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Serum Apolipoprotein A-Iv Level in HCV Related Liver Diseases

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Abstract

Background: *Hepatitis C virus (HCV) is a globally prevalent pathogen and chronic infection eventually causes cirrhosis leading to hepatocellular carcinoma (HCC) and ultimately death. Accurate assessment of liver fibrosis has become increasingly important as it allows for individualized management. Liver plays a key role in the metabolism of plasma apolipoproteins. Apolipoprotein A-IV (apoA-IV) is a component of intestinally derived, triglyceride-rich lipoproteins.*

Objective: *The present work was designed to study the level of apo A-IV in HCV related liver diseases.*

Methods: *Sixty treatment-naive patients with HCV related liver disease were enrolled in the study. They were classified as follow: twenty patients with chronic HCV infection (stage of fibrosis ≤ F3), twenty patients with HCV related liver cirrhosis, twenty patients with HCV related HCC and ten normal subjects as control. Serum level of apoA-IV was measured using enzyme linked immunosorbant assay (ELISA). Liver biopsies from patients with chronic HCV were examined to assess histological activity grade and fibrosis stage according to METAVIR scoring system and triphasic CT for HCC patients was performed.*

Results: *Apo A-IV level was significantly lower in liver cirrhosis than chronic hepatitis patients with a cutoff point of <36.4 (p<0.001). The receiver operating characteristic (ROC) curve showed an area under the curve (AUC) of 0.990 (P < 0.001), a sensitivity of 100% and a specificity of 90% for diagnosis of cirrhosis. Also, it was significantly lower in liver cirrhosis than HCC patients with a cutoff point ≤25.1 (p<0.001). The ROC showed AUC of 0.876, a sensitivity of 80.0 % and a specificity of 93.33% for diagnosis of HCC.*

Conclusion: *Serum apo A-IV showed bi-modal elevation in HCV related liver diseases and could be used as a noninvasive marker for liver cirrhosis in patients with chronic HCV infection and could be a promising marker for early detection of HCC in HCV hepatic cirrhosis.*

Key words: *(Apolipoprotein A-IV, Hepatitis C Virus, Liver cirrhosis, Hepatocellular carcinoma)*

INTRODUCTION

Hepatitis C virus continues to be a major cause of concern and a huge burden on public health systems worldwide. The WHO estimates that a minimum of 3 per cent of the world's population is chronically infected with HCV. [1] However, within this chronically infected population the disease outcomes

vary, it can be mild or severe and can lead to scar tissue formation. Chronic infection eventually causes cirrhosis leading to HCC and ultimately death. [2] Globally, at least one third of HCC cases are attributed to HCV infection and 350,000 people die from HCV related diseases per year. [3] With the advancement in the treatment of patients with chronic liver diseases, the accurate assessment of liver fibrosis has become increasingly important as it allows for individualized management. The lack of accurate, reproducible and easily applied methods for assessment of hepatic fibrosis has been the major limitation for both the clinical management and research in liver diseases. [4] For the past 50 years; liver biopsy has been considered to be the gold standard for staging of liver fibrosis. However, many recent studies clearly highlight several crucial drawbacks of liver biopsy.[5] In recent years, interest in identifying and describing liver fibrosis by using non invasive surrogate markers has been on the rise. [6]

Under normal physiological conditions, liver ensures homeostasis of lipid and lipoprotein metabolism. Hepatic cellular damage and HCC impairs these processes, leading to alterations in plasma lipid and lipoprotein patterns.[7] Understanding the tight association between HCV life cycle and lipoprotein metabolism is very important because HCV infection is a unique model wherein the virus causes chronic infection while coexisting with the host and simultaneously taking over the host's metabolism.[8] In addition, the anomalous lipid metabolism caused by HCV infection may lead to liver injury and hepatocarcinogenesis.[9] However, clinical studies have indicated that HCV induced over expression of lipogenic genes may exert a strong influence on inflammation and fibrosis progression of the infected liver, rather than causing the lipid accumulation observed in hepatic steatosis. [10] Mature apo A-IV is a 377 amino acids peptide and contains about 6% carbohydrate. It is present in plasma in various isoforms. Its gene is located on chromosome 11. It is synthesized in the intestine. [11]

SUBJECTS AND METHODS

This study was conducted on 60 patients with HCV related liver disease. The patients were classified into three groups: Twenty patients with chronic hepatitis C (CHC) who were diagnosed by presence of circulating anti HCV antibody and histopathological finding consistent with chronic hepatitis (stage of fibrosis ≤ F3), twenty patients with HCV related liver cirrhosis (based on clinical, laboratory and/or radiological finding of cirrhosis), twenty patients with HCV related HCC (based on having focal hepatic lesion(s) in a background of cirrhosis evidenced by triphasic CT and the characteristic criteria of HCC) and ten normal individual were enrolled in the control arm. The human apo A-IV ELISA kit (Wuhan EIAab Science Co., Ltd. optics valley, Wuhan, China) was measured in the serum of all enrolled cases. [12]

Patients were excluded from the study if they had HBV infection; concomitant schistosomiasis; history of alcohol consumption; other known causes of chronic liver disease; cardiac, respiratory or renal diseases; any kind of malignancy or known chronic diseases such as diabetes mellitus, or connective tissue disorders. Patients who had received anti-viral or immunomodulatory therapy were also excluded. The study was conducted in accordance with the provisions of the Declaration of Helsinki and Good Clinical Practice guidelines. An informed consent was obtained from all subjects prior to procedures initiation.

Once admitted into the study, the base line assessment included: age, gender, symptoms and signs of chronic liver disease, liver and spleen size, serum albumin, serum bilirubin, serum aspartate and alanine aminotransferases (AST and ALT respectively), serum gamma glutamyl transpeptidase (GGT), prothrombin time, international normalized ratio and serum creatinine. HCV antibodies, hepatitis B surface antigen and hepatitis B core antibody using ELISA and HCV RNA levels in serum using real time polymerase chain reaction assay. Abdominal

ultrasonographic examination was used to assess: liver echo-pattern and presence of hepatomegaly. While triphasic CT was employed to assess focal hepatic lesions to establish HCC diagnosis.

Histopathological examination:

Core liver biopsies were obtained from all patients with CHC. All specimens were fixed in a 10% formalin solution and embedded in paraffin. Tissue sections were stained with: ***Hematoxylin-Eosin and Trichrome Stains*** for assessment of necroinflammation grade and fibrosis stage according to the METAVIR scoring system composed of a two-letter and two-number coding system: [13] A = Histological activity was graded as A0 = no activity, A1 = mild activity, A2 = moderate activity and A3 = severe activity. F = Fibrosis stage was defined as F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = portal fibrosis with rare septa, F3 = numerous septa without cirrhosis and F4 = cirrhosis.

Statistical analysis of the data [14]

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0.[15] Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum) mean, standard deviation and median. Comparison between different groups regarding categorical variables was tested using Chi-square test. When more than 20% of the cells showed count less than 5, correction for chi-square was conducted using Monte Carlo correction. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test and D'Agstino test, also Histogram and QQ plot were used for vision test. Normal data distribution entailed parametric tests while non-parametric tests were applied in presence of abnormally distributed data. For normally distributed data, comparison between the different groups was done using F-test (ANOVA) and Post Hoc test (Tukey) while for abnormally distributed data, comparison was done using Kruskal Wallis

test and pair wise comparison was assessed using Mann-Whitney test. Significance of the obtained results was judged at the 5% level.

RESULTS

Characteristics of studied patients are presented in tables I. Table II showed the statistical comparisons between patients with CHC, cirrhosis, HCC and healthy subjects as regards the serum level of apo A-IV. Apo A-IV level was significantly lower in liver cirrhosis than chronic hepatitis patients with a cutoff point of < 36.4 (p<0.001). As shown in figure 1, the ROC curve showed an AUC of 0.990 (P < 0.001), a sensitivity of 100% and a specificity of 90% for diagnosis of cirrhosis. Also, it was significantly lower in liver cirrhosis than HCC patients with a cutoff point ≤25.1 (p<0.001). The ROC showed AUC of 0.876, a sensitivity of 80.0 % and a specificity of 93.33% for diagnosis of HCC. (as shown in figure 2) There was no significant correlation between apo A-IV level and age, gender, liver profile tests, AFP, lipid profile, CRP, hepatic histopathological changes nor the BCLC.

Table I: Characteristics of studied patients

	Chronic HCV (n = 20)		Liver cirrhosis (n = 20)		HCC (n = 20)	
	No.	%	No.	%	No.	%
Gender						
Male	8	40.0	12	60.0	11	55.0
Female	12	60.0	8	40.0	9	45.0
Age (years)	44.15 ± 6.33		59.05 ± 5.69		62.85 ± 4.51	
ALT (U/L)	38.35 ± 15.97		49.65 ± 22.97		48.45 ± 17.73	
AST (U/L)	42.35 ± 14.43		63.95 ± 25.89		58.65 ± 25.04	
Serum albumin (g/dL)	3.75 ± 0.30		2.67 ± 0.48		2.71 ± 0.44	
Total Bilirubin (mg/dL)	0.88 ± 0.33		2.22 ± 1.61		2.45 ± 1.67	
Direct Bilirubin (mg/dL)	0.34 ± 0.24		1.36 ± 1.14		1.72 ± 1.46	
GGT (U/L)	24.85 ± 7.52		23.45 ± 7.86		25.60 ± 9.28	
Prothrombin activity (%)	93.12 ± 4.72		58.03 ± 12.96		63.83 ± 13.56	
Alpha feto protein (ng/mL)	4.42 ± 1.97		6.46 ± 2.36		1071.38 ± 1970.87	
HbA1C (%)	5.89 ±0.36		5.89 ± 0.36		5.80 ± 0.45	
C reactive protein (mg/L)	2.48 ± 1.62		2.96 ± 1.60		2.76 ± 2.20	
Total cholesterol (mg/dL)	125.60 ± 21.04		124.90 ± 18.40		141.90 ± 31.58	
Tri glyceride (mg/dL)	104.0 ± 25.33		102.25 ± 22.24		89.45 ± 22.06	
HDL (mg/dL)	25.45 ± 7.79		25.65 ± 7.67		26.50 ± 7.03	
LDL (mg/dL)	55.60 ± 19.85		51.40 ± 25.11		70.20 ± 30.99	

ALT: alanin aminotransferase, AST: aspartate aminotransferase, GGT: gamma-glutamyltransferase, HbA1c:glycated hemoglobin, HDL : High density lipoprotein , LDL : Low density lipoprotein

Table II: Comparison between the four groups according to apolipoprotein A-IV serum level

	Chronic HCV (n = 20)	Liver cirrhosis (n = 20)	HCC (n = 20)	Control (n = 10)	KWχ^2	P
Apolipoprotein A-IV(ng/ml)						
Min. – Max.	37.80 – 98.70	15.50 – 46.10	24.10 – 69.80	25.20 – 56.20	46.847[*]	<0.001[*]
Mean ± SD.	68.19 ± 17.63	25.01 ± 7.91	37.59 ± 11.10	39.13 ± 8.97		
Median	71.05	23.10	36.90	38.65		
p Cont.	<0.001[*]	<0.001[*]	0.428			
Sig.bet. Grps.	p₁<0.001[*], p₂<0.001[*], p₃<0.001[*]					

KW: Kruskal Wallis test, grps was done using Mann Whitney test

p_{cont}: p value for comparing between group control and each other group

p_1: p value for comparing between chronic HCV and liver cirrhosis

p_2: p value for comparing between chronic HCV and HCC

p_3: p value for comparing between liver cirrhosis and HCC

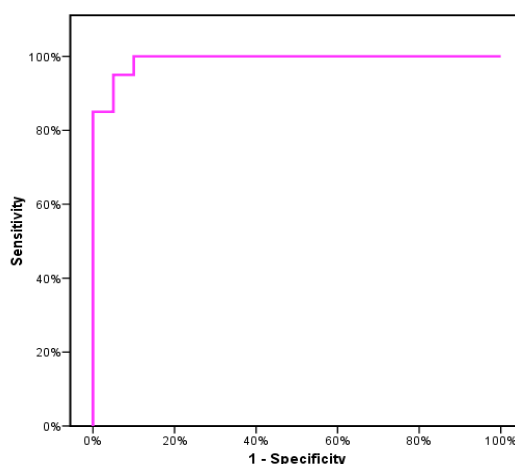*: Statistically significant at $p \leq 0.05$

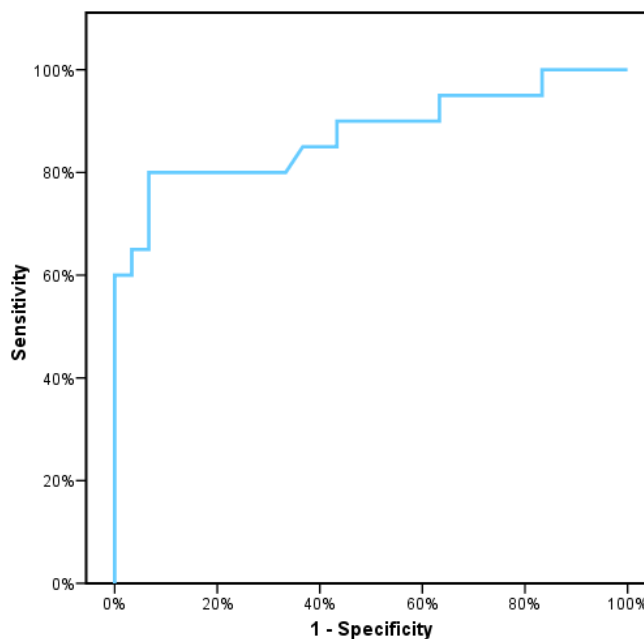**Figure (1):** ROC curve for Apolipoprotein A-IV between chronic HCV and cirrhotic patients

Figure (2): ROC curve for Apolipoprotein A-IV between HCV related cirrhosis and HCC

DISCUSSION

In the present study, apo A-IV level greatly reduced in the liver cirrhosis patients, compared with the chronic hepatitis patients and reduced level of apo A-IV in liver cirrhosis group compared to HCC group. In agreement with these results, Seishima et al, found that there was a decreased intestinal apo A-IV and its mRNA levels in a carbon tetrachloride (CCl4)-induced cirrhosis rat model. This may be related to the decreased ability to absorb fat in cirrhosis. [16] Also, Pleguezuelo et al, compared its level in plasma between cirrhotic patients with and without HCC. They found that apo A-IV was significantly higher in patients without HCC. Their study revealed that levels of apoA-1 and apoA-IV were the only factors independently associated with HCC. Interestingly, apoA-IV was associated with lower risk of HCC. ROC curve for this equation using a cut-off level of 0.35 showed an area under the ROC of 0.91; with 89% sensitivity and 91% specificity for diagnosis of HCC. [17]They conducted their study on patients with liver cirrhosis secondary to (HBV, HCV and alcoholic liver disease) in contrast to our study which was conducted on HCV related cirrhosis and HCC.

Using two-dimensional polyacrylamide gel electrophoresis, Sugimoto et al attempted to isolate novel specific biomarker in the sera of patients with chronic hepatitis, cirrhosis and HCC. One of the differentially expressed proteins was apo A-IV isoform. No change was found in the expression pattern of this isoform in either normal or chronic hepatitis sera, whereas a significant reduction in the expression level of this protein was observed in the sera of LC and HCC patients. [12]

Metabolic syndrome has closely linked to the development of human HCC. By using the HBV X (HBx) transgenic mouse model, Teng et al studied the dynamic evolution of serum and liver profiles of lipids and global cDNA expression at different stages of HBx tumorigenesis. They observed that the lipid (triglycerides, cholesterol, and fatty acids) profiles revealed a biphasic response pattern during the progression of HBx tumorigenesis: a small peak at early phase and a large peak or terminal switch at the tumor phase. By analyzing cDNA microarray data, the early peak correlated to the oxidative stress and pro-inflammatory response, which then resolved at the middle phase and were followed by the terminal metabolic switch in the tumor tissues. Five lipid

metabolism-related genes, the arachidonate 5-lipoxygenase, lipoprotein lipase, fatty acid binding protein 4, 1-acylglycerol-3-phosphate O-acyltransferase 9, and apo A-IV were identified to be significantly activated in HBx transgenic HCCs and further validated in human HBV-related HCCs. Inhibition of these lipid genes could reverse the effect of HBx on lipid biosynthesis and suppress HBx-induced cell proliferation in vitro. These results support the concept that metabolic syndrome plays an important role in HBV tumorigenesis. The dysregulation of lipid metabolic genes may predict the disease progression to HCC in chronic hepatitis B patients. [18]

Level of apo A-IV is altered in HCC patient because lipids and lipoprotein metabolism could be regulated by cytokines. For instance, interleukin-6 (IL-6), tumor necrosis factor (TNF-α), IL-1 may inhibit TG synthesis.[19]Tumor cells are known to produce large amounts of pro-inflammatory cytokines known to suppress plasma TG levels. Argiles, et al, reported that IL1 profoundly affects lipid metabolism by delaying intestinal absorption and decreasing tissue uptake. [20] IL-1 and IL-6 significantly decreased microsomal triglyceride transfer protein mRNA levels in HepG2 cells.[21]Apo A-IV has been also incriminated in other types of cancers. Zervos et al found that apo A-IV gene was up-regulated in familial pancreatic cancer PCA. [22]

Li et al, [23] studied the influence of apoA-IV on the secretion of cytokines stimulated by lipopolysaccharide (LPS) in the cells. The macrophage RAW264.7 cells were pre-treated with apo A-IV (20μg/ml) for one hour, followed with LPS (500ng/ml) for 20 h. The culture medium was collected and RayBio® Mouse Inflammation Antibody Array G Series I was used to measure the cytokines. ApoA-IV was found to reduce Fas ligand, Fractalkine and Eotaxin-2. Both apo A-IV and LPS increased the cytokines GCSF, IL-2, IL-3, IL-4, IL-6, IL-9, IL-12P40/P70, GCSF, TIMP-1, LIX, SDP-1, TCA-3, TECK, TIMP-2, sTNF-RI, sTNF-RII, GM-CSF, RANTES, I-TAC, KC,

MCP1,TNF-α, TECK, IL-17 and Leptin. Notably, TNF-α and TECK induced by LPS were significantly inhibited by apo A-IV. Interestingly, apo A-IV enhanced IL-10 and IL-1α by itself and that induced by LPS.

This significant change in apo A-IV serum level in liver disease could be due to the peculiar relationship between HCV infection and lipids as HCV virions circulate in serum bound to lipoproteins and lipids have been shown to modulate (and, indeed, are essential to) the HCV life cycle. As a result, lipid metabolism is overall altered, conferring an idiosyncratic profile to HCV infection. [24] Most Apo, lipids and lipoproteins, are synthesized in the liver. Thus hepatocellular injury or chronic liver diseases including HCC may result in abnormal pattern of these molecules in plasma. [25]Although the precise physiological function of apoA-IV has yet to be deduced, its altered level in chronic hepatitis group compared to the control group may be due to its anti inflammatory anti oxidant role which can be proved through several studies which had demonstrated its anti atherogenic role. Wong et al found that apo A-IV was found to be protected against atherosclerosis without an increase in HDL concentration and displayed reduced oxidative markers. Apo A-IV was found to have accumulated in the damaged arterial wall of these animals thus acting as an antioxidant in situ. [26]

Recalde et al suggested that human apolipoprotein (h-apoA-IV) exerts its protective role by inhibiting the lipopolysaccharide (LPS)-induced stimulation of monocytes. The systemic proinflammatory response was less strong in LPS-stimulated human apolipoprotein A-IV in apoE-deficient mice(h-apoA-IV/E0) mice than in LPS-stimulated apoE-deficient (apoE0) mice. [27]Also, Khovidhunkit et al suggested that apo A-IV and A-V are positive acute-phase proteins that increase in the serum during inflammation. They found that Interleukin-6 increased apo A-IV and apo A-V mRNA levels in Hep3B cells.[28] Spaulding et al found that apo A-IV could limit the cellular apoptotic response to two different oxidant chemicals: *tert*-butyl

hydroperoxide (TBH), a model hydroperoxide, and diamide, a cell-permeantthiol oxidant, A-IV resembles N-acetyl cysteine (NAC) in its protective ability[.(29)]

After low-number transplantation of islets of Langerhans into the liver of streptozotocin-diabetic rats, the hepatocytes in the acini are exposed to a local hyperinsulinemia, whereas the remaining tissue is affected by hypoinsulinemia. In this model, insulin induces alterations that resemble preneoplastic foci of altered hepatocytes (FAH) and develop into hepatocellular tumors in later stages of carcinogenesis. In rodents, apo A-IV is synthesized in the small intestine and the liver. Whereas intestinal production is mainly influenced by lipid intake and chylomicrone formation, little is known about mechanisms regulating hepatic A-IV synthesis. As it is known that insulin modulates lipoprotein metabolism in different ways, they investigated the effect of insulin on hepatocytic A-IV mRNA expression in this model. After Laser microdissection of FAH and quantitative RT-PCR (LightCycler), a 3.2 to 7.4-fold increase of A-IV mRNA in the FAH[.(30)]

CONCLUSION

Measurement of serum apo A-IV can be a useful parameter in the follow up of HCV patients. Serum apo A-IV could be considered as noninvasive marker for liver cirrhosis with cut off point>36.4, 100% sensitivity and 90% specificity. It could predict the presence of HCC in HCV infected patient with liver cirrhosis with cut off point ≤25.1 with sensitivity 80.0 % and specificity 93.33%.

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