Screening of Hepatitis B- and C- Viral Markers in Iraqi Patients with Proteinuria

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ABSTRACT:

BACKGROUND:

Viral hepatitis may lead to nephropathy as one of its multiple extra hepatic manifestations. Symptomatic proteinuria as detected by dipstick, and qualitative urine collection are simple tests in practice as well as useful cardinal test of underlying renal abnormalities. The aim of this study was to elucidate the impact of hepatitis B virus (HBV) and/or hepatitis C virus (HCV) infections on the occurrence of symptomatic proteinuria amongst adults and pediatrics.

PATIENTS & METHODS :

A prospective study included (143) adult and pediatric Iraqi patients presented with symptomatic proteinuria, and 108 (age- and sex-matched) apparently healthy individuals (as control group) who were serologically screened for HBV & HCV markers using third generation Enzyme linked immunosorbent assay (ELISA) techniques, screening for HIV by ELISA and other related immunological and biochemical profile.

RESULTS :

It was found that the prevalence of hepatitis B surface antigen (HBsAg), anti hepatitis B core antibody (anti-HBc-IgM), anti hepatitis B surface antibody (anti-HBs) and anti hepatitis C antibody (anti-HCV) in the proteinuria group as compared to control group, were (7.0% vs. 0.9%, $P<0.05$) for HBsAg, (2.8% vs. 0% ,$P>0.05$) for anti-HBc-IgM, (20.3% vs. 23.1%, $P>0.05$) for anti-HBs antibody, and (6.3% vs. 0%, $P<0.01$) for anti-HCV.

CONCLUSION :

Our study demonstrated a significant association between proteinuria and HCV, and HBV infection in the adult and pediatric population. The interpretation of serological patterns of viral hepatitis markers in patients with newly diagnosed proteinuria are important, it might suggest that detailed urinalysis and qualitative urine protein assessment is mandatory when managing patients with HCV or with HBV infections.

KEY WORDS : hepatitis b virus / hepatitis c virus/ proteinuria .

INTRODUCTION:

Proteinuria has been shown to be an early diagnostic marker of kidney damage and can predict the progression of renal disease in patients with diabetes as well as cardiovascular morbidity and mortality in diabetic and general population [1,2].

Patients with proteinuria who seek medical advice are screened for viral hepatitis markers[1,2]. Screening with a dipstick for proteinuria is often a satisfactory first approach to evaluation of kidney and provides a sensitive marker of many types of kidney disease from early to advanced stages. Moreover, it is also a useful and simple test to disorder [3]. Viral hepatitis infection *per se* may predict the severity of proteinuria and can be used to guide further investigations of possible renal lead to nephropathy. This uncommon extra hepatic manifestation might be induced either by the direct cytopathic effect of virus or by the interplay between viral, host and environmental factors [4-6]. The association between HBV infection and renal involvement, mainly membranous nephropathy, was first reported in 1971 [7]. Since then, many reports have addressed the correlation between HBV infection and nephropathy [8-10]. However, the natural history and the pathogenesis are not well understood, but are believed to be mediated by deposition of immune complexes of HBV antigens in the glomeruli [11]. Hepatitis C virus (HCV) has been shown to be a lymphotropic as well as a hepatotropic virus [5]. Replication of HCV in diseased extrahepatic organs and tissues may have cytopathic effects. It, therefore, may either trigger latent autoimmunity or induce de novo an

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autoimmune disease [12]. Renal involvement has been postulated to be a distinct extrahepatic manifestation in chronic hepatitis C (CHC) infection [13-16].

This study was aiming to investigate some clinical, biochemical, and immunological parameters associating the occurrence of proteinuria as well as the percentage of association of the serological markers of HBV and HCV infections with the pediatric and adult patients with proteinuria.

PATIENTS AND METHODS:

Between November 2004 and August 2005, 143 adults and pediatrics patients with newly diagnosed proteinuria (presented with edema, haematuria, acute deterioration in renal function). from two hospitals in Baghdad (Specialized Surgical Hospital /Baghdad Medical City and, Central Children Hospital), adults subgroup were 79 patients with newly diagnosed proteinuria, their ages ranged from 18 years to 75 years, and there were 64 pediatric subgroup patients presented with proteinuria, and their ages ranged from 1.5 to 14 year. Inclusion criteria were urine protein excretion >1000 mg/m2 per day for adults and protein excretion >1000 mg/m2 per day or > 40 mg/m2 per hour, or spot urine Protein/Creatinine ratio >1.0, for pediatrics) according to Recommendations from a Pediatric Nephrology panel established at the National Kidney Foundation conference on Proteinuria (PARADE) criteria[17]. Exclusion criteria were, presence of diabetes mellitus, patients on dialysis, or with significant renal failure (serum creatinine > 4.5 mg/dl).

Control group were 108 age and sex matched healthy subjects, with no history of renal or liver diseases, and negative proteinuria on urine analysis on two occasion, and normal renal function test (serum creatinine <1.4 mg/dl). Adults subgroup were 63 (30.6 ± 9.81 years, 39 male and 24 female), pediatrics subgroup were 45 (10.2 ± 3.2 years, 26 male and 19 female).

The study designed to compare the prevalence of hepatitis serological markers in proteinuria patients group (no.143) with healthy control group(no.108), and both groups were in turn subdivided into adults and pediatrics subgroups.

The following data were gathered from patients and control groups; age, gender, clinical data, type of presentation of glomerular disease, infections history and concomitant infections, therapy received, extra renal manifestations, biochemistry data, hematocrit and hemoglobin, platelets, leucocytes, percentage of eosinophils, creatinine and creatinine clearance, proteinuria, sediment, uric acid, cholesterol, total proteins, albumin, bilirubin, GOT, GPT, GGT, LDH, alkaline phosphatase, IgG, IgA, IgM, ANA, anti-DNA, C3,C4, cryoglobulinemia and proteinogram.

Urinalysis was performed with spontaneously voided fresh urine in the morning. A test dipstick paper was used for the detection of proteinuria. Urine 24 hour collection was used for quantitative protein assay. When urine collection is not available urine spot protein / creatinine ratio was used.

Hepatitis B surface antigen, Hepatitis B core IgM,, Anti Hepatitis B surface antibodies, Anti-HCV total antibodies were detected using a third-generation, ELISA kit, (RANDOX, UK). Hepatitis E V IgM antibodies were detected using ELISA (Biokit. SPAIN). Anti-HIV antibodies with Tetra ELISA (Biotest. Germany). Hepatitis AV IgM antibodies detected with ELISA, (BIOTEC Laboratories Ltd. UK). ANA and Anti-ds-DNA were detected using ELISA. (Biomaghreb).

The data are expressed as mean and standard deviation and the corresponding range. Statistical Inter-group comparisons were done by using the Student's t test and Mann-Whitney test. The chi-squared test was used for qualitative variables. Values with a p value < 0.05 were statistically significant.

RESULT:

A total of 143 non diabetic patients involved in this study, 79 adults (age 31.1 ± 11.6 years, 57 male and 22 female), and 64 were pediatric patients, (age 8.0 ± 2.7 years, 40 male and 24 female). The biochemical characteristics are shown in (Table1).

The prevalence of HBsAg (as a marker of HBV infection) was found in 7.0 % (10/143) in the whole (pediatric & adults) study group compared with 0.9 %(1/108) in the (age & sex matched) apparently healthy control group, statistically the difference was significant (P<0.05).

Anti-HBc-IgM were positive in 2.8 % (4 / 143), in the proteinuria group, whereas none in control group were positive. Statistically, the difference was non-significant (P > 0.05). In addition, 20.3 % (29 / 143) of proteinuria patients (compared to 23.1 %; 25/108 of apparently healthy individuals) were positive for anti-HBs antibodies. Statistically, the difference also was non-significant (P > 0.05).

The prevalence of anti-HCV amongst patients with proteinuria was 6.3% (9/143), which was significantly higher than that of control group (0 /108, zero %) (P < 0.001).

While the prevalence of Anti HAV-IgM antibodies did not significantly differ between proteinuria group and control group (3.5% vs. 0.9%, P>0.57). Anti HEV antibodies seropositivity did not significantly differ between proteinuria group and control group (2.1% vs. 0%, P> 0.05).
HIV screening with ELISA were negative in both patients and control group. as shown in Table (2).

Table 1 :Biochemical characteristics of 134 non diabetic patients with proteinuria

Variables	Patients group n(143)	Control group n (108)	P-Value
Blood Urea(mg /dL)	46.9±19.6	35.7±7.6	0.015
Serum creatinine(mg /dL)	1.4±0.5	0.83±0.17	0.001
24hr urine protein(Adults)gr/24hr	1.9±1.0	no proteinuria	
Total .S.protein(gm /L)	5.3±1.3	7.1±0.6	0.001
S.albumin(gm /L)	2.94±0.79	4.4±0.7	0.001
S.globulin(gm /L)	3.2±1.6	2.1±0.4	0.14
SGOT(U/L)	13.3±7.8	7.6±1.7	<0.001
SGPT(U/L)	14.5±11.1	7.8±1.8	<0.001
Alkaline phosphatase(U/L)	67.1±38.6	59.5±17.5	0.33
Fasting plasma glucose(mg /dL)	112.1 ± 24.9	102.1 ± 23.2	0.2
Triglycerides (mg /dL) in adults	191.3 ± 150.8	142.6 ± 106.5	<0.001
Cholesterol (mg /dL) in adults	243.6 ± 37.8	206.9 ± 29.1	<0.001

Table 2: Seroprevalence of ELISA – hepatitis markers (HbsAg, Anti-HBc IgM, AntiHBs antibody, Anti HCV antibody, Anti HAV IgM, Anti HEV antibody) and HIV antibody markers among patients with proteinuria compared with control group

Group	HBs-Ag +ve n(%)	Anti-HBc -IgM+ve n(%)	Anti-HBs +ve n(%)	Anti-HCV+ve n(%)	Anti-HAV+ve -IgM n(%)	Anti-HEV+ve n(%)	Anti-HIV+ve n(%)
Proteinuric patient (143)	10 (7.0%)	4 (%2.8)	29 (20.3%)	9 (6.3%)	5 (3.5%)	3 (2.1%)	0 (0%)
Control(108)	1 (0.9%)	0 (0%)	25 (23.1%)	0 (0%)	1 (0.9%)	0 (0%)	0 (0%)
P-value	0.019	0.078	0.625	0.008	0.11	0.131	1.0
Significance	Sig. (P<0.05)	Non Sig. (P>0.05)	Non Sig. (P>0.05)	Highly Sig. (P<0.01)	Non Sig. (P>0.05)	Non Sig. (P>0.05)	Non Sig. (P>0.05)

The seroprevalence of HbsAg in adults patients subgroup (6.3%) was higher than that (0.9%) in adult control subgroup, statistically, the seroprevalence of HBsAg was significantly constraint to adults patients subgroup (P<0.05), while the prevalence of HBsAg did not significantly differ between pediatric patients subgroup and pediatrics control subgroup (0.7% vs, 0.0%, P>0.05).
Regarding the parameter of anti-HBc-IgM, its prevalence in pediatrics patients subgroup were (0.7%) while in adults patients subgroup were (3.5%). Neither pediatrics control nor adults control subgroups had shown any positivity to anti-HBc-IgM ELISA test. Statistically, results revealed that the prevalence of anti-HBc-IgM was significantly constraint to adult patients subgroup at (P<0.05) and goes with chronic infection or carrier state.

The seroprevalence of anti-HBs in pediatrics patients subgroup were lower (16.1 %) than that of pediatrics control subgroup (18.5%), statistically the difference was non-significant (P>0.05), and the seroprevalence of anti-HBs in adult patients subgroup were lower (4.2%) than that of adult control subgroup (4.6%), statistically the difference was also not significant (P> 0.05).
In adults patients subgroup, the prevalence of anti-HCV were 5.6%, while in the adult control subgroup no positive case was found, and a highly significant difference was noticed (P< 0.01).
The seroprevalence of anti-HCV in pediatrics patients subgroup was 0.7% whereas in children control group, no positive case was recorded, and statistically the difference was non-significant (P>0.05).(Table3)
The presence of more than one viral markers in the same patients were unusual finding in the present study, two patients are positive for both HBsAg

and Anti HBc-IgM (means current or acute infection), one patient were positive for HBs Ag , HBc-IgM, and Anti-HCV. All patients with positive viral markers show no evidence (clinical and synthetic liver function test) suggestive of chronic liver disease.

Table 3:The seroprevalence of hepatitiss B & C markers among adult subgroup and pediatric subgroup compared with their control subgroups.

Study Groups	HBs-Ag +ve n(%)	Anti-HBc -IgM +ve n(%)	Anti-HBs+ve n(%)	Anti-HCV+ve n(%)
Adults Patients no(79)	9 (6.3%)	5 (3.5 %)	6 (4.2%)	8 (5.6%)
Control Adults no (63)	1 (0.9%)	0	5 (4.6%)	0
Pediatrics Patients no (64)	1(0.7%)	1(0.7%)	23 (16.1%)	1(0.7%)
Control Pediatrics no(45)	0	0	20 (18.5%)	0

Immunological study for adult patients subgroup shows ANA +ve in seven patients, Anti Ds-DNA were positive in five of them with clinical features suggestive of Systemic Lupus Erythmatosus, all viral seromarkers were negative except HBs Ag +ve in one patient only.

Immunological assay for adults patients subgroup were conducted including (C3, C4, IgA, IgG, IgM) as a part of the workup for adult patient with significant proteinuria, and compared with adults control subgroup showed that C3 were significantly lower in proteinuria group (98.07±40.97 vs, 122.41±34.46, P<0.002). while no significant difference in the level of C4, IgA, and IgG, between adult patients and adult control subgroups.

The level of IgM was found to be significantly higher in adult patients subgroup than in adult control (186.56±77.51 vs, 115.99±43.78 , P<0.002).(Table 4).

Table 4: Immunological serum markers in proteinuria adult subgroup compared with adult control

Variables	Adult patients subgroup n(79)	Adult control subgroup n (63)	P-Value
ANA +ve n,%	7(8.8%)	1(1.2%)	<0.01
Anti ds-DNA +ve n,%	5(6.3%)	0(0%)	<0.01
C3 g/L	98.07±40.97	122.41±34.46	<0.002
C4 g/L	35.21±12.73	35.96±11.48	0.75
IgA g/L	172.98±63.30	157.91±47.70	0.43
IgM g/L	186.56±77.51	115.99±43.78	<0.002
IgG g/L	813.63±389.96	984.59±310.77	0.11

DISCUSSION:

During the past decade, proteinuria has taken on a new importance, and is shown to be a cardinal sign and an independent risk factor for the outcome of both kidney and cardiovascular disease [(1)]. Not only is proteinuria associated with glomerular injury and loss of its normal perm selective properties, but experimental data have demonstrated that protein–tubular cell interactions also have inflammatory and fibrogenic consequences that can contribute to interstitial damage and fibrosis [(18)]. Most screening methods of a qualitative character commonly use a commercial dipstick, which measures total protein or albumin. These dipsticks, which are of course simple to use, usually afford high specificity, but low sensitivity, Thus, dipstick - proteinuria assessment remains a useful and simple test to predict the severity of proteinuria and can be used to guide further investigation of possible renal disorder [(3)]. Adult patients with symptomatic proteinuria (edema, hematuria, unexplained renal failure) who involved in the present study have a proteinuria >1gm/m2/24 hr and this level is more in favor of glomerular proteinuria, rather than orthostatic proteinuria or tubular proteinuria which is milder and transient form of proteinuria [(19)]

The diagnostic evaluation of the child with dipstick-positive proteinuria depends in part upon the presence or absence of symptoms and qualitative urine protein collection >1 gm/m2/day,

but this is not always possible in pediatrics patients, the alternative way is urine spot > 1 mg protein/mg creatinine ratio, or calculate one hour urinary protein, >40 mg/m2/1 hr urine collection, this approach is consistent with the recommendations of the 2000 Pediatric Nephrology panel established at the National Kidney Foundation conference on Proteinuria, Albuminuria, Risk, Assessment, Detection and Elimination (PARADE) [17].

Hepatitis B virus-related nephropathy, mainly membranous nephropathy, has also been observed for decades [7-10]. In patients seropositive for HBeAg and HBeAg seroconversion to anti-HBe leads to abrogation of proteinuria in most cases [20]. Treatment with Alpha-interferon has been demonstrated to ameliorate proteinuria amongst patients with chronic HBV [21]. HBV vaccine given as part of routine immunization, even with low coverage rates, was highly effective in reducing the incidence of HBV-related nephropathy in children [22].

HCV-associated nephropathy has been postulated to be a distinct extrahepatic manifestation, which might be related to interplay between intrinsic renal disease, autoimmune abnormality and host susceptibility [4,13,16]. It occurs largely in the context of cryoglobulinemia [13]. There are also quite number of cases who do not have cryoglobulinemia but still present with one of the major HCV-associated nephropathy, namely membranoproliferative glomerulonephritis, membranous nephropathy and focal segmental glomerulosclerosis, the latter two generally not associated with cryoglobulinemia [16]. Previous studies have shown that in persons with HCV infection, higher incidence of microalbuminuria and proteinuria did occur, and the positive rate of proteinuria was approximately two times higher in patients with chronic hepatitis C than in those with other forms of liver disease [14,15].

The prevalence of HBV in glomerulonephritis have a lot of geographical variations. In a Chinese study for patients with IgA Nephropathy and detected HBs-antigenemia in 34 % (17/50), HBeAg in 6 % (3/50) , anti-HBe was positive in 20 % (10/50), anti-HBc in 52 % (26/50) and anti-HBs in 20 % (10/50) of those cases [23]. Whereas in France which is, a low endemic country, the frequency of hepatitis B virus infection in patients with glomerulonephritis, ranged between zero % in Caucasian population and 1.4 % in patients came from highly or intermediately endemic regions [24].

In the Middle East, Iraq and the United Arab Emirates have intermediate endemicity for HBV, while Jordan, Oman, Palestine, Yemen and Saudi Arabia have high endemicity [25]. Using counter immunoelectrophoresis (CIEP), the carrier rate of HBsAg among the Arab Population of Iraq was found to be 2.8% [26].

The occurrence of HCV infection in the general population in different parts of the world varies from (0.2 % - 18 %). The areas of higher prevalence include countries in the Far east, Mediterranean countries and certain areas in Africa and Eastern Europe [27]. In other parts of the world, such as Northern Europe, the prevalence of anti-HCV was 0.2% -0.8%, whereas in Southern Europe and Japan, the prevalence of anti-HCV was 1.2% -1.5%. In Middle East countries, high prevalence of anti-HCV antibodies among normal population where (2.4%) anti-HCV positivity were found in Yemen, (1.9 %) in Sudan and (19 %) in Egyptian blood donors [28].

The prevalence of Anti-HCV among patients with glomerulonephritis is widely different. and the frequency of Anti-HCV positivity could range between zero and 100% in the different types of renal diseases[29]. In Italy, the prevalence of Anti-HCV positivity among patients with biopsy-proven glomerulonephritis was 13 % (38/248) where the anti-HCV rate was significantly higher in patients with cryoglobulinaemic membranoproliferative and mesangioproliferative GN [30].

Our results demonstrated that prevalence of HCV infection was significantly higher among those with proteinuria than those without (6.3% vs. 0% P <0.008).

The prevalence of HBV infection presented as HBs Ag was higher among patients with proteinuria than those without(7% vs, 0.9% P<0.05). A similar study conducted in Taiwan, shows that the prevalence of positive anti-HCV amongst subjects with proteinuria was 9.6%, which was significantly higher than that of subjects without proteinuria (6.2%, P< 0.001). On the contrary, the prevalence of HBsAg seropositivity did not differ between proteinuria and non-proteinuria subjects(13.0% vs. 13.8%, P>0.57) [31].

Although higher prevalences of Anti HAV IgM, and Anti HEV IgM were detected in the present study, no statistically significant differences were found between the prevalence of Anti HAV IgM, and Anti HEV IgM in the proteinuria and control groups (3.5% vs,0.9%, P>0.11 and 2.1% vs, 0.0%,P> 0.131 , respectively). Acute and chronic viral hepatitis has been shown to be associated with various extrahepatic manifestations [32]. In this

respect, infections with hepatitis B and C viruses were notoriously considered as potential etiologies of different glomerular diseases. In addition, glomerulonephritis (GN) or other immune complex (IC)-related diseases were found to be associated with HAV infection. However, hepatitis E virus might be excluded from the list of etiological agents of GN, since it is a self-limiting viral infection that almost followed by recovery without chronicity [33]. Up to our knowledge, this is the first attempt in Iraq, to explore the role and to assess the prevalence of both HAV and HEV in cases with proteinuria, thus, the causes of such findings in the present study are uncertain and need further studies, at molecular and pathological levels, to find supportive evidences for their role in such pathological entity.

This study also showed a significantly high prevalence of anti–HBs antibodies in pediatric than in adult patients with proteinuria and in control group . This could be explained by the acquired antibodies through the vaccination, or by increased exposure to the risk factors for attracting HBV infection through frequent hospitalization and repeated injections where such explanations could be supported by the findings in other studies[31,34].

ELISA for HIV antibody were negative in both patients and control groups, and it was important to have this test in order to exclude any HIV-associated nephropathy.

CONCLUSION:

In conclusion, our results demonstrated that HCV and HBV infection in adults and pediatric patients played a significant role in association with proteinuria. It might suggest that detailed urinalysis and qualitative urine protein assessment is mandatory laboratory investigation when managing patients having HCV- and / or HBV- acute or chronic infection or carrier state. We failed to observe any significant association between HAV infection and HEV infection and proteinuria in the current study.

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