifically, including MBL. This disagreement may be due to the discrepancy in the investigated populations or sample size differences.

Though the MBL was higher in chronic HCV patients, we found that it has a negative correlation with the level of viremia detected by PCR, in the same direction Mohamed *et al.* [9] proved the same

correlation as ours, this finding support that the MBL has an important role in the innate immunity which has been proven by many researchers [10 – 12].

The positive correlation between the MBL level in chronic HCV patients, and the levels of AFP is another finding was detected in our study, and this result reinforce that of Esmat *et al.* [4], they found the same correlation in Egyptian patients, moreover they postulate that the level of both (MBL and AFP) increased as the advancement of the fibrosis stage, these results go with previous studies that concluded that there is a strong association between the high MBL levels and the disease progression [8 &13].

We could conclude that the increase in serum MBL in chronic HCV patients may play a role in host defense immunity and regulation of inflammatory process against HCV infection.

References

1. Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. Hepatol.. 2013; 57(4):1333-42.

2. Umar M, Khan AG, Abbas Z, Arora S, Elewaut A, Esmat G, Foster G, Fried M, Goh K, MD, Hamama TBK, Imawari M, Isakov V, Krabshuis J, LaBrecque D, LeMair A, MD, Malfertheiner P, Ryder S, Schiedermaier P, Stimac D, Tandon R, Villamil F, Zapata R, Ferenci P.. Diagnosis, management and prevention of hepatitis C. Prevention. 2013; 10:4.

3. Zhao M-f, Wan T-h, Song G-z, He J-l, Chen Z. Association between Mannose-Binding Lectin Gene Polymorphisms and Hepatitis B Virus Infection: A Meta-Analysis. PloS one. 2013; 8(10):e75371.

4. Esmat S, Omran D, Sleem GA, Rashed L. Serum mannan-binding lectin in egyptian patients with chronic hepatitis C: its relation to disease progression and response to treatment. Hepatitis monthly. 2012; 12(4):259.

5. Kilpatrick D, Delahooke T, Koch C, Turner M, Hayes P. Mannan-binding lectin and hepatitis C infection. Clin & Exp Immunol. 2003; 132(1):92-5.

6. Alves Pedroso M, Boldt A, Pereira-Ferrari L, Steffensen R, Strauss E, Jensenius JC, Ioshii SO, Messias-Reason I. Mannan-binding lectin MBL2 gene polymorphism in chronic hepatitis C: association with the severity of liver fibrosis and response to interferon therapy. Clin & Exp Immunol. 2008; 152(2):258-64.

7. Brown KS, Ryder SD, Irving WL, Sim RB, Hickling TP. Mannan binding lectin and viral hepatitis. Immunology letters. 2007; 108(1):34-44.

8. Yuen MF, Lau CS, Lau YL, Wong WM, Cheng CC, Lai CL. Mannose binding lectin gene mutations are associated with progression of liver disease in chronic hepatitis B infection. Hepatol. 1999; 29(4):1248-51.

9. Mohamed AA, Elbedewy TA, Faried A, Ismael OE, Kotb NS, Ramadan N, Zaki NA, El Shimy A. Pretreatment serum mannose-binding lectin as an early predictor for sustained virological response to pegylated interferon/ribavirin combination therapy in Egyptian patients with hepatitis C virus. Universal J Pharmacy 2015, 04 (01): 49-56

10. Tarr AW, Urbanowicz RA, Ball JK. The role of humoral innate immunity in hepatitis C virus infection. Viruses. 2012; 4(1):1-27.

11. Kilpatrick D. Mannan-binding lectin and its role in innate immunity. Transfusion Medicine. 2002; 12(6):335-52.

12. Holmskov U, Malhotra R, Sim RB, Jensenius JC. Collectins: collagenous C-type lectins of the innate immune defense system. Immunology today. 1994; 15(2):67-74.

13. Brown K, Keogh M, Tagiuri N, Grainge M, Presanis J, Ryder S, Irving WL, Ball JK, Sim RB, Hickling TP. Severe fibrosis in hepatitis C virus-infected patients is associated with increased activity of the mannan-binding lectin (MBL)/MBL-associated serine protease 1 (MASP-1) complex. Clin & Exp Immunol. 2007; 147(1):90-8.