*Research Article*

# Association of Hepatitis C Virus Infection with Proteinuria and Glomerular Filtration Rate in Egyptian patients

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**Abstract**
HCV infection should be considered as a systemic disorder which is often associated with a number of extrahepatic manifestations such as porphyria cutanea tarda, lymphoproliferative disorders, cryoglobulinemia and Glomerulopathies. We aimed to review the prevalence of proteinuria in HCV infection in Egyptian patients. ***Methods:*** A total 1023 patients were screened for HCV, confirmed by PCR for HCV-RNA and subjected to more detailed clinical, biochemical and histological study. patients were subjected to complete urine analysis for detection of Proteinuria which confirmed with urine albumin creatinine ratio of more than 30 mg/g. **Results:** Of the 1023 eligible participants, the prevalence of clinical proteinuria among patients with HCV (with mean age 45.93 ± 13.164 years) was 5.6 % (58 patients out of 1028), individuals with HCV have no increased risk for renal disease reflected by undetectable changes in creatinine level (mean 0.908 ±0.6999). **Conclusion**: HCV infection is associated with proteinuria and normal GFR. The biological mechanism of the observed association needs further study.
**Keywords:** hepatitis C virus, proteinuria, serum creatinine.

## Introduction

The prevalence of anti-HCV from population-based studies is used to compare HCV infection levels globally. Hepatitis C virus infected an estimated 185 million people worldwide and is a significant cause of morbidity and mortality. Also, in developed countries, HCV predominantly infects people who inject drugs[1].

As for the geographical distribution of anti-HCV in persons aged 10-50 years: the Nile Delta and Upper Egypt have rates of 28% and 26% respectively.[2]
A significant relationship between HCV and proteinuria was suggested in otherwise healthy individuals.[3,4], Additionally, anti-HCV antibody positivity was observed to be significantly associated with proteinuria.[5]

A study concluding that patients with HCV-positivity had a 40% higher likelihood of renal insufficiency (serum creatinine levels ≥ 1.5 mg/dl) compared to HCV Ab negative patients. [6]

## Methods

The study comprised the evaluation of a total 1028 patients that screened for serum HCV antibodies using a 3rd generation ELISA technique, Seropositive cases were confirmed to have HCV-RNA by poly-merase chain reaction.

All included patients were subjected to complete urine analysis for detection of proteinuria, Proteinuria then confirmed with urine albumin creatinine ratio of more than 30 mg/g.

Samples analyzed immediately after collection, or stored in aliquots at 4°C or –20°C for 7 days, if particulates were present, sample was centrifuged and the clear supernatant was used for the assay. Equilibrate all components to room temperature.

### *Protein Determination*

1. Samples were run in duplicate, 20 µL of each sample transferred into four separate wells: two Sample wells and two Internal Standard wells. 5 µL

$dH_2O$ was added to Sample wells, and 5µL of Standard to the Internal Standard wells. 25µL of $dH_2O$ was transfered into two wells. This will be the Blank in duplicate.

2. 200 µL of PR Reagent was added to each protein determination wells.
3. Incubated 10min at room temperature, and then read the optical density at 600 nm for Protein.

***Creatinine Determination***

1. Samples were run in duplicate, 20 µL of each sample transferred into four separate wells: two Sample wells and two Internal Standard wells. 5µL $dH_2O$ was added to Sample wells, and 5 µL of Standard to the Internal Standard wells. 25µL of $dH_2O$ was transfered into two wells. This will be the Blank in duplicate.
2. Sufficient Working Reagent (WR) was Prepared for all wells by mixing, for each creatinine determination well, 50µL CR Reagent A, 50µL CR Reagent B, and 150µL $dH_2O$. Transfered 200µL of WR into each creatinine determination well.
3. Incubated 10min at room temperature, and then read the optical density at 530 nm for Creatinine determination.

***Calculation***

Protein concentration of a Sample is calculated as

$$[\text{Protein}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{OD_{STANDARD} - OD_{SAMPLE}} \times 10000 \times n \quad (\mu g/dL)$$

Creatinine concentration of a Sample is calculated as

$$[\text{Creatinine}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{OD_{STANDARD} - OD_{SAMPLE}} \times 25 \times n \quad (mg/dL)$$

Protein Creatinine Ratio of a Sample is calculated as

$$\text{Protein Creatinine Ratio} = \frac{[\text{Protein}]}{[\text{Creatinine}]} \quad (\mu g/mg)$$

## Statistical analysis

Continuous variables were reported as means ± standard deviation. Comparisons be-tween patients with proteinuria and patients without proteinuria were performed with in depended samples t-test and covariance analysis.

Comparisons between patients with proteinuria and patients without proteinuria were analyzed using paired samples t-test. Whereas comparisons among groups of treatment were performed using ANOVA test.

The Pearson correlation coefficients (r) were applied to measure the correlation for continuous normally and non-normally distributed variables

## Results

This table shows the incidence of proteinuria was (58) patients out of (1028) with 5.6%, this was detected with dipstick of fresh urine sample.

**Table (1):** Incidence of Patients with Proteinuria among Study Population.

| Variable | Frequency | Percent |
|---|---|---|
| **Negative Protenuria** | 970 | 94.4% |
| **Positive Protenuria** | 58 | 5.6 % |
| **Total** | 1028 | 100.0 |

**Table (2):** Incidence and Grades of proteinuria compared to A/C ratio among patients study

| Variable | | N | Mean ± SD | minimum | maximum |
|---|---|---|---|---|---|
| A.C ratio | Protenuria (+) | 38 | 405.68 ± 92.617 | 300 | 642 |
| | Protenuria (++) | 17 | 898.06 ± 996.007 | 300 | 3540 |
| | Protenuria (+++) | 3 | 4135.67± 1979.529 | 2000 | 5909 |

**Table (3):** Comparison between patients with proteinuria and and those without proteinuria as regards clinical and laboratory data using independent samples t test

| Variable | Pre-Treatment Protenuria | N | Mean | SD | Std. Error Mean | p-value |
|---|---|---|---|---|---|---|
| Age | (-) PROTENURIA | 970 | 45.83 | 13.198 | 0.424 | 0.334 |
| | (+) PROTENURIA | 58 | 47.55 | 12.591 | 1.653 | |
| ALT | (-) PROTENURIA | 970 | 45.39 | 24.572 | 0.789 | 0.826 |
| | (+) PROTENURIA | 58 | 44.66 | 20.776 | 2.728 | |
| AST | (-) PROTENURIA | 970 | 45.55 | 22.463 | 0.721 | 0.466 |
| | (+) PROTENURIA | 58 | 43.35 | 20.604 | 2.705 | |
| Albumen | (-) PROTENURIA | 970 | 4.272 | 0.4809 | 0.0154 | 0.755 |
| | (+) PROTENURIA | 58 | 4.252 | 0.5510 | 0.0723 | |
| Bilirubin | (-) PROTENURIA | 970 | 0.758 | 0.2347 | 0.0075 | 0.034 |
| | (+) PROTENURIA | 58 | 0.826 | 0.2504 | 0.0329 | |
| Hemoglobin | (-) PROTENURIA | 970 | 13.19 | 1.575 | 0.051 | 0.378 |
| | (+) PROTENURIA | 58 | 13.00 | 1.698 | 0.223 | |
| Platlets | (-) PROTENURIA | 970 | 213.41 | 60.095 | 1.930 | 0.283 |
| | (+) PROTENURIA | 58 | 204.74 | 53.624 | 7.041 | |
| Creatinine | (-) PROTENURIA | 970 | 0.829 | .2144 | 0.0069 | 0.001 |
| | (+) PROTENURIA | 58 | 2.231 | 2.4812 | 0.3258 | |
| PCR | (-) PROTENURIA | 970 | 1792605.88 | 3177332 | 102017 | 0.161 |
| | (+) PROTENURIA | 58 | 1201666.19 | 1811263 | 237830 | |

This table shows there is significant difference between the two groups as regard CREATENIN level (P-value=0.001) and BILRUBIN (P-value=0.034) however there is no significant difference as regard AGE, serum ALBUMIN, AST, ALT, HB and PCR

## Discussion

Results of our study however, has found the prevalence of clinical proteinuria among patients with HCV (with mean age 45.93± 13.164 years) was 5.8 % (58 patients out of 1028), with 3.8 % having mean A/C ratio of 405.68±92.617 and 1.7% has mean A/C ratio of 898.06±996.007 and only 0.3% of patients has mean A/C ratio of 4135.67± 1979.529.

This result is similar to that reported by the Third National Health and Nutrition Examination Survey (5.1% among patients without diabetes, hypertension, cardio-vascular disease, or elevated serum creatinine levels).

This also in agreement with[3] found Hepatitis C seropositivity was associated with albuminuria only among those who were older than 40 yr, and the association was strongest for those who were 60 yr and older (P = 0.01 for trend across age categories). Among people who were aged 20 to 39, chronic HCV was negatively associated with albuminuria.

This is in contrast with[8], who found that Infection with HCV alone showed a Significant positive correlation with protei-nuria (OR, 1.14; 95% CI, 1.003-1.300; P _ 0.04) and[9] who also found that Anti-HCV-positive rate amongst protei-nuria subjects was significantly higher than nonproteinuria subjects (9.6% vs. 6.2%, P<0.001). Also the prevalence of protei-nuria amongst anti-HCV-positive subjects (10.2%) was significantly higher than that in HBsAg-positive subjects (6.4%, P= 0.004) and in HBsAg-negative or anti-HCV-negative subjects (7.0%, P=0.004).
This discrepancy might be explained by long-term infection in some studies, the definition of renal insufficiency, or the viral genotype.

In our study there was no significant difference between Female and male regarding prevalence of proteinuria (56.9% Vs. 43.1%). This is also in agreement with the Third National Health and Nutrition Examination Survey.
In our study, we did not find an increased risk for renal disease in HCV as reflected by undetectable changes in creatinine level (mean 0.908 ±0.6999).

Our study confirmed that there is no significant difference between proteinuric group and nonprotienuria group regarding AGE, ALT, AST, ALBUMIN, PCR level, however there is significant difference between the two groups regarding serum creatinine (P- value 0.001) and bilirubin level (P- value 0.034).

Our study results of proteinuria was not associated with diabetes where diabetic patients were excluded and 51 patients out of 58 was Cryoglobulin negative.
Therefore, our results suggested that HCV was associated with protinuria indepen-dently of cryoglobulinemia or diabetes status.

## Conclusions

Eradication of the virus is, undoubtedly, a key target in the therapeutic approach to HCV-related extrahepatic features.
However, the scenario has suffered a disruptive change with the appearance of DAA, which have emerged as game-changers in HCV therapy; therefore, it may be anticipated that the therapeutic approach to HCV patients presenting with EHMs will also change dramatically.

The current clinical experience about the use of the new DAAs in extrahepatic disease is limited (170 cases reported in the last 2 years from uncontrolled studies, principally in CV patients and some isolated cases of B cell lymphoma, while there is no evidence on their use in other EHMs).

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