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Full Length Research Paper

Incidence of bacterial infection in chronic hepatitis C virus (HCV) patients with cirrhosis and association between toll-like receptor 4 D299G gene polymorphism and Gram- negative bacterial infections in the patients

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This study aimed to investigate the incidence of bacterial infection in chronic hepatitis C virus (HCV) patients with cirrhosis and association between toll-like receptor 4 D299G gene polymorphism and Gram-negative bacterial infections in the patients. 100 HCV cirrhotic patients with ascites and 20 age- and sex-matched healthy subjects as control were included. Conventional culture methods were used to identify the causative organism of infection. Toll-like receptor 4 D299G polymorphism was detected by PCR- RFLP (polymerase chain reaction – restriction fragment length polymorphism). Patients were divided into: Group I: 100 HCV cirrhotic patients with ascites. They were subdivided into: Group I (a): Patients with Toll-like receptor 4 D299G polymorphism, Group I (b): Patients without polymorphism. Group II (control): 20 healthy subjects. This study showed significant higher incidence of infections in cirrhotic patients with Toll-like receptor 4 D299G polymorphism which play a role in the development of bacterial infection in cirrhotic patients that makes down-regulation of TLR4 response one of the immune mechanisms predisposed to Gram negative bacterial infection in cirrhotic patients.

Key words: TLR-4 gene polymorphism, cirrhosis, bacterial infection.

INTRODUCTION

Hepatitis C virus (HCV) is increasing health problems all over the world. It affects millions of people worldwide and is considered a leading cause of liver diseases including cirrhosis and hepatocellular carcinoma. Bacterial infections are considered the most frequent and severe complications in HCV patients with cirrhosis leading

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to significant morbidity and mortality (Negro et al., 2014; Sadik et al., 2015). Development of bacterial infection in cirrhotic patients may be predisposed by change in the mechanism of antimicrobial defense (Mencin et al., 2009). It is known that the host genetic background can influence the outcome of HCV infection. Several polymorphisms were found to be associated with HCV infection. Mutations that alter the ability of innate immune receptors to bind their pathogen-associated molecular pattern (PAMPS) may also affect host susceptibility to infection (Schroder and Schumann, 2005). Toll-like receptors (TLRs) are a family of transmembrane receptors with extra cellular leucine-rich receptors and an intra-cellular signaling domain (Medvedev et al., 2013). TLR4, one of the most important and well-studied TLRs, is located on chromosome 9q32-33. TLR4 is known to recognize bacterial LPS but this receptor has also been found to recognize fusion protein from the respiratory syncytial virus (RSV) and the envelope protein of mouse mammary tumor virus (MMTV) (Bali et al., 2013). Reports have also shown that TLR4 can be stimulated by HCV nonstructural protein NS5A and thereby results in the secretion of IFNs and IL-6 from hepatocyte and B cells. The activation of TLR2 and TLR4 signaling in hepatocyte leads to upregulation of proinflammatory cytokines and chemokines, and recruitment of inflammatory cells to the liver (Bart-Ferwerda et al., 2008). In association of CD14 monocytes and its co-receptor MD-2 TLR4 triggers the inflammatory response to LPS of Gram negative bacteria and is a key factor in eliciting the systemic inflammatory response that can lead to sepsis, organ failure and septic shock (Mish and Hawn, 2008). The aim of this study is to investigate the incidence of bacterial infection in chronic HCV patients with cirrhosis and association between toll-like receptor 4 D299G gene polymorphism and Gram-negative bacterial infections in those patients.

METHODOLOGY

After approval of ethical committee in Tanta Faculty of Medicine and a written consent from all participants, this study was carried out on 100 HCV cirrhotic patients with ascites and 20 age- and sex-matched healthy subjects considered as control group. All patients were admitted to Tropical Medicine Department, Tanta University Hospital, Tanta, Egypt, during the period between Januaruy 2017 and January 2018. All patients and control were subjected to full history taking, clinical examination, routine laboratory investigations (liver function tests, complete blood picture, kidney function tests) and abdominal ultrasound for diagnosis of cirrhosis and real time – PCR for diagnosis of HCV.

Sampling

6 ml blood was taken under complete aseptic precautions and divided into two portions the first for routine assay and bacteriological study, and the other for the molecular study.

Inclusion criteria

HCV cirrhotic patients with ascites (child – Pugh C).

Exclusion criteria

(1) Patients taking immunomodulatory drugs.
(2) HCV coinfection with human immune deficiency virus or hepatitis B virus infection.
(3) Advanced hepatocellular carcinoma or extra hepatic malignancy.
(4) Recent history of previous infection (within previous 6 weeks).
(5) Hepatic encephalopathy in the previous 6 weeks.
(6) Treatment with antibiotics in the previous 6 weeks.
(7) Gastrointestinal bleeding in previous 7 days.

The patients were divided into:

Group I: 100 HCV cirrhotic patients with ascites that were subdivided according to presence of Toll-like receptor 4 D299G polymorphism into:

Group I (a) : Patients with Toll-like receptor 4 D299G polymorphism.
Group I (b) : Patients without polymorphism.

Group II: 20 healthy subjects as control.

PCR- RFLP (polymerase chain reaction – restriction fragment length polymorphism) was done to determine the presence of the Toll-like receptor 4 D299G polymorphism

Bacteriological study

Conventional culture techniques were used to identify the causative organism of bacterial infection which was confirmed by biochemical reactions.

Genomic DNA extraction and polymorphism genotyping

8 ml of peripheral blood was collected in EDTA tube and centrifuged at 3500 g for 10 min. Genomic DNA was extracted from buffy coat fraction using QIA mp DNA blood minikit (Qiagen Inc. Valencia, CA, USA) . The primer sequences used for TLR-4 Asp299Gly genotyping were 5-GATTAGCATACTTAGACTACCTCCATG-3 and 5-GATCAACTTCTGAAAAAGCATTCCCAC-3. The polymerase chain reaction (PCR) consisted of an initial denaturation at 95 C, 30 s at 57 C and 1 min at 72 C. Once the amplification was confirmed, the PCR product was digested for 1 h at 37 C with restriction enzyme NcoI. The change of A to G at position 896 produced a site for the restriction enzyme NcoI. The digested fragments of PCR amplification were analyzed by electrophoresis on 2.5 % agarose gel (Zhang et al, 2013)

Statistical analysis

Statistical package for social sciences (SPSS) package (version 9.0) was used for data analysis.

RESULTS

There was no statistical difference between cirrhotic patients and control as regard age and gender (P> 0.05). Table 1 showed statistically significant difference

Table 1. Clinical and laboratory data in all groups.

Variable	Cirrhosis before AB (n=100)	Cirrhosis after AB (n=100)	Control (n=20)	P
Age	53.57±4.12	54.83±9.31	49.37±10.68	0.753
Gender (m/f)	58/42	58/42	11/9	1.000
Bilirubin (mg/dl)	1.8±0.4	1.6±1.0	0.8±0.5	<0.001*
ALT (IU/L)	59.5±47.3	66.4±22.8	24.0±15.2	<0.001*
AST (IU/L)	70.8±63.0	83.5±35.7	33.2±14.6	<0.001*
Albumin (g/dl)	3.2±0.7	3.1±0.5	4.8±0.3	<0.001*
PT (seconds)	14.5±2.8	13.7±0.8	11.6±0.4	<0.001*
Platelet(10^3/mm^3)	157.7±87.5	105.4±24.5	241.3±34.8	<0.001*
Hb (gm/dl)	9±0.5	9.5±0.7	12±1.3	< 0.001*
WBC(cell/cmm)	3.6±1.1	2.7±0.71	4.5±1.4	<0.001*
S.creatinine (mg/dl)	2.21±0.15	1.52±0.22	o.73±0.19	<0.001*

All data are expressed in terms of mean ± standard deviation. P< 0.05 *significant. ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; PT, Prothrombin time; AB, Antibiotic.

Table 2. Type of infections in cirrhotic patients with and without polymorphism.

Variable	Polymorphism (n=10)	No polymorphism (n=90)	P
	N (%)	N (%)	
SBP	5 50	10 11.1	0.030*
Gram -ve organism	4 40	4 4.4	0.029*
Gram +ve organism	1 10	6 6.7	0.029*
UTI	4 40	7 7.8	0.049*
Gram -ve organism	4 40	5 5.5	1.000
Gram +ve organism	0 00.00	2 2.3	1.000
Chest infections	3 30	3 3.3	0.023*
Ellulitis	3 30	2 2.2	0.006*
Encephalopathy	6 60	15 16.7	0.037*
Bacteremia	4 40	4 4.4	0.004*
Gram -ve organism	3 30	2 2.2	0.945
Gram +ve organism	1 10	2 2.2	0.945

P <0.05 * Significant.

between cirrhotic and control as regard serum billirubin, ALT, AST, albumin, platelets count, prothrombin time, heamoglobin level white blood cells and creatinine. Serum creatinine was significantly higher in cirrhotic before antibiotic than after antibiotic ($p < 0.001$).

Table 2 revealed that 10 out of 100 (10%) cirrhotic patients are presented with Toll-like receptor 4 D299G polymorphism. Spontaneous bacterial peritonitis (SBP) infection was significantly higher in TLR-4 D299G polymorphism patients than those without polymorphism (50% Vs 11.1%) ($P < 0.05$). SBP caused by Gram negative bacteria in 40% of patients with polymorphism compare to 4.4% of those without. This was statistically significant ($P < 0.05$). Urinary tract infection was significantly higher in 40% of polymorphism group compare to 7.8% of patients without polymorphism $P<0.05$. The infection was mainly caused by Gram negative bacteria in both groups. Encephalopathy was found in 60% of patients with TLR-4 D299G polymorphism compare to 16.7% of patients without polymorphism. This was statistically significant ($P < 0.05$). The incidence of other types of infection as chest infection, cellulitis and bacteremia were significantly higher in polymorphism patients ($P < 0.05$).

Table 3. Microbilogical profile of infection of the studied group.

Site of infection	Peritonitis (%)	UTI (%)	Chest (%)	Cellulitis (%)	Encephalitis (%)	Bacteraemia (%)
Klebsiella	2 (2)	1 (1)	1 (1)	1 (1)	6 (6)	2 (2)
E.coli	3 (3)	3 (3)	1 (1)	1 (1)	5 (5)	0 (0)
Staph.aureus	5 (5)	2 (2)	1 (1)	0 (0)	2 (2)	3 (3)
Citrobacter	3 (3)	4 (4)	1 (1)	2 (2)	3 (3)	2 (2)
Enterococci	2(3)	1 (1)	2 (2)	1 (1)	5 (5)	1 ()

Table 4. Correlation between the TLR-4 polymorphosm and bacterial infection in cirrhotic patients.

Variable	R	P
TLR-4 gene polymorphism / infection	0.565	0.019*

P <0.05 * Significant.

Table 3 shows bacteriological profile of infection of the studied group. Table 4 positive correlation was found between TLR-4 gene polymorphism and incidence of infection in cirrhotic patients (r = 0.565, P = 0.019).

DISCUSSION

It has long been considered that immune response is impaired in patients with cirrhosis and that this predisposes bacterial infection. TLRs provide the most important early critical response to invading organism (Jover et al., 2009). TLR variant genotypes are associated with significantly increased serum levels of their specific antigenic ligands (LTA, LPS and bacterial DNA, respectively).

The results of this study showed significant higher incidence of bacterial infection in cirrhotic patients with ascites and TLR4 gene polymorphism than in cirrhotic patient without polymorphism. These findings came in accordance with the results reported by Argente et al. (2010) who observed a significant trend toward a higher incidence of bacterial infection and a significantly higher number of infections per patient in cirrhotic group withTLR4 D299G polymorphism.

In this study, TLR4D299G polymorphism was detected in 10% of cirrhotic patients with ascites. This result was nearly similar to the result of Argente et al. (2010), they reported incidence of about 9% of TLR4 D299G polymorphism in Child –Pugh C cirrhotic patients. Also, we revealed high incidence of encephalopathy in TLR4 D299G polymorphism group compared to cirrhotic group without polymorphism. This also was in accordance with the study of Argente et al. (2010), they reported higher incidence of encephalopathy in TLR-4 D299G polymorphism patients than in patients without polymorphism. They observed that this complication showed a trend toward a higher frequency during the study period in polymorphism group.

There are several explanation for high incidence of encephalopathy in these type of patients. Polymorphism itself could be a consequence of the high number of infections in these patients (Fernandez et al., 2007). This could also account for the higher serum creatinine levels in patients with TLR-4 D299G polymorphism, as they presented a tendency towards a higher incidence of infections as the cause of cirrhotic hospitalization (Jover et al., 2009). It could therefore be that a different inflammatory response related to the presence of the TLR-4 D299G polymorphism favors the development of encephalopathy as one of the most important complication (Frances et al., 2008). The results of this study showed a significant positive correlation between TLR4 gene polymorphism and incidence of infections in cirrhotic patients. These findings were also reported by Argente et al. (2010); they reported a significant correlation between TLR4 gene polymorphism and incidence of encephalopathy in cirrhotic patients. In other diseases, this significant positive correlation was observed by Yin et al. (2010), they reported TLR4 gene polymorphism is associated with increased risk of urinary tract infection in adults especially with acute cystitis and urethritis. This study showed significant impairment of TLR4 expression in PBMCs of cirrhotic patients in this study. This was in agreement with Tazi et al. (2006) and Testro et al. (2010), they revealed significant down regulation of TLR4 expression in PBMCs of cirrhotic patients. In contrast to this study finding, Stadlbauer et al. (2008) and Riordan et al. (2003) reported increased expression of TLR4 in monocytic cells of cirrhotic patients which showed return to normal level after antibiotic use. This disparity may be associated with the different etiology of the liver cirrhosis and/or different severe degrees of cirrhosis. A significant inverse correlation was detected between TLR4 expression and incidence of infection. These findings were similar to those reported

by Testro et al. (2010). All previous findings raised the possibility that, the high rate of Gram negative infections in patients with decompensated cirrhosis is not purely due to translocation of enteric organisms , but is contributed to an impairment of the TLR-4 dependent innate immune response. One potential explanation for these effects is that in response to repeated exposure to high concentration of Gram negative bacterial products and endotoxin, the TLR-4 dependent innate immune response is down regulated in an attempt to prevent chronic unopposed inflammation. In contrast to healthy control people, endotoxemia in cirrhotic patients does not lead to the typical systemic reaction, suggesting altered immune response to circulatory endotoxin in these patients (Manigold et al., 2003)

Conclusion

Toll-like receptor 4 D299G polymorphism has a role in the development of bacterial infection in cirrhotic patients that makes down-regulation of TLR4 response as one of the immune mechanisms predispose to Gram negative bacterial infection in cirrhotic patients.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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