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Impact of chronic hepatitis C infection on diabetic patients with proteinuria

Azza M Abdu Allah[1], Nesreen G Elhelbawy[1], Ayman Alsebaey[2]

[1]Medical Biochemistry and [2]Hepatology Departments
Faculty of Medicine and National Liver Institute, Menoufia University, Egypt

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Keywords

- HCV
- DM
- Albuminuria

Abstract

Background: Hepatitis C (HCV) and diabetes mellitus (DM) are major health burden with various systemic complications. Both affect the kidney with increasing the incidence of progression to end stage renal disease. **Aim:** Usefulness of tumor necrosis factor (TNF), high-sensitivity CRP (hs-CRP) and interleukin-18 (IL-18) as differentiating markers of proteinuria in HCV diabetic patients. **Methods:** Eighteen as control and 72 patients with DM were enrolled. Diabetic patients were divided into 4 equal groups; diabetic patients with microalbuminuria negative for HCV (n=18), positive for HCV (n=18), diabetic patients with macro-albuminuria negative for HCV (n=18), positive for HCV (n=18). TNF, hs-CRP and IL-18 were measured in all groups. **Results:** TNF was not useful for distinguishing microalbuminuria (174.61 ±64.89 vs. 229.33 ±101.31 pg/mL; p=0.093) or macroalbuminuria (69.78 ±22.34 vs. 78.00 ±20.57 pg/mL; p=0.259) in HCV diabetic patients. The hs-CRP had a paradox relationship where higher levels were found in HCV diabetic patients with microalbuminuria (6.42 ±0.76 vs. 4.43 ±0.70 mg/L; p=0.001) unlike those with macroalbuminuria (5.25 ±0.69 vs. 6.72 ±1.56 mg/L; p=0.003). IL-18 increased in microalbuminuria (619.72 ±72.53vs 148.54 ±5.69 mg/L; p=0.001) or macro-albuminuria (609.22 ±52.05 vs. 352.44 ±12.33 pg/mL; p=0.001) in HCV diabetic patients. **Conclusion:** IL-18 is a promising

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Corresponding author: Azza M Abdu Allah. Department of Medical Biochemistry, Faculty of Medicine and National Liver Institute, Menoufia University, Egypt Email: azza.abdallah@yahoo.com, Mob #: +201117294576

Introduction

Hepatitis C virus (HCV) is a RNA virus that infects more than 150 million people all over the world. It is a global burden on health resources. There are various routes of infection e.g. addiction, recipients of infected blood products, etc. HCV has 6 genotypes that differ in the geographical distribution and response to antiviral therapy (1,2).

In fact HCV is not a simple infection in which the virus lives in the liver with local effects only. Actually it is a systemic or multi facet disease in which it affects the liver causing liver fibrosis that progress to cirrhosis, decompensating and ultimately hepatocellular carcinoma. Outside the liver there are lots of extrahepatic manifestations like cryoglobinemia, glomerulonephritis, neuropathy, diabetes mellitus (DM), lymphoma, thyroid dysfunction, Sjogren's syndrome, porphyria cutaneatarda, lichen planus and recently cardiomyopathy (3,4)

HCV is a culprit of various forms of glomerulonephritis e.g. membranoproliferative glomerulonephritis (MPGN)with type II mixed cryoglobulinemia, membranous nephropathy, focal segmental glomerulosclerosis, fibrillary glomerulonephritis,immunotactoidglomerulopathy, IgA nephropathy, renal thrombotic microangiopathy, vasculitic renal involvement and interstitial nephritis(5,6). A close bidirectional relationship is found between HCV and DM (7) where diabetes mellitus patients have higher prevalence of HCV(8) and in addition HCV-related liver disease patients have also higher prevalence of DM(9).

Tumor necrosis factor (TNF) is a cytokine that is implicated in regulation of inflammation, survival, apoptosis and cell migration, proliferation and differentiation (10 It is seen in association with HCV (11 or DM related kidney injury (12).

Interleukin-18 (IL-18) is a cytokine produced mainly by macrophages. It is involved in diabetic nephropathy (13).With IL-12 it induces immunity following infection with microbial products like lipopolysaccharide (LPS) (14.It is also associated with HCV infections (15).

This study aimed to assess the usefulness of TNF, high-sensitivity CRP (hs-CRP) andIL-18 as differentiating markers of proteinuria in HCV diabetic patients.

Subjects and Methods

After institutional review board approval, this study was conducted at Faculty of medicine and National Liver Institute hospitals, Menoufia University, Egypt. An informed consent was obtained from all enrolled patients.

This study was conducted on a total number of 72 patients and 18 as control group. They were divided into four equal groups; diabetic patients with microalbuminuria negative for HCV (n=18), positive for HCV (n=18), diabetic patients with macroalbuminuria negative for HCV (n=18), positive for HCV (n=18).Chronic hepatitis is defined as hepatitis that lasts more than 6 months with persistent positive HCV PCR(EASL, 2016). Microalbuminuria is defined as persistent albuminuria of 20 to 200 mg/24 hours on at least three occasions in absence of urinary tract infection. Macroalbuminuria is defined as persistent albuminuria ≥300 mg/24 hours (16).

Diabetic mellitus type II was diagnosed by the following criteria: a fasting plasma glucose level of 126 mg/dL (7.0 mmol/L) or higher, or a 2-hour plasma glucose level of 200 mg/dL (11.1 mmol/L) or higher during a 75-g oral glucose tolerance test (OGTT), or a random plasma glucose of 200 mg/dL (11.1 mmol/L) or higher in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis (17).

Patients with following criteria were excluded; other causes of liver diseases e.g. HBV and HCV co-infection, evidence of advanced decompensated liver disease, hepatocellular carcinoma or extrahepatic malignancy.

All patients underwent thorough history taking, complete physical examination, body mass index calculation [body weight (kg)/height^2 (m)], liver function tests, renal function tests, CBC, INR and abdominal ultrasonography. In all patients TNF, hs-CRP and IL-18 were measured.

Sampling: Urine of 24 hours was collected for detection of microalbuminuria. Blood samples were drawn after an overnight fast under complete aseptic condition. Three ml of blood was placed in a plain tube for serum separation and two ml blood was placed in EDTA tube for HbA1c determination. The obtained serum was stored immediately at −20°C for future analysis. Methods of Assay: The diagnostic rapid test kit was used to analyze the samples for HCV antibodies. This is a rapid chromatographic immunoassay for the qualitative detection of antibody to HCV in serum (Acon laboratories, USA). Fasting blood sugar, HbA1c, total cholesterol, triglyceride, and HDL-C concentrations were determined by Synchron *Cx9. LDL-c concentration was calculated according to the Friedewald formula. Microalbuminuria was detected by Cayman's HAS EIA Kit (competitive assay) Cayman Chemical Company, Ann Arbor, Michigan 48108 USA. Human TNF-alpha was detected by ELISA, (Abazyme. 85 Pine Grove St. Needham, MA 02494 USA). Serum IL-18 and hsCRP are measured using solid-phase enzyme-linked immunosorbent assay (Immunospec corporation catalog No.E29-056).

Statistical analysis

Data was statistically analyzed using IBM® SPSS® Statistics® version 21 for Windows. Data are expressed as mean ±standard deviation. P-values <0.05 were considered statistically significant. Comparisons between two groups were performed using the Student's t-test for parametric data, and Mann-Whitney test for nonparametric data. Chi-squared test ($\chi 2$) and Fisher exact test for categorical data analysis.

Results

The comparison between DM-related microalbuminuria negative and positive for HCV association showed that both groups were matched for sex and they were not different from the control group. Patients with HCV association were older than those negative for HCV and the control (65.39 ±5.53 vs. 57.11 ±5.25 years; p=0.001) and (65.39 ±5.53 vs. 61.22 ±6.16 years; p=0.04) respectively. Meanwhile patients negative for HCV were younger than the control (57.11 ±5.25 vs. 61.22 ±6.16 years; p=0.038) (Table1).

Patients with HCV association had significant lower BMI than those negative for HCV and the control (22.44 ±5.67 vs. 28.26 ±2.6 kg/m^2;

p=0.001) and (22.44 ±5.67 vs. 28.09 ±1.96 kg/m^2; p=0.001) respectively. But there was no significant difference between Patients negative for HCV and control (Table1).

Patients with HCV association had significant lower fasting blood glucose than those negative for HCV and the control (92.72 ±6.31vs. 169.72 ±9.45 mg/dl; p=0.001) and (92.72 ±6.31vs. 98.55 ±3.16 mg/dl; p=0.002) respectively. In contrast patients negative for HCV had significant higher fasting blood glucose than the control (169.72 ±9.45 vs. 98.55 ±3.16 mg/dl; p=0.001)(Table1).

Furthermore patients with HCV association had significant lower HbA1c than those negative for HCV (5.10 ±0.78 vs. 7.98 ±0.79%; p=0.001).On the other hand patients negative for HCV had significant higher values than the control (7.98 ±0.79 vs. 4.93 ±0.56%; p=0.001).But there was no significant difference between Patients with HCV association and control (Table1).

The cholesterol level was significantly higher in both patients negative and positive for HCV than the control. Patients who are positive for HCV had significant higher cholesterol level than those negative for HCV (229.44 ±13.02 vs. 215.78 ±5.15 mg/dl; p=0.001). On contrary they had significant lower blood triglycerides (135.17 ±22.66 vs. 287.11 ±45.88mg/l; p=0.001). There was non-significant difference between both groups regarding HDL but LDL was significantly higher in those positive for HCV than negative ones (158.94 ±13.69 vs. 118.02 ±5.81mg/l; p=0.001)(Table1).

Both patients negative and positive for HCV had significant higher TNF than the control (174.61 ±64.89 vs. 12.50 ±11.72 pg/ml; p=0.001) and (229.33 ±101.31 vs. 12.50 ±11.72 pg/ml; p=0.001) respectively. Unfortunately TNF did not differ in negative and positive for HCV (Table1, Figure 1).

Concerning hs-CRP both patients negative and positive for HCV had significant higher values than the control (4.43 ±0.70 vs 0.49 ±0.23 mg/l; p=0.001) and (6.42 ±0.76 vs 0.49 ±0.23 mg/l; p=0.001) respectively. The hs-CRP was significantly higher in patients positive for HCV than negative ones (6.42 ±0.76 vs. 4.43 ±0.70 mg/l; p=0.001) (Table1, Figure 2).

Respecting IL-18 both patients negative and positive for HCV had significant higher values than the control (373.41 ±50.98vs148.54 ±5.69pg/ml; p=0.001) and (619.72 ±72.53vs 148.54 ±5.69 mg/l; p=0.001) respectively. The IL-18 was significantly higher in patients positive for HCV than negative ones (619.72 ±72.53 vs. 373.41 ±50.98pg/ml; p=0.001) (Table1, Figure 3).

The comparison between DM-related macroalbuminuria negative and positive for HCV association showed that control and the studies patients were matched for age and sex (Table 2).

The control group compared to patients negative and positive for HCV had significant lower fasting blood glucose (98.55 ±3.16 vs. 161.06 ±21.16 and 172.22 ±19.12 mg/dl), HbA1c (4.93 ±0.56 vs. 8.00 ±0.6 and 8.05±1.03mmols/mol), cholesterol (201.11 ±22.35 vs. 266.78 ±19.97 and 241.67 ±15.01mg/dl), triglycerides (101.83 ±24.18 vs. 291.61 ±39.21 and 136.67 ±28.31mg/l), LDL (126.52 ±23.10 vs. 168.12 ±19.92 and 170.78 ±14.18mg/dl), TNF (12.50 ±11.72vs. 69.78 ±22.34 and 78.00 ±20.57pg/ml), hs-CRP (0.49 ±0.23 vs.

6.72 ±1.56 and 5.25 ±0.69mg/l) and IL-18 (148.54 ±5.69 vs. 352.44 ±12.33 and 609.22 ±52.05pg/ml) . But BMI (28.09 ±1.96 vs. 25.01 ±3.35 and 24.50 ±2.75kg/m^2) and HDL (54.12 ±5.81 vs. 40.33 ±8.77 and 43.56 ±5.09mg/l) were significantly higher (Table 2).

There were no significant difference between patients negative and positive for HCV regarding BMI, fasting blood glucose, HbA1c, HDL, and LDL. Patients positive for HCV had significant lower blood cholesterol (241.67 ±15.01 vs. 266.78 ±19.97mg/L; p=0.001) and triglycerides (136.67 ±28.31 vs. 291.61 ±39.21mg/L; p=0.001) than negative ones (Table 2).

There was no significant statistical difference between patients negative and positive for HCV regarding TNF (Table 2, figure 1).Unlike hs-CRP which was significantly higher in negative patients (6.72 ±1.56 vs. 5.25 ±0.69 mg/l; p=0.003) (Table 2, figure 2).Patients positive for HCV had significant higher serum IL-18 than negative ones (609.22 ±52.05 vs. 352.44 ±12.33pg/ml; p=0.001) (Table 2, figure 3).

Table 1 Comparison of diabetic patients with microalbuminuria negative and positive for HCV

	Control	DM microalbuminuria		P		
		-ve HCV	**+ve HCV**			
	N=18 (a)	**N=18 (b)**	**N=18 (c)**	**a×b**	**a×c**	**b×c**
Age(Y)	61.22 ±6.16	57.11 ±5.25	65.39 ±5.53	0.038	0.04	0.001
Male (Female)	8 (10)	8 (10)	10 (8)	1	1	0.740
BMI	28.09 ±1.96	28.26 ±2.60	22.44 ±5.67	0.829	0.001	0.001
FBS(mg/dl)	98.55 ±3.16	169.72 ±9.45	92.72 ±6.31	0.001‡	0.002‡	0.001‡
HBA1C%	4.93 ±0.56	7.98 ±0.79	5.10 ±0.78	0.001	0.453	0.001
Cholesterol(mg/dl)	201.11 ±22.35	215.78 ±5.15	229.44 ±13.02	0.023‡	0.001‡	0.001
Triglycerides(mg/dl)	101.83 ±24.18	287.11 ±45.88	135.17 ±22.66	0.001	0.001	0.001‡
HDL(mg/dl)	54.12 ±5.81	40.33 ±6.65	43.44 ±6.20	0.001	0.001	0.156
LDL(mg/dl)	126.52 ±23.10	118.02 ±5.81	158.94 ±13.69	0.164‡	0.001‡	0.001‡
TNF(pg/ml)	12.50 ±11.72	174.61 ±64.89	229.33 ±101.31	0.001‡	0.001‡	0.093‡
hs-CRP(mg/l)	0.49 ±0.23	4.43 ±0.70	6.42 ±0.76	0.001‡	0.001‡	0.001
IL18(pg/ml)	148.54 ±5.69	373.41 ±50.98	619.72 ±72.53	0.001‡	0.001‡	0.001
‡Mann Whitney Test						

Table 2 Comparison of diabetic patients with macro-albuminuria negative and positive for HCV

	Control	DM macro albuminuria		P		
		-ve HCV	+ve HCV			
	N=18 (a)	N=18 (b)	N=18 (c)	a×b	a×c	b×c
Age(Y)	61.22 ±6.16	61.50 ±5.02	63.44 ±4.87	0.883	0.238	0.246
Male (Female)	8 (10)	8 (10)	11 (7)	1	0.505	0.505
BMI	28.09 ±1.96	25.01 ±3.35	24.50 ±2.75	0.005‡	0.001	0.624
FBS(mg/dl)	98.55 ±3.16	161.06 ±21.16	172.22 ±19.12	0.001‡	0.001‡	0.106
HBA1C%	4.93 ±0.56	8.00 ±0.60	8.05 ±1.03	0.001	0.001‡	0.557‡
Cholesterol(mg/dl)	201.11 ±22.35	266.78 ±19.97	241.67 ±15.01	0.001	0.001	0.001
Triglycerides(mg/dl)	101.83 ±24.18	291.61 ±39.21	136.67 ±28.31	0.001	0.001	0.001
HDL(mg/dl)	54.12 ±5.81	40.33 ±8.77	43.56 ±5.09	0.001	0.001	0.081‡
LDL(mg/dl)	126.52 ±23.10	168.12 ±19.92	170.78 ±14.18	0.001	0.001‡	0.648
TNF(pg/ml)	12.50 ±11.72	69.78 ±22.34	78.00 ±20.57	0.001‡	0.001‡	0.259
hs-CRP(mg/l)	0.49 ±0.23	6.72 ±1.56	5.25 ±0.69	0.001‡	0.001‡	0.003‡
IL18(pg/ml)	148.54 ±5.69	352.44 ±12.33	609.22 ±52.05	0.001‡	0.001‡	0.001‡
‡Mann Whitney Test						

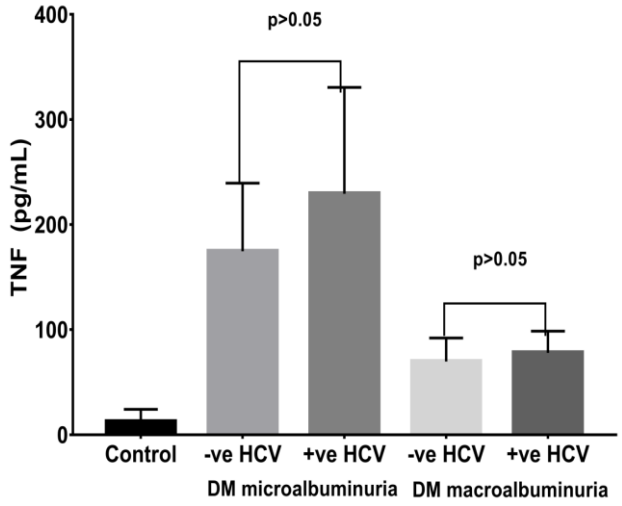

Figure (1): The relationship between TNF and HCV negative and positive cases with either micro or macroalbuminurea

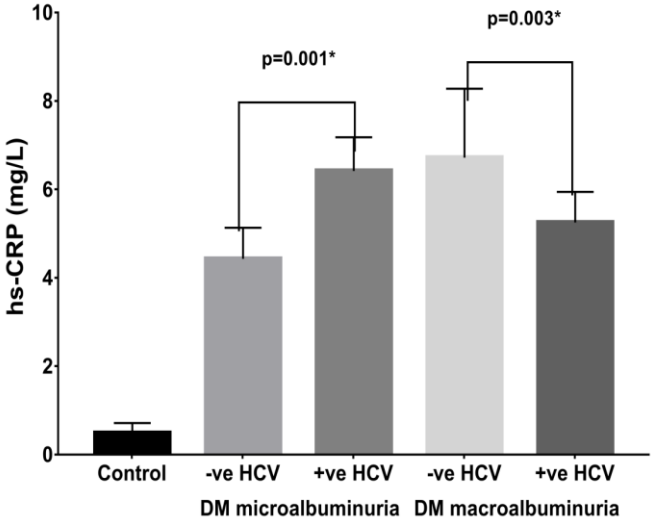

Figure (3): The relationship between hs-CRP and HCV negative and positive cases with either micro or macroalbuminurea

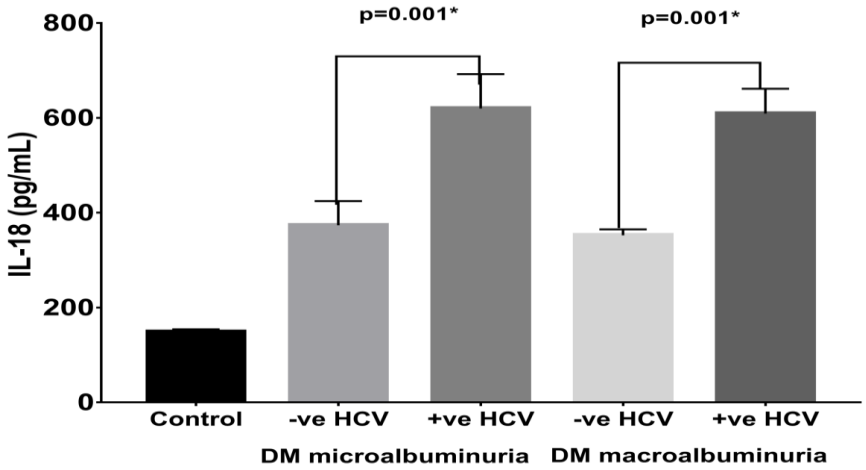

Figure (3): The relationship between IL-18 and HCV negative and positive cases with either micro or macroalbuminurea

Discussion

HCV is a global challenging health problem. Most infected patients progress to chronicity. It affects the liver mainly causing inflammation, steatosis, fibrosis, cirrhosis and in some patients hepatocellular carcinoma (1,2). The drawbacks of HCV infection are affecting all the body systems. HCV is a major cause of cryoglobinemia related vasculitis and various types of glomerulonephritis (6).The prevalence of positive HCV in hemodialysis patients ranges from less than 5 to 60% (18,19). HCV is linked to the progression and severity of chronic kidney disease (20). A recent meta-analysis found decreases survival in hemodialysis patients positive for HCV (21).

DM is usually complicated with micro or macrovascular diseases. The microvascular includes retinal, renal and neuropathic disease meanwhile the macro-vascular includes coronary artery and peripheral vascular disease.

Diabetic nephropathy is a clinical syndrome characterized by the following: persistent albuminuria (>300 mg/d or >200 μg/min) that is confirmed on at least 2 occasions 3-6 months apart, progressive decline in the glomerular filtration rate and elevated arterial blood pressure (22). It remains the most common cause for end stage renal disease(23).

An intimate relationship is found between HCV and DM (7).HCV may causes insulin resistance (24). DM patients have higher prevalence of HCV infection(8). Patients with HCV-related liver disease have higher prevalence of DM (9).

In this study we tried to find that the HCV infection increases the burden over the kidney in diabetic patients. Moreover, we tried to assess the role of TNF, hs-CRP and IL-18 in such condition. *Abdel Aziz et al.,(25)* studied the impact of HCV infection on the microvascular complications in diabetic patients (25). Patients with chronic HCV had higher serum cholesterol, triglycerides.,HbA1c and urinary albumin excretion.

In the current study TNF was not useful for distinguishing microalbuminuria or macroalbuminuria in HCV diabetic patients. The hs-CRP had a paradox relationship where higher levels were found in HCV diabetic patients with microalbuminuria unlike those with macroalbuminuria. IL-18 was the best marker where being of higher values in microalbuminuria or macroalbuminuria in HCV diabetic patients.

In conclusion; IL-18 is a promising marker for distinguishing micro and macroalbuminuria in HCV diabetic patients.

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