Renal Involvement in Asymptomatic Cases of HCV Infection

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Abstract: Background: About 150 million people are chronically infected with hepatitis C virus and more than 350 000 people die every year from hepatitis C-related liver diseases**.** HCV glomerulonephritis is well documented as the most important extrahepatic manifestation of HCV infection. Renal manifestations are usually associated with long standing HCV infection (i.e. more than 10 years). At this time it will be associated with concurrent clinical and laboratory features of chronic active hepatitis or liver cirrhosis. Other authors claimed that it can occur earlier and it might be the presenting feature of HCV infection while the patient is hepatic symptoms free**.** So, we aimed to study renal involvement of HCV infection in hepatic symptoms free patients. **Patients and methods: One hundred** HCV +ve cases with asymptomatic compensated liver were collected from the outpatient clinic of internal medicine in Ain Shams University Hospital***(Group A)***and were compared to **25** healthy control subjects ***(Group B)***for: clinical examination, abdominal U/S, FBG, diagnostic tests for hepatitis virus C and B including quantitative PCR for virus C, serum bilirubin, aminotransferases (ALT and AST), albumin, PT and INR, eGFR, urea, creatinine and complete urinalysis including P/C ratio. **Results:** AST and ALT were higher ***(p<0.001)*** and PT and INR were more prolonged ***(p<0.01)*** in patients group than in the control group with mean levels within normal reference range. There was no significant correlation between Liver function tests and viral load. There was a significant negative correlation between age of patients and AST ***(p<0.05),*** ALT ***(p<0.001)*** and serum albumin ***(p<0.001)***.There was also a significant negative correlation between age and viral load ***(p<0.01).*** Creatinine ***(p<0.01)*** and P/C ratio ***(p<0.001)*** were higher while eGFR was lower ***(p<0.001)*** in patients than in the control group with mean levels within normal reference range. There was no significant correlation between kidney function tests and viral load. There was a significant correlation between creatinine and age of patients ***(p<0.01)***. Also, eGFR correlated inversely significantly to age of patients ***(p<0.001)***. Abnormal urinary sediments were significantly more encountered in patients group than in control group ***(p<0.05).*** **Conclusion:** Both liver and kidney are involved early in the course of HCV infection with deterioration of liver and kidney functions. The deterioration is not apparent by current laboratory tests except when compared to normal subjects. Ultrasonography is also invaluable, age of the disease is difficult to be determined, viremia is misleading and the problem is great because of the huge population of patients. We recommend more sensitive tests for early diagnosis of HCV and scheduled score sheets for better interpretation of laboratory tests. Early treatment should be considered. We recommend, also, performing this study on larger sample size to have more reliable information.

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1.Introduction:

About 150 million people are chronically infected with hepatitis C virus (HCV)and more than 350,000 people die every year from hepatitis C-related liver diseases***(1).*** In addition to liver disease, HCV infection might be associated with a variety of extrahepatic manifestations e.g. SjÖgren's syndrome, sialadenitis, idiopathic pulmonary fibrosis, polyartritis nodosa, porphyria cutenea tarda, lichen planus, lymphoproliferative disorders, mooren's corneal ulcer and others ***(2,3,4***).

HCV infection has been reported in association with distinct histological patterns of glomerulonephritis. Membranoproliferative glomerulonephritis (MPGN) associated with type II cryoglobulinaemia is the most predominant type of HCV related glomerulonephritis. Characterization of the cryoprecipitate demonstrated HCV core antigen bound to specific immunoglobulin G (Ig G), which was in turn bound to rheumatoid factor (Ig M)***(5,6).*** Less common glomerulonephritis have been also reported in HCV infected patients like MPGN without cryoglobulinaemia, membranous glomerulonephritis, focal segmental glomerulosclerosis (FSGS), mesangial proliferative GN with IgA deposition, renal thrombotic microangiopathy and immunotactoid glomerulonephritis ***(3,4).***

Particular renal manifestations of HCV associated glomerular disease include proteinuria, up to nephrotic range, microscopic hematuria and renal failure which might be the presenting symptom. Renal manifestations may occur in the absence of liver disease or cryoglobulinaemia and in this

situation, diagnosis of HCV is made as a retrospect after a series of renal evaluation tests ***(5,6,7,8).***

Renal manifestations are usually associated with long standing HCV infection (i.e. more than10 years). At this time it will be associated with concurrent clinical and laboratory features of chronic active hepatitis or liver cirrhosis. Other authors claimed that it can occur earlier and it might be the presenting feature of HCV infection while the patient is hepatic symptoms free ***(4,5).***

So, we aimed to study renal involvement of HCV infection in hepatic symptoms free patients.

2. Patients and methods:

In our cross sectional study, 100 HCV+ve cases (64 males and 36 females) were collected from the outpatient clinic of internal medicine in Ain Shams University Hospital. These cases did not come to the hospital seeking medical advice , but, were discovered accidentally while they were preparing their health care files for different purposes, e.g. travelling abroad, and found to be HCV Ab +ve with asymptomatic compensated liver**(Group A)** . **Twenty five** healthy subjects (15 males and 10 females) were included as a control group **(Group B).**

All patients and control group were subjected to full history taking, full clinical examination, fasting blood sugar (FBS), calculation of body mass index (BMI) and abdominal ultrasonography.

HCV Ab testing (3rd generation ELISA) and quantitative polymerase chain reaction (PCR) for HCV RNA were done to ensure the HCV status. Viremia was interpreted as follows:

- >100 to 1×10^4 copies/ml = Very weak viremia.
- $>1\times10^4$ to 1×10^5 copies/ml = Mild viremia.
- $>1\times10^5$ to 1×10^6 copies/ml = Moderate viremia.
- $>1\times 10^6$ copies/ml = High viremia.

Hepatitis B Surface Antigen (HBsAg), hepatitis B core antibody (HBcAb) and serum antischistosomal antibodies were done to exclude hepatitis B and schistosomal infections.

Liver function tests, done, included serum liver enzymes, aspartate aminotransferase(AST) and alanine aminotransferase(ALT),Serum total bilirubin, serum albumin, prothrombin time (PT) and international normalized ratio (INR).

Kidney function tests were also performed and included serum urea and creatinine, complete urinalysis, urinary total protein/creatinine (P/C) ratio and estimated glomerular filtration rate (eGFR) using the MDRD equation recommended by the national kidney foundation($GFR=170\times SCr^{-0.999}\times SUN^{-0.170}\times SAlb+0.318\times age^{-0.176}\times 1.180$ or 0.762 if female).

Exclusion criteria:

Patients with diabetes mellitus, hepatitis B virus infection, and HIV infection, chronic kidney disease, past history of interferon therapy or schistosomiasis were excluded to ensure that the obtained results of kidney function tests were not interfered by another disease.

Statistical analysis:

Statistical analysis was performed using Statistical Package for Social Science (SPSS) version 19 on a personal IBM compatible laptop. Quantitative parametric data were expressed as ***mean± standard deviation (SD),*** while qualitative non-parametric data were expressed as numbers and ***percentages***. ***Student t test*** was used for comparison of two independent groups for parametric data, while ***Paired t test*** was used for comparison of two dependent groups. ***Pearson correlation*** was used for possible association between two different variables. ***Chi square test*** was used for binomial and descriptive data. ***P value*** was considered significant when <0.05 and highly significant when <0.01 or <0.001. P value was considered insignificant when >0.05.

3. Results:

In our cross sectional study, we included 100 HCV seropositive patients and 25 completely normal subjects as a control group.

As we can see from (table 1) both groups showed homogeneity to each other as regards age, gender, BMI, blood pressure and fasting blood glucose which makes them liable for comparison.

Table (1): Comparison of HCV+ve group to the control group as regards personal data

	HCV +ve cases (Group A) (N=100)	Control group (Group B) (N=25)	t	P
Age	44.51±6.95	41.76±8.18	1.707	0.09
sex	64♂,36♀	15♂, 10♀	X^2 0.138	0.711
BMI	26.54±1.78	26.76±1.34	0.588	0.558
BP ***systolic***	122.9±11.57	123.6±11.5	0.271	0.787
diastolic	74.85±8.8	77.8±7.23	1.549	0.124
FBS	77.56±9.77	81.32±8.73	1.76	0.082

Table (2): Liver function tests in HCV+ve cases compared to the control group

	HCV +ve cases (Group A) (N=100)	Control group (Group B) (N=25)	t	P
AST (total) • Normal • High	35.53±6.77 79% (33.03±4.99) 21% (44.95±3.46)	29.12±6.5	4.256	000
ALT (total) • Normal • High	35.4±6.74 76% (32.64±5.03) 24%(44.13±2.95)	27.8±5.4	5.256	000
Total bilirubin	0.63±0.21	0.63±0.24	0.143	0.886
S. Albumin	4.31±0.18	4.43±0.25	2.869	0.005
PT	13.19±0.69	12.78±0.36	2.845	0.005
INR	1.077±0.058	1.04±0.03	2.857	0.005
HCV degree of viremia : • Very weak • Mild • Moderate • High	 70% 21% 6% 3%	 0 0 0 0		

Table 3: Comparison between normal and high AST groups among HCV+ve patients

	Normal AST N=79 Mean± SD	High AST N=21 Mean± SD	t	p
Serum AST	33.03±4.99	44.95±3.46	10.293	000
Serum ALT	34.13±6.5	40.19±5.46	3.92	000
Age	44.58±6.97	44.24±7.04	0.201	0.841
BMI	26.57±1.85	26.44±1.57	0.294	0.770
HCV RNA titre	245791.14± 614948.91	331876.19± 412710.58	0.605	0.546
Serum bilirubin	0.629±0.216	0.61±0.2	0.376	0.708
Serum albumin	4.314±0.18	4.276±0.181	0.815	0.397
Prothrombin time	13.133±0.644	13.381±0.757	1.478	0.143
INR	1.0743±0.0575	1.0957±0.065	1.457	0.143
FBS	77.53±9.68	78.1±10.35	0.234	0.816
Serum creatinine	0.859±0.168	0.9±0.173	0.974	0.332
Serum urea	26.54±3.74	27.38±3.92	0.902	0.369
P/C ratio	0.1641±0.1199	0.1867±0.1361	0.747	0.457
e GFR	96.902±16.328	94.093±18.642	0.682	0.497

Table 4: Comparison between normal and high ALT groups among HCV+ve patients

	Normal ALT N=76 Mean± SD	High ALT N=24 Mean± SD	t	p
Serum AST	33.79±6.31	41.04±5.1	5.122	000
Serum ALT	32.64±5.03	44.13±2.95	10.602	000
Age	44.86±7.04	43.42±6.67	0.883	0.379
BMI	26.61±1.75	26.32±1.92	0.686	0.494
HCV RNA titre	227993.42± 423769.37	377475± 912476.03	1.107	0.271
Serum bilirubin	0.62±0.22	0.642±0.184	0.441	0.660
Serum albumin	4.313±o.186	4.283±0.163	0.705	0.483
Prothrombin time	13.164±0.675	13.25±0.737	0.529	0.598
INR	1.077±0.05845	1.0846±0.06359	0.544	0.587
FBS	77.12±9.54	79.33±10.49	0.968	0.335
Serum creatinine	0.862±0.171	0.888±0.165	0.645	0.520
Serum urea	26.43±3.62	27.63±4.18	1.353	0.179
P/C ratio	0.1683±0.1242	0.1704±0.1221	0.073	0.942
e GFR	96.3633±16.7399	96.15±17.0995	0.054	0.957

Liver function tests in (Table 2) showed that serum liver enzymes (ALT and AST) were significantly higher in seropositive cases than in the control group ***(p<0.001).***In spite of this high significant difference, the mean values of both ALT and AST in HCV+ve cases remained within the normal reference range. 21% of patients had AST higher than the normal reference range, but when compared to the remaining 79%, with normal level of AST, there was no significant difference in any parameter (Table 3). The same for ALT, 24% of cases had high levels of ALT but there was no significant difference when compared to the rest of the group (all p values were***>0.05***) (Table 4).Serum albumin was significantly lower in patients group than in the control group ***(p<0.01).*** Mean level of serum albumin remained within normal reference range.PT and INR were found to be more prolonged in patients group than the control group with highly significant p values ***(p<0.01)*** for both PT and INR. Mean levels of PT and INR remained within normal reference ranges. Serum bilirubin was insignificantly different between both groups ***(p>0.05).***HCV degree of viremia showed highly significant inverse relationship to age of patients ***(P<0.01) (***Table 6).Otherwise, we found no significant correlation between the degree of viremia and hepatic or renal function tests.

Table 5: Kidney function and urinalysis in HCV+ve cases compared to the control group

	HCV +ve cases (Group A) (N=100)	**Control group (Group B) (N=25)**	**t**	**P**
Serum Urea	26.72±3.77	27.72±3.95	1.174	0.243
Serum Creatinine	0.868±169	0. 75±0.13	3.187	0.002
Estimated GFR:	96.312±16.74	112.5±24.6	3.907	000
• Normal>90ml/m/1.73m²	74%	100%		
• Reduced<90ml/m/1.73m²	26%	0%		
Urinary P/C Ratio:	0.1688±0.1231	0.08±0.03	3.570	0.001
• Normal (<0.2)	82%	100%		
• Increased (>0.2)	18%	0%		
Urinalysis:			**X²**	**P**
Hematuria and albuminuria				
• Normal	54%	80%	6.874	0.076
• Hematuria	31%	20%		
• Albuminuria	7%	0%		
• Hematuria + Albuminuria	8%	0%		
Sediments				
• Normal sediments.	54%	80%	5.598	0.018
• Abnormal sediments.	46%	20%		

Kidney function tests in (table 5) showed that **S. Creatinine** was significantly higher in patients than in control group ***(p<0.01)*** whereas, no significant difference detected in case of **S.Urea** ***(p>0.05).*** Both means of urea and creatinine remained within the normal reference range. Estimated **GFR** was significantly lower in patients group than in control group ***(p<0.001***).In spite of having 26% of cases with reduced eGFR <90 ml/m/1.73m², the mean value of eGFR remained within the normal reference range. **Urinary protein/creatinine ratio** in urine was significantly higher in patients than in control group ***(p<0.001).*** In spite of having 18% of patients with above normal ratio, the mean value of p/c ratio remained within normal reference range. The degree of proteinuria did not correlate to sex, BMI or blood pressure in patients or control group. Abnormal **urinary sediments** were significantly more encountered in patients group more than control group ***(p<0.05)*** with no relation to gender neither in patients group nor in the control group.

Age of the patients, (table 6), correlated inversely significantly to serum liver enzymes (ALT and AST) (***p<0.001*** for ALT and ***<0.05*** for AST). Also, it correlated inversely significantly to serum albumin ***(p<0.001)*** but, it did not correlate to the prothrombin time ***(P>0.05).***

A significant positive correlation was found between age and serum creatinine ***(p<0.01)*** but not with blood urea ***(p>0.05***). Another significant inverse correlation was found between age and eGFR ***(p<0.001).*** No correlation was found between age and p/c ratio ***(p>0.05).***

Table *6:* Correlations of age of patients to different parameters

Age correlation to..	r	P
Serum AST	-0.229	0.022
Serum ALT	-0.080	0.431
Serum albumin	-0.395	0.000
Prothrombin time	-0.189	0.060
INR	-0.186	0.046
HCV RNA viral load	-0.258	0.004
Serum creatinine	0.267	0.007
Estimated GFR	-0.318	0.001
Protein/creatinine ratio	-0.032	0.751

Table 7: Abdominal ultrasonography findings in patients and control groups

Groups / U/S findings	Patients Group (Group A) (N=100)	Control Group (Group B) (N=25)	TOTAL (N=125)	X^2	P
Fatty change	2 (2%)	3 (12%)	5 (4%)	**(Pearson)**	
Hepatomegaly	14(14%)	2 (8%)	16 (12.8%)	12.307	0.015
Hepatomegaly +Fatty change	2 (2%)	2 (8%)	4 (3.2%)	**(likelihood ratio)**	
hepatosplenomegaly	0 (0%)	1 (4%)	1 (0.8%)		
Normal liver	82 (82%)	17 (68%)	99 (79.2%)	9.966	0.041
Normal kidneys	100 (100%)	25 (100%)	125 (100%)		

Abdominal ultrasonography showed a significant difference between patients group and control group as regards liver abnormalities (hepatomegaly) **(p<0.05)** (table 7).

Table 8: Minimum, maximum and normal values of important parameters in patients group (N=100)

	Minimum	maximum	Mean± SD	Normal Reference value
AST	24	51	35.53±6.77	<40 (U/L)
ALT	22	51	35.40±6.74	<40 (U/L)
Serum total bilirubin	0.3	1.1	0.625±0.211	0.2-1.2 (mg/dl)
Serum albumin	3.9	4.7	4.306±0.180	3.8-5 (g/dl)
Prothrombin time	12.5	14.5	13.185±0.688	11-18 (seconds)
INR	1.02	1.19	1.0788±5.948	0.9-1.2
Serum creatinine	0.6	1.3	0.868±0.169	0.5-1.5 (mg/dl)
Blood urea	19	36	26.72±3.77	15-40 (mg/dl)
Estimated GFR	60.48	148.8	96.3121±16.74	100-130 (ml/m/1.73m²)
Protein/creatinine ratio	0.05	0.58	0.169±0.123	<0.2

Table 8shows to what extent the deviation from normal reference values was trivial. Serum bilirubin, serum albumin, Prothrombin time, INR, serum creatinine and blood urea were all within the normal reference ranges. AST and ALT when being outside the normal range they did not exceed 2 folds of normal. Lowest eGFR did not pass to stage 3 chronic kidney disease. The highest value of P/C ratio was 0.58.

4. Discussion:

Renal involvement in HCV +ve cases is one of the commonest extra hepatic complications of HCV infection. About 150 million people are chronically infected with hepatitis C virus (HCV)and more than 350,000 people die every year from hepatitis C-related liver diseases***(1).***It is important to discover the disease early to try to prevent progress of renal involvement or at least to delay it as much as possible. In our study, 100 asymptomatic HCV seropositive patients were compared to 25 seronegative healthy subjects as a control group to evaluate the impact of virus C on the kidney in asymptomatic HCV infection

It was impossible to determine the time of onset of HCV infection and hence the duration of infection.

So, we used the current age of the patients as an indicator, this method was used by many authors ***(4,5).***Most of patients, up to 80%, who have chronic HCV infection, are asymptomatic until evidence of hepatic failure becomes clinically apparent. The rate of progression to cirrhosis is usually slow; it takes about 20 years or more to develop serious complications of HCV infection ***(9,10).*** This explains why our patients were asymptomatic.

Liver function tests:

Mean serum **aminotransferase *levels*** were higher in patients group than in the control group ***(p<0.001)*** but the mean levels were still within normal. Only 21% (AST) and 24% (ALT) of patients had raised aminotransferases above normal but not reaching even double normal values. Elevated levels of aminotransferases did not correlate to the level of viremia or to any of the parameters of liver function tests. Elevated enzymes above normal range and elevated mean levels of enzymes are markers of chronicity; both of them are indicators of the known natural history of HCV infection characterized by fluctuating liver enzymes and liver cell damage ***(11-14).*** **Serum albumin** was significantly decreased, ***Prothrombin time*** was prolonged and ***INR*** was increased significantly inpatients group than in control group **(all *p values <0.01),** but all mean values were still within normal reference ranges which denotes early hepatic dysfunction inpatients group. Hepatic function tests did not correlate to the ***level of viremia***. 91% of patients had low to mild viremia because, through the natural history of the disease, as time elapses, the virus becomes detectable in liver of patients more than in their blood stream. This also explains the only significant inverse correlation of viremia to age of patients. Our results coincides with other studies who added that viremia and progression of HCV infection is multifactorial depending upon the age of infection, male sex, excess iron, increased alcohol consumption, co-infection with hepatitis B, Immunity of patients and genotype of the virus ***(14-19)***

Kidney function tests:

We found that mean ***serum creatinine*** was higher in seropositive cases than the control group ***(p<0.01),*** however, values of creatinine in patients group were found to be within normal range. Seropositive cases had also lower mean ***e.GFR*** than that of the control group ***(p<0.001)*** in spite of the fact that only 26% of cases had e.GFR <90ml/min/1.73m². These results drive us to the fact that normal urea and creatinine in HCV +ve patient does not mean a normal kidney especially when we add the results of urinalysis. Urinary ***P/C ratio*** was found to be higher in seropositive cases than in the control group ***(p<0.001).***In spite of the fact that only 18% of cases had significant proteinuria, mean P/C ratio remained within normal reference range. ***Asymptomatic microscopic hematuria*** and ***proteinuria*** were encountered more in patients group than in the control group ***(p<0.01*)**.*Abnormal urine sediments were also encountered more in seropositive cases than in control group ***(p<0.05).***But the total number of urinary sediment abnormalities is only 46% of total number of cases against 20 % in the control group and does not correlate to the age of patients, which makes them insufficient and inconsiderable for accurate diagnosis of renal involvement.

Serum creatinine and e.GFR showed highly significant correlation to age of patients ***(p<0.01)*** which proves that the kidney is involved early in the course of HCV infection in a progressive form, provided that patients were asymptomatic and they do not have a pre-existing renal disease, serum investigation levels are still within normal range and with no detectable structural changes proved by ***ultrasonography***. Our results agree with many studies ***(4,20).*** **Bandi**, reported that renal involvement can occur early in the course of the disease and occasionally is the presenting symptom of HCV infection ***(4).*** **Tsui et al,** found a correlation between age and proteinuria but he was involving diabetic cases ***(5).***

We are adding a vote to **Ramos et al,** who suggested treatment of mild early cases of renal affection by HCV with low dose interferon and other known options of anti HCV drugs ***(21).***

So, we conclude that, both liver and kidney are involved early in the course of HCV infection with deterioration of liver and kidney functions. The deterioration is not apparent by current laboratory tests except when compared to normal subjects. Ultrasonography is also invaluable as an apparently normal kidney by ultrasonography may not be normal by laboratory tests. Age of the disease is difficult to be determined, viremia is misleading and the problem is great because of the huge population of patients.

We recommend the use of scheduled score sheet for better interpretation of laboratory tests, to stop using equations for these patients and use real tests e.g. laboratory creatinine clearance instead of estimated GFR, reevaluation of the role of biopsies in such cases, wide screening of risk groups for HCV infection using 3rd generation ELISA and to consider early treatment to stop activity of the disease in this golden early stage. We recommend, also, performing this study on larger sample size to have more reliable information.

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