Original Article

Hepatitis C Virus among Iraqi Patients on Renal Dialysis, Some Immunological Profiles

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Summary:

Back ground: Chronic hepatitis C (HCV) is the most common chronic liver disease at present, and HCV infection is found with variable prevalence in dialysis populations in different parts of the world.

Objective: The aim of this study was to determine the concentration of sialic acid and immunoglobulins level in the sera of patients with chronic renal failure whom infected with Hepatitis C virus, and the effect of hemodialysis on them.

Patients&Methods: Regarding to this aim, total sialic acid levels (TSA) and immunoglobulins level were studied on the blood samples of 20 patients with chronic renal failure + Hepatitis C virus (positive group) and 20 patients with chronic renal failure (negative group) and 20 healthy volunteers. Serum TSA determinations were carried out by the resorcinol method.

Results: In (positive group), the mean of TSA concentration (after hemodialysis treatment) was 68 ± 2.9 mg/dl, and the mean of TSA concentration in (negative group) was 110.7 ±7.5 mg/dl, while the mean TSA level in healthy control group was 58.3 ± 1.7 mg/dl. Also, this study aimed to assess serum concentration of immunoglobulins: IgG, IgM and IgA. Serum IgG was increased in patients of (positive group) and (negative group) with respect to control healthy (6.1, 5.5, 5.4 respectively) ($p<0.05$).There was no significant difference between the third groups in serum IgM concentration (6.6, 6, 6.2 respectively).Also in serum IgA concentration (5.8, 6, 5.5 respectively).

Conclusion: We conclude that the TSA levels in the first groups both were found to be significantly elevated as compared to control levels ($p<0.05$), and Hepatitis C virus are linked to selective increase of serum IgG.

Key Words: Hepatitis C virus, Hemodialysis, Sialic acid, Immunoglobulins.

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Introduction:

The liver and kidneys have the primary responsibility of filtering waste and toxic substances from the body and blood stream. Both organs play a vital, hand-in-hand role in processing nutrients, eliminating waste, and regulating the blood, making them crucial to overall health (1). Organ damage of the kidneys and liver is either acute (short-term, days to weeks) or chronic (long-term, years). The type of Hepatitis C that leads to kidney problems is chronic, when the liver suffers permanent damage and cannot perform its functions properly (2). Chronic hepatitis C (HCV) is the most common chronic liver disease at present, and HCV infection is found with variable prevalence in dialysis populations in different parts of the world (2,3). The high rate of HCV transmission among chronic kidney disease patients may be due to direct exposure to infectious blood and/or blood products because of inadequate infection control (4). The prevalence of HCV increases with age, the number of blood transfusions received, the mode of dialysis and the time on dialysis (3,5). Probable association between HCV infection and renal disease was first reported in .1990Subsequently) ,Johnson et.al. 1999) hepatitis C patient is unable to adequately break down essential proteins that are carried through the blood to the kidneys to be eliminated. If demonstrated a strong association between HCV infection and membranoproliferative glomerulonephritis (MPGN) (6). The liver of a the kidneys are unable to eliminate the normal amount of these proteins, the impairment affects the overall functionality of the kidneys as a problematic result (4). Our defense against bacteria and viruses, the immune system is essential maintaining health. The strength of our immune system is one factor to determine wether Hepatitis C becomes of an acute case or developed chronic illness. HCV often causes persistent infection despite the presence of neutralizing antibodies against the virus in the sera of hepatitis C patients. The virus infects both hepatocytes and B cells through the binding of its envelope glycoprotein E2 to CD81, the putative viral receptor. The infection becomes chronic when the virus evades cells and establishes itself in the body. At this point, the immune system becomes weakened and ineffective against Hepatitis C. However, the immune system partially controls the viral infection but due to long-lasting inflammatory milieu, hepatic damage occurs (7). Serum immunoglobulin concentrations are commonly elevated in patients with liver disease. Immunoglobulin class increase may vary depending on cause of liver disease. Hepatitis C virus is, together with alcohol, a leading cause of chronic liver disease (8).

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Subjects, Material & Methods:

Patients:

Total serum sialic acid level (TSA) and immunoglobulin levels were studied from the blood samples of patients with end-stage renal disease. A total of 40 patients with chronic renal failure attending Al-Kindy Teaching Hospital/ Baghdad during the period March-July 2011. Their age range was 30-50 years. The patients were divided into three groups:

20 patients with chronic renal failure who had been treated by dialysis (negative group), 20 chronic renal failure patients who had been treated by dialysis and had been infected with chronic Hepatitis C virus (positive group), and 20 serum samples from healthy volunteers.

Material & Methods:

Measurement of Total Serum Sialic Acid (TSA): Determination of total serum sialic acid for control, patients and calibration samples was performed by the resorcinol method described by Svennerholm 1957(9).

Twenty µL for each concentration {6,12,24,48}-µg / ml of calibration samples or plasma samples were put in clean and sterile test tube, 980 µL distill water was added for each tube, the solution mixed and put in an ice bath. One ml of resorcinol reagent (coloring, SA) was added to each tube. The tubes were put in water bath at $100c^0$ for 15min, and then transferred into ice bath for 10min, 2ml of (Butyl acetate / Methanol) solution was added with a good Mixing, the sample centrifuged at 3000 r/min for 10min, and reading the color extraction at wavelength 580 nm. Assessment of Immunoglobulin levels: The concentration of IgG, IgM and IgA immunoglobulins were measured by using the method of (Single Radial Immunodiffusion) (RID) (Mancini, et al.,1965) (10) with commercially available plates, RID plates for 12 test (Biomaghrib).Each plate contains monospecific antiserum directed against the protein listed on the label in an agarose gel layer. The results were analyzed using the computer programme SPSS (Statistical package for Social Sciences) vrsion 13. Their data were presented in terms of means ± standard errors (S.E), and differences between means were assessed by ANOVA and LSD tests.

Results:

Table (1-1). Serum levels of sialic acid, IgG, IgM, and IgA in patients and control.

Groups	No.	Sialic acid Mean±S.E.	IgG Mean±S.E.	IgM Mean±S.E.	IgA Mean±S.E.
Positive patients(+ve)	20	68 ± 2.9 A	6.1 ± 0.2 A	6.6 ± 0.2 *	5.8 ± 0.2 *
Negative patients(-ve)	20	110.7± 7.5 B	5.5 ± 0.2 *	6 ± 0.2 *	6 ± 0.3 *
Control healthy	20	58.3 ± 1.7 AB	5.4 ± 0.03 A	6.2 ± 0.2	5.5 ± 0.1

Different letters: Significant difference (P□0.05) between means.
*: No Significant difference (P□0.05) between means.

Discussion:

In chronic renal failure with Hepatitis C virus patients (positive group), the mean of TSA concentration was (68 mg/dl) and the mean of TSA concentration in chronic renal failure patients (negative group) was (110.7 mg/dl). The TSA level in both positive and negative groups was found to be significantly elevated as compared to control level (58.3 mg/dl). It has been thought that this increase may be due to the increased synthesis and catabolism of glycoproteins and glycolipids in chronic renal failure patients (12). Our results agreed with the findings of (E.Uslu, et al.,2002)(13), that there is an increase in serum sialic acid level in chronic renal disease and end stage renal failure requiring hemodialysis, the improved serum sialic acid level probably reaches a definite value. Serum sialic acid level is minimally influenced by hemodialysis performed with a hemophane membrane, and the hemodialysis procedure causes increased cytokine production (13). Hemodialysis did not provide clearance of sialic acid; to the contrary, it caused an insignificant increase in serum sialic acid levels. Uraemic toxins are retention solutes that accumulate in the serum/plasma of patients with a reduced kidney function and contribute to a variety of metabolic and functional disturbances, such as a diminished immune defense(14). The mean of serum immunoglobulin IgG level was significantly increased in chronic renal failure patients with hepatitis C virus (positive group) (6.1mg/dl) than the corresponding mean in patients with chronic renal failure group (negative group) (5.5 ml/dl) and control healthy group (5.4 mg/dl) as shown

in (Table 1-1). Immunoglobulin G (IgG) mediates pro- and anti-inflammatory activities through the engagement of its Fc fragment (Fc) with distinct Fcg receptors (FcgRs). One class of Fc-FcgR interactions generates pro-inflammatory effects of immune complexes and cytotoxic antibodies. IgG acquires anti-inflammatory properties upon Fc sialylation, which is reduced upon the induction of an antigen-specific immune response (15). Our results agreed with (Quintela,G.,A., et al.,2003), that is hepatitis C virus was linked to a selective increase of serum IgG, even in cases with mild or minimal liver disease(8). The means of serum immunoglobulin IgM level in (positive group) and (negative group), as well as, control healthy group showed no significant difference (6.6, 6 and 6.2 mg/dl, respectively) (Table1-1), because, IgM antibody is the predominant antibody in primary immune response (16), and because IgM antibody response is usually present during acute viral infections, and it wanes as a subsequent IgG response arises. Nevertheless, IgM antibody to viral antigen frequently persists if chronic infection evolves (17), showed that 22% of end stage renal disease (ESRD) patients with anti-HCV IgG activity showed anti-HCV IgM core antibody in the serum. Anti-HCV IgM activity seems to correlate positively with HCV viraemia in (ESRD) population. Testing for this antibody may be useful as a serological marker to indicate the presence of ongoing HCV infection (17), (18). Serum immunoglobulin IgA level mean showed no significant difference between the three investigated groups (positive group: 5.8 mg/dl; negative group: 6 mg/dl; control healthy group: 5.5 mg/dl) (Table1-1). Transmission of HBV and HCV infections are common among chronic renal failure (CRF) patients on dialysis. The immune dysfunction caused by CRF makes patients more vulnerable to the cytopathic effects of HBV and HCV infections (4).

Predominant deposition of immunoglobulin A (IgA) in the renal mesangium is seen in patients with HBV associated mesangial proliferative glomerulonephritis (MGN) (6). Other forms of immune complexes mediated glomerular diseases like MGN, IgA nephropathy and focal segmental glomerulosclerosis (FSGS) described among HCV infected individuals, may have similar pathogenic mechanisms as their idiopathic counterparts (19).

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