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Value of toll-like receptor 3 (TLR3) in chronic kidney disease patients with hepatitis C virus infection

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Abstract

Background

Hepatitis C virus (HCV) infection is a severe public health problem with a worldwide predominance of 3%. An estimated 5–20% of infected patients develop cirrhosis annually, and 1–4% of them develop hepatocellular carcinoma. In Egypt, the prevalence of HCV antibody and HCV RNA are 10.0 and 7.0%, respectively, in the 15–59 years age group. In children of 1–14 years old, the prevalence of HCV antibody and HCV RNA are 0.4 and 0.2%, respectively. HCV infection is a cause of chronic kidney disease (CKD) and results in a significant problem in hemodialysis (HD) patients. Toll-like receptors (TLRs) might have an essential role in the pathogenesis of renal diseases. They include TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12, and TLR13, though the latter two are not found in humans. Recently, it is shown that the mouse and human kidney tubular epithelial cells appear to express TLR3 constitutively and upregulate its expression upon challenge with its legend. For this reason, it appears conceivable that this receptor might play an essential role in this organ in health and disease.

Aim

The present study was carried out to determine the value of TLR3 in CKD patients with HCV infection. Also, we aimed to study the levels of TLR3 for Child–Pugh different classes to determine its impact on the prognosis of liver disease in those patients.

Patients and methods

All participants were selected from the National Institute of Urology and Nephrology. Their ages ranged between 40 and 62 years. Our study included 130 individuals of which 105 patients were selected from a dialysis unit with the end-stage renal disease on regular HD three times/week for at least 1 year and 25 healthy participants as a control group. They were divided into three groups. The control group (GI) consisted of 25 clinically healthy participants matched for age and sex; GII constituted 45 HCV negative patients; and GIII had 60 HCV positive patients. GIII was subclassified into GIIIA, GIIIB, and GIIIC, each of which consisted of 20 patients according to the Child–Pugh scoring. All patients and control samples were subjected to the following tests: Antibody screening for HCV using the ELISA technique and quantitative HCV RNA by PCR. Detection of TLR3 was done using the ELISA technique. Other parameters such as Complete Blood Picture (CBC), Prothrombin Time (PT) (INR), urea, creatinine, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), total bilirubin, alkaline phosphatase, albumin, cholesterol, and triglycerides levels were also estimated.

Results

The percentage of HCV-positive cases among a studied group of patients on HD was 57.2%, and the percentage of negative cases was 42.8%. There was no significant difference between GI, GII, and GIII as regards age and duration of dialysis. There was a highly significant difference between GIII as regards TLR3, INR, urea, total bilirubin, albumin, and triglycerides compared with GI and GII. There was a highly significant positive correlation of TRL3 with creatinine and PCR RNA and a significant negative correlation with albumin and cholesterol. We also found that there was a highly significant difference between GIIIC as regards INR, total bilirubin, and albumin compared with GIIIA and GIIIB. TLR3 showed no significant difference between GIIIA, GIIIB, and GIIIC.

Conclusion

TLR3 may have a role in the pathogenesis of HCV infection-related CKD and can be used as a new marker for the development of HCV related end-stage renal disease.

Keywords: Child–Pugh classification, chronic kidney disease, hemodialysis, hemodialysis, hepatitis, toll-like receptors 3

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INTRODUCTION

Renal failure patients on hemodialysis (HD) are at high risk for blood-borne infections by prolonged vascular access and the potential for exposure to contaminated equipment, failure in infection control measures. Also in some developing countries, the rapidly growing HD patients are treated using limited resources thereby increasing the chances of nosocomial transmission, as indicated by high hepatitis C virus (HCV) prevalence and incidence [1]. Infection due to hepatitis viruses is one such infection, which is a significant cause of morbidity and mortality in HD patients and poses a problem in the management of patients in renal dialysis units [2].

The prevalence of HCV infection among dialysis patients is much higher than healthy blood donors or the general population [3]. The prevalence varies markedly from one geographical area to another, genotype 1a and 1b being the most frequently encountered ones in Europe, USA, and Japan. HCV subtypes 2a and 2b are commonly found in North America, subtype 2c in Northern Italy, while genotype 4 is predominant in North Africa (especially in Egypt) and the Middle East; genotypes 5 and 6 are commonly reported in South Africa and Hong Kong, respectively. Genotype 3a is endemic to Southeast Asia, and seems to be dominant in intravenous drug users in Europe and the USA [4].

The Child–Pugh score Table A (sometimes the Child–Turcotte–Pugh score) is used to assess the prognosis of chronic liver disease, mainly cirrhosis. Although it was initially used to predict mortality during surgery, it is now used to determine the prognosis, as well as the necessary strength of treatment and the necessity of liver transplantation. The score employs five clinical measures of liver disease. Each measure is scored 1–3, with 3 indicating most severe derangement [5].

A role in HCV-associated renal injury has been recently suggested for toll-like receptors (TLRs). They are primary proteins expressed on immune and nonimmune cells, as critical components of the innate immune system. They recognize the molecular patterns associated with microbial pathogens and induce an immune response. TLRs are single, membrane-spanning, noncatalytic receptors usually expressed in macrophages, dendritic cells, and other sentinel cells which recognize structurally conserved molecules derived from microbes. They are recognized by TLRs, which activate immune cell responses [6].

TLR3 is a receptor that has initially been described to induce antiviral responses by recognition of viral components such as single-stranded RNA (ssRNA) or double-stranded RNA (dsRNA) [7]. Some studies have suggested that TLR inhibitors have therapeutic potential and also the use of TLR ligands as vaccine adjuvants [8].

So, the present study was done to determine the value of TLR3 in the pathogenesis of HCV infection-related chronic kidney disease patients, to find a link between the increased risk of HCV infection and those patients. Also, we aimed to study the levels of TLR3 for Child–Pugh different classes to determine its impact on the prognosis of liver disease in those patients.

PATIENTS AND METHODS

One hundred and thirty (64 men and 66 women) participants of both sexes were selected from National Institute of Urology and Nephrology, of which 105 patients (40–62 years) were selected from the dialysis unit with end-stage renal disease (ESRD) and 25 healthy individuals as a control group. All participants signed an informed written consent with their acceptance for participating in the present study after explaining to them the aim and the value of our work. All participants were subjected to full history taking and examination. History of encephalopathy and nutrition was taken in detail in group (G) III patients. The studied participants were classified into control group (GI), which consisted of 25 clinically healthy participants matched for age and sex (GII) 45 HCV negative renal patients, and (GIII) 60 HCV-positive renal patients with the ESRD. GIII was subclassified into three subgroups (GIIIA, GIIIB, and GIIIC) each consisted of 20 patients. All patients underwent periodic HD three times per week for at least 1 year, and HBV, HCV, and HIV screening were periodically done for them. Those with hypertension, diabetes mellitus, HBV and HIV infection, malignant tumors, and autoimmune diseases were excluded. Any controversy between the results of ELISA and PCR were excluded from the study. A sample of 5 ml fresh blood were collected from the median cubital vein under aseptic precautions into vacutainers, allowed to clot at 37°C in a water bath, and centrifuged at 4000 rpm for 10 min and the serum was collected, separated, and stored at −20°C till analysis was performed. HD patient samples for HCV RNA detection had been collected before the dialysis procedure to avoid the risk of false-positive PCR results because of the presence of heparin in the blood [9].

All control and patients samples were subjected to the following tests

(1) CBC (using Cell Dyn, USA).
(2) Prothrombin time (INR) (Dad behring DFT, Germeny).
(3) Serum urea, creatinine, ALT, AST, alkaline phosphatase (ALP), total bilirubin, albumin, cholesterol, and triglyceride levels were performed (using Siemens, USA).
(4) Antibody screening for HCV by ELISA (using Axiom, Germeny).
(5) Quantitative PCR was done for all studied

Table A: The Child-Pugh score

Measure	1 point	2 points	3 points
Total bilirubin (mg/dl)	<2.0	2.0-3.0	>3.0
Serum albumin (g/dl)	>3.5	2.8-3.5	<2.8
Prothrombin time (s)	<4.0	4.0-6.0	>6.0
Ascites	None	Mild (or suppressed with medication)	Moderate to severe (or refractory)
Hepatic encephalopathy	None	Grade I-II	Grade III-IV

participants (Thermacycle; Bio-Rad, USA).

(6) Detection of TLR3 was done using ELISA kit (Human TLR3 ELISA Kit, CD283, ab131557; USA).

(7) The Child–Pugh score was calculated as follows:

Interpretation

The chronic liver disease is classified as Child–Pugh classes A–C, table b using the added score from above [10].

Statistical methods

Data were analyzed using SPSS, version 23 (IBM Corp., Armonk, New York, USA). Categorical variables were presented as number (or fraction) and percentage and between-group differences were compared using the Pearson χ^2-test. Continuous numerical variables were presented as mean ± SD, and intergroup differences were compared using one-way analysis of variance with the application of the Tukey–Kramer test for multiple pairwise comparisons if there was a statistically significant difference among the groups. Correlations were tested using the Pearson correlation. Two-sided *P* value of less than 0.05 was considered statistically significant.

RESULTS

This study included 130 participants who were divided into three groups. The GI included 25 healthy adults who were age and sex matched. The GII included 45 HCV-negative patients with renal failure (GII). The GIII included 60 HCV-positive patients with renal failure (GIII). Among the total number of HD patients included in our study, the percentage of HCV-positive patients was 57.2%, and the percentage of HCV negative was 42.8%. After statistical analysis of our results, we found that there was no significant difference between GI, GII, and GIII as regards age and duration of dialysis. However, there was a highly significant difference between GIII compared with GI and GII as regards TLR3, INR, urea, total bilirubin, albumin, triglycerides, and hemoglobin. Also, there was a significant difference for creatinine, ALP, and cholesterol between GII and GIII compared with GI (Table 1 and Fig. 1). Also, we found a highly significant difference between GIIIC with GIIIA and GIIIB as regards INR, total bilirubin, and albumin although TLR3 showed no significant difference between them (Table 2 and Fig. 2).

Moreover, a correlation study was done between TLR3 and all parameters studied in GIII. Our correlation results showed a strong significant positive correlation of TLR3 with HCV RNA (*r*: 0.783) (Fig. 3) and creatinine (*r*: 0.730) (Fig. 4) (*P* < 0.0001). Also, there is a significant negative correlation between TLR3 with albumin (*r*: −0.102) (Fig. 5) and cholesterol (*r*: −0.307).

Table B: Interpretation of Child -Pugh Score

| Points	Class	One-year survival (%)	Two-year survival (%)
5-6	A	100	85
7-9	B	81	57
10-15	C	45	35

However, there was no significant correlation between TLR3 and each of the following parameters: age (*r*: 0.022), duration of dialysis (*r*: 0.019), urea (*r*: 0.014), ALT (*r*: 0.017), and triglycerides (*r*: 0.017) (Table 3).

DISCUSSION

HCV infection represents a major medical and epidemiologic challenge in patients with ESRD on renal replacement therapy with dialysis or transplantation [11]. HD patients are especially at high risk for blood-borne infections in light of continued vascular access and potential exposure to contaminated equipment. It has been estimated that, among patients on HD, the prevalence of HCV infection varies considerably from less than 5% to nearly 60% in different areas of the world [12]. However, due to the introduction of routine screening and increased thoughtfulness regarding prevention of spread, the prevalence of HCV infection has declined in many dialysis centers, but it remains unacceptably high, ranging from 8 to 10% even in industrialized countries [13].

TLRs may be considered a 'Swiss Army knife' of the immune system, ready to respond in a multitude of infectious disease states. TLRs have a vital role in the innate immune response which might also trigger secondary immune responses, including the activation of endothelial cells in the kidney. These responses lead to the production of adhesion molecules and cytokines and to macrophage infiltration, which can lead to infection-induced organ damage [8]. Recently it has been shown that TLR3 is expressed in the mouse and human kidney tubular epithelial cells. These cells appear to express TLR3 constitutively and upregulate its expression upon challenge with its ligands. Thus, it seems plausible that this receptor might play an essential role in this organ in health and disease. TLR3 is a receptor that has initially been described to induce antiviral responses by recognition of viral components such as ssRNA or dsRNA [7].

In our study, we tested the TLR3 levels in HCV-positive and HCV-negative patients undergoing renal dialysis together with the control which showed a high significance difference in GIII as compared with GI and GII (*P* < 0.0001). Also, there is a highly significant positive correlation (*r*: 0.783) between TLR3 and PCR RNA in HCV-positive patients, but TLR3 showed
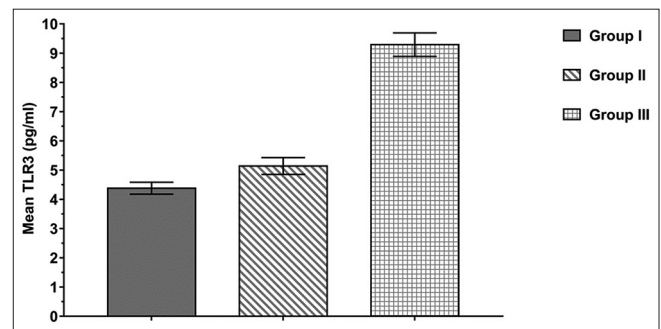

Figure 1: Mean TLR3 in the three study groups. Error bars represent the mean SEM. TLR, toll-like receptor.
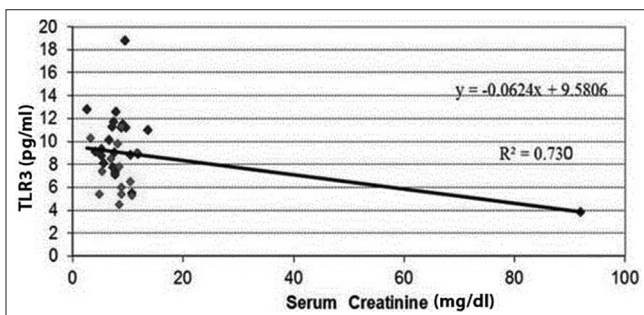
Table 1: Comparison of data of the three study groups

Variables	Group I ($n=25$)	Group II ($n=45$)	Group III ($n=60$)	F/χ^2	P^a
Age (years)	46.47±8.29	48.0±6.34	47.38±5.69	0.4567	0.634
Duration of HD (years)	0.00±0.00	2.68±1.25	2.91±1.92	2.359	0.004
INR	1.01±0.26	1.04±0.34	3.69±4.48[†,‡]	12.2	<0.0001
Urea (mg/dl)	28.36±7.31	124.31±45.39[†]	124.41±40.62[†,‡]	62.44	<0.0001
Creatinine (mg/dl)	0.75±0.16	8.79±2.98[†]	10.35±14.41[†]	8.405	0.0004
Total bilirubin (mg/dl)	0.43±0.36	0.51±0.47	2.92±2.14[†,‡]	43.13	<0.0001
ALT (IU/l)	40.56±7.98	36.08±12.01	34.57±20.29	1.254	0.289
AST (IU/l)	20.40±4.49	22.71±10.86	23.60±34.49	0.1513	0.860
ALP (IU/l)	94.12±21.76	290.04±258.77[†]	210.97±152.84[†]	9.074	0.0002
Albumin (g/dl)	4.22±1.089	3.43±0.41[†]	3.22±0.54[†]	21.37	<0.0001
Cholesterol (mg/dl)	188.4±28.80	157.1±28.80[†]	175.2±49.03	5.539	0.005
Triglycerides (mg/dl)	106.8±28.80	145.5±28.80[†]	195.5±49.03[†,‡]	49.86	<0.0001
Hemoglobin (g/dl)	14.08±0.78	10.58±1.99[†]	10.27±1.39[†]	57.77	<0.0001
TLR3 (pg/ml)	4.38±1.03	5.14±1.94	9.29±3.12[†,‡]	53.17	<0.0001
HCV RNA (copies/ml)	0/20	0/45	60/60	125.0	<0.0001[b]

Data are presented as mean±SD or fraction. ALP, alkaline phosphatase; HCV, hepatitis C virus; HD, hemodialysis; TLR, toll-like receptor. [a]One-way analysis of variance (analysis of variance) unless otherwise specified. [b]Fisher's exact test. [†]Statistically significant difference versus group I (Tukey-Kramer test). [‡]Statistically significant difference versus group II (Tukey-Kramer test).
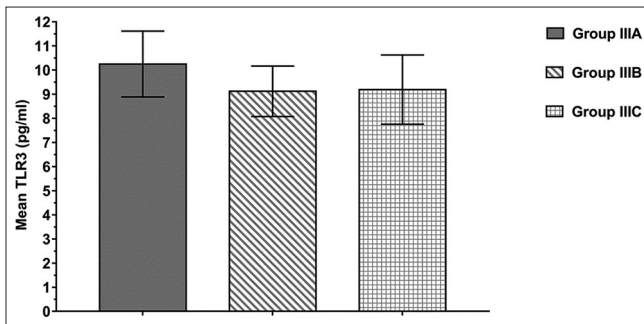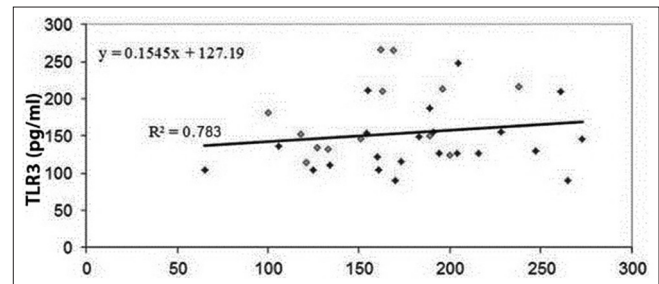

Figure 2: Mean TLR3 in GIIIA, GIIIB, and GIIIC error bars represent mean SEM. G, group; TLR, toll-like receptor.
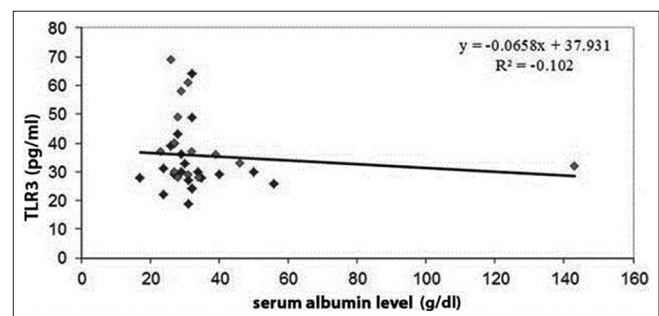

Figure 3: Correlation between TLR3 and HCV RNA level in HCV-positive patients (GIII). TLR, toll-like receptor.

Figure 4: Correlation between TLR3 and creatinine level in HCV-positive patients (GIII). HCV, hepatitis C virus; TLR, toll-like receptor.

Figure 5: Correlation between TLR3 and albumin level in HCV-positive patients (GIII). HCV, hepatitis C virus; TLR, toll-like receptor.

no significant difference in GIII subgroups GIIIA, GIIIB, and GIIIC. Our results are comparable with those obtained by Wornle *et al.* [6], who revealed an increased expression of TLR3 specifically in microdissected glomeruli of patients with HCV-related membranoproliferative glomerulonephritis, but not in non-HCV membranoproliferative glomerulonephritis which suggest a link between TLR3 and HCV-related glomerular disease. TLRs recognize various pathogen-associated molecular patterns, such as lipopolysaccharides, lipoproteins, peptidoglycans, and unmethylated cytidine–guanosine dinucleotide motifs that are present in bacterial and viral DNA and viral dsRNA and play an essential role in the activation and regulation of the innate immune system and inflammation. TLRs might have an essential role in the pathogenesis of renal diseases; their exaggerated activation is associated with ischemic kidney damage, acute kidney injury, end-stage renal failure, acute tubulointerstitial nephritis, acute renal transplant rejection, and delayed allograft function [14].

Table 2: Comparison of group IIIA, group IIIB, and group IIIC

Variables	Group IIIA (*n*=20)	Group IIIB (*n*=20)	Group IIIC (*n*=20)	*F*	*P*a
Age (years)	47.59±7.32	46.51±6.12	48.37±5.19	0.444	0.644
Duration of HD (years)	2.23±1.15	3.11±1.92	2.83±1.68	1.549	0.221
INR	2.35±1.69	4.23±1.29†	6.81±0.77†,‡	58.830	<0.0001
Urea (mg/dl)	134.52±46.13	151.55±39.61	148.74±43.69	0.194	0.825
Creatinine (mg/dl)	10.56±12.62	11.61±15.41	10.91±13.41	0.030	0.971
Total bilirubin (mg/dl)	1.16±1.32	2.63±1.11†	3.68±0.49†,‡	29.910	<0.0001
ALT (IU/l)	34.29±20.33	33.56±21.63	35.51±22.81	0.042	0.959
AST (IU/l)	22.96±33.67	23.22±34.32	22.65±31.92	0.001	0.999
ALP (IU/l)	206.52±137.87	213.11±151.14	211.67±163.62	0.015	0.985
Albumin (g/dl)	3.62±0.92	3.29±0.54	2.32±0.14†,‡	23.67	<0.0001
Cholesterol (mg/dl)	179.14±36.77	164.69±53.25	174.82±22.84	0.701	0.500
Triglycerides (mg/dl)	198.68±51.47	191.24±49.03	203.54±31.78	0.380	0.686
Hemoglobin (g/dl)	11.04±1.51	10.39±2.12	10.11±2.36	1.106	0.338
TLR3 (pg/ml)	10.25±6.11	9.12±4.67	9.19±6.41	0.240	0.787
HCV RNA (copies/ml)	2.31±0.93	1.35±1.12	1.11±2.82	2.402	0.100

Data are presented as mean±SD. ALP, alkaline phosphatase; HCV, hepatitis C virus; HD, hemodialysis; TLR, toll-like receptor. ªOne-way analysis of variance (analysis of variance). †Statistically significant difference versus group IIIA (Tukey-Kramer test). ‡Statistically significant difference versus group IIIB (Tukey-Kramer test).

Table 3: Correlation between toll-like receptor 3 and other quantitative variables

Variables	Pearson *r*
Age	0.022
Duration of dialysis	0.019
Urea	0.014
Creatinine	0.730**
ALT	0.017
AST	0.010
ALP	0.012
Albumin	−0.102**
Cholesterol	−0.307*
Triglycerides	0.017
HCV RNA	0.783**

Serum HCV-RNA (copies/ml). ALP, alkaline phosphatase; HCV, hepatitis C virus. *Statistically significant correlation at the *P*<0.05 level. **Statistically significant correlation at the *P*<0.001 level.

In the study of Anna Gluba *et al.* [15], who investigated the role of the TLR receptors in renal diseases found that different types of TLRs recognize specific pathogenic agents. TLR3 recognizes dsRNA, TLR4 binds lipopolysaccharides of Gram-negative bacteria, TLR5 binds bacterial flagellin, and TLR7 binds ssRNA, which is found in HIV-1, vesicular stomatitis virus, influenza virus, and some small interfering RNAs; TLR8 recognizes imidazoquinolines and ssRNA; and TLR9 binds bacterial and viral cytidine–guanosine dinucleotide motif. The binding of a ligand to a TLR activates numerous signaling pathways (which induce the production of inflammatory cytokines, chemokines, and interferons (IFNs). TLRs activate nuclear factor κB, mitogen-activated protein kinase 8 and mitogen-activated protein kinase 13, extracellular signal-regulated kinase 1 and extracellular signal-regulated kinase 2, IL-6, and IFN regulatory factors and promote the production of tumor necrosis factor, IL-1β and IL-12 TLR3,

TLR4, TLR7, TLR8, and TLR9. It stimulates antiviral responses through IFN-α and IFN-β. Sato *et al.* [16] suggested a correlation between the expression levels of TLRs and cytokines, and chronic HCV infection; TLR3 recognizes dsRNA and induces type 1 IFN synthesis.

Collectively, suppressed expression of TLR3 in cells transfected with entire HCV may be responsible for continuous HCV infection, although a part of the HCV gene enhances its expression. Pugh and colleagues initially used the Child–Pugh scores to predict mortality during surgery in patients presenting with bleeding esophageal varices. However, at present, it is used to assess the prognosis of chronic liver disease, including HCV infection which unfortunately leads to cirrhosis. Moreover, it estimates the necessary strength of treatment and the necessity of liver transplantation. In our study, we classified our HCV patients to three classes A, B, and C to weigh these values. Our correlation study between Child–Pugh different classes and other parameter studies showed that there was a definite significant correlation with INR, total bilirubin, and albumin, lightening the impact of these three essential items. Statistical tests did not consider any significant difference between each class and TRL3 levels in the same group, predicting a relation between the influences of both of them in the condition of HCV patients [17].

Chaurasia and colleagues, suggested that the score can be more successful when used with another published one which is the model for end-stage liver disease to estimate the severity of cirrhosis and determine the priority for liver transplantation.

We assume together with Durand and Valla [18] that the five variables of the Child–Pugh score were neither adequately nor accurately selected, and anticipated that not all are independent predictors of prognosis. Moreover, we are pointing to another aspect which is that the Child–Pugh score does not take

into account specific critical other variables such as serum creatinine in particular, which have been shown to have a determinant impact on the prognosis of cirrhosis. Many studies confirmed that albumin and prothrombin time are somewhat redundant, including both variables in a single score may result in overweighting their influence. They added that the cutoff value for each variable has been roughly chosen and that there is no evidence that moving from one class to the next translates into a proportional change in mortality risk. This is typically the same when considering serum bilirubin cutoff levels. They concluded that this ceiling effect of discrete classes does not exist with continuous variables. Also, the limits for qualitative clinical variables such as ascites and encephalopathy are still vague. They may be influenced by subjective personal interpretation [19].

Interestingly enough, the Child–Pugh score does not take into consideration the cause of cirrhosis and the possibility of stopping or slowing the damaging process to the liver. This limitation is especially relevant in patients having cirrhosis related with viral replication [19]. Inconsistent with our results, Al-Anazi *et al.* [20] studied the single-nucleotide polymorphisms within the TRL3 gene and their association with HCV-related disease risk. They investigated their research in the Saudi Arabian population and showed that distinct genetic variants of TLR3 are associated with HCV infection and HCV liver disease progression.

The mean serum blood urea and creatinine showed a significant difference ($P < 0.0001$) ($P = 0.0004$) in GII and GIII as compared with the GI control group, respectively. Also, we found a strong significant positive correlation between TLR3 and serum creatinine level ($r = 0.730$). Hosny *et al.* [21] confirm our results, who found that serum creatinine was slightly lower in HCV-negative HD patients as compared with HCV-positive HD patients.

Mean serum albumin showed a highly significant difference ($P < 0.0001$) between GII and GIII as compared with GI. Also, we found a significant negative correlation between TLR3 and serum albumin level of HCV-positive patients ($r=-0.102$).

Our results were by those of Sezer *et al.* [22] and Tutal *et al.* [23], who found that albumin levels were lower in HCV positive than in HCV-negative HD patients. They have speculated that hypoalbuminemia as a negative acute phase reactant and may due to increased inflammation in infected patients rather than hepatic dysfunction. In contrast to our study, Sabry *et al.* [24] reported that albumin mean levels were within normal in both groups and minimally higher in HCV-negative patients, in a nonsignificant way.

Mean serum ALT and AST showed no significant differences ($P = 0.289$ and 0.860) in GIII and GII as compared with GI. These results were in agreement with Tutal *et al.* [23], who indicated that ALT levels were normal and similar between HCV-positive and HCV-negative HD patients. In contrast,

Hosny *et al.* [21] reported that HCV-positive patients showed significantly higher levels than HCV-negative patients as regards ALT ($P = 0.031$), but HCV-positive patients showed significantly higher levels as regards AST ($P = 0.017$). The typical clinical problem is to evaluate the importance of mildly elevated serum aminotransferase values which are depressed by 20–50% in 10–90% of dialysis patients. The reason is unknown; many explanations have been advanced, including inhibition of levels of aminotransferases activity in dialysis patients with uremic toxins [25].

Mean serum ALP showed highly significant differences in GII and GIII as compared with GI ($P = 0.0002$). Altered patterns of mineral metabolism, including ALP, are often observed in ESRD patients on HD [26]. Serum ALP is commonly an enzyme from bone and liver, as well as kidney, intestine, or leukocytes [27]. Notably, Kalantar-Zadeh *et al.* [28] demonstrated an increased risk of all-cause mortality associated with higher baseline and time-varying ALP levels in HD patients, without including adjustments for elevated serum phosphorus and calcium levels, which have been associated with higher mortality risk.

Mean serum triglycerides showed a highly significant difference ($P < 0.0001$) in GII and GIII as compared with GI. Also, it was higher in GIII as compared with GII. These findings concurred with those reported by [29]. They found that the serum triglyceride level was more significant in the HCV group. Importantly, the HCV load regardless of the genotype correlated directly with the triglyceride and very low density lipoprotein levels with (r: 0.73) and (r: 0.84), respectively. Serum triglyceride level may play an essential role in viral replication; these data further suggest that therapies directed at lowering plasma triglyceride levels may enhance the efficacy of current antiviral treatment regimens [30].

Mean serum cholesterol showed a significant difference ($P = 0.005$) in GII as compared with GI. Moreover, there is a significant difference between GIII and GII and a significant negative correlation between TLR3 and serum cholesterol level in GIII (r: 0.307). Our results were in agreement with those who reported that the total cholesterol levels were lower in chronic hepatitis C patients compared with healthy participants ($P < 0.005$). Cholesterol downregulation within treated cells may be linked to sphingomyelinase activation, because the removal of sphingolipids from the plasma membrane displaces cholesterol from lipid rafts, increasing the intracellular cholesterol pool, and this increase acts as a signal to downregulate the mevalonate pathway. Reduced levels of cellular cholesterol led to the inhibition of viral secretion in all three viral systems [31].

CONCLUSION

TLRs may be considered as a 'Swiss Army knife' of the immune system, ready to respond to a multitude of infection and disease states. TLRs have a vital role in the innate immune response. These receptors might also trigger secondary immune

responses, including the activation of endothelial cells in the kidney and lead to the production of adhesion molecules, cytokines, and to macrophage infiltration, which can lead to infection-induced organ damage. Patients on renal dialysis are more liable to HCV infection than the average population. TLR3 has a role in the pathogenesis of HCV infection in dialysis patients. Thus, this parameter may be considered as a new marker for the development and severity of complications in renal disease patients.

Recommendation

Further research work is needed to determine the exact role of these receptors and to evaluate strategies to prevent TLR-mediated local inflammation. This is to support some other studies that have suggested that TLR inhibitors have therapeutic potential. Also, TLR ligands might be used as a vaccine adjuvant shortly. Antagonists to TLR3 may prevent renal complications in patients with HCV infection.

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Conflicts of interest
There are no conflicts of interest.

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