Bacteriological and Immunological profiles for Patients with Viral Hepatitis in Babylon Province-Iraq

Mohammed A.K. AL-SAADI*, Ilham A. AL-SAEDI, Zainab Mahdi

College of Medicine, University of Babylon, Hilla, Iraq

Abstract : Aims: A cross-sectional study was conducted to investigate the bacterial profile as well as to assess some immunological markers in patients with HBV and HCV infections.
Methods: The study Included (70) patients with viral hepatitis with age ranged between (14-61) years old from both sexes (37 males and 33 females) with in a period of November 2015 to March2016.Blood and urine samples were collected from each subjects who were inpatient in Merjan Teaching Hospital in Babylon province. Twenty apparently healthy subjects were involved in this work as control. All specimens (blood and urine) were subjected to identify the aerobic bacterial types associated with viral hepatitis by biochemical and serological tests. Complement components (C3 and C4) and interferon –gamma were estimated as immune markers by ELISA technique.
Results: The results showed that (28.57%) of blood specimens and (62.8%) of urine specimens were positive for bacterial growth of both Gram-positive and Gram-negative. No significant differences (p < 0.05) were estimated between hepatitis patients and control for all immune markers.
Conclusion: Bacterial infections were detected in viral hepatitis patients as a result of   a state of immunosuppressive state.
Key Words : Bacterial Infection, Hepatitis, IFN-γ, Complement component (C3, C4).

Introduction

Hepatitis is an inflammation of the liver. The condition can be self-limiting or can progress to fibrosis (scarring), cirrhosis or liver cancer. Hepatitis viruses are the most common cause of hepatitis in the world but other infections, toxic substances (e.g. alcohol, certain drugs), and autoimmune diseases can also cause hepatitis. There are 5 main hepatitis viruses, referred to as types A, B, C, D and E[1].

The pathogenesis of hepatitis B virus (HBV) infection and its individual clinical courses are largely influenced by the host's immune response directed against the virus. In contrast to infection with hepatitis C virus (HCV), most patients infected with hepatitis B apparently clear viral infection. For patients with self-limited acute hepatitis B, it has recently been demonstrated that T cells reactive with hepatitis B core protein (HBc) predominantly belong to the Th1 subset, with production of interferon-gamma (IFN-γ)[12].

Iraq is a developing country, where hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are still prevalent, with an HBV carrier rate of 2%−5%. Although Iraq includes HBV vaccination in its Expanded Programme on Immunization, the coverage rate is less than 80%[3,4]. Hospital-acquired HBV and HCV infections continue to occur despite increased awareness of the problem among the medical community[5].

Infection of the urinary tract, bacteremia and pneumonia are the most common types of infection in liver disease patients[6].

Hepatitis B virus itself does not cause a cytopathic effect. Antigen-antibody complexes cause some of the early symptoms (e.g., arthralgias, arthritis, and urticaria) and some of the complications in chronic hepatitis (e.g., glomerulonephritis, cryoglobulinemia, and vasculitis). Hepatitis C virus infects hepatocytes primarily, but there is no evidence for a virus-induced cytopathic effect on the liver cells. Rather, death of the hepatocytes is probably caused by immune attack by cytotoxic T cells[7].

Liver failure is a potentially life-threatening condition that occurs in the setting of extensive injury to hepatocytes[8]. One of the most consequences of liver failure is the increasing rate of host susceptibility to bacterial infections[9].

This study aimed to illustrate the bacterial profile in patients with viral hepatitis as a first study in Babylon province -Iraq.

Methods:

A cross-sectional study Included (70) patients with clinically diagnosed viral hepatitis with age ranged between (14-61) years old from both sexes (37 males and 33 females) with in a period of November 2015 to March 2016. Those subjects were inpatients in Merjan Teaching Hospital in Babylon province, Blood and urine samples were collected from each subject. Moreover, serum was collected from 20 apparently healthy subjects as control for immune parameters.

Bacterial Identification

The isolates were identified by vitek instrument according to the manufacture company (Biomerieux/ Franch).

Immunological study

- Level of Serum Interferon-γ (IFN-γ)was carried out according to the instruction of company (Elabscince/ China) by using ELISA technique.
- Complement Components (C3 and C4) Level were determined by radial immune-diffusion assay plates according to the manufacture company (Liofilchem/ Italy).

Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Science Software (SPSS, version 22). Variables were described using frequency distribution and percentage for the subjects according to their characteristics and mean; standard deviation (SD) for continuous variable. Independent sample t-test was used. The p-value of < 0.05 was statistically significant.

Ethical approval

The necessary ethical approval from ethical committee in Merjan Teaching Hospital was obtained. Moreover, all subjects involved in this work were informed and the agreement required for doing the experiments and publication of this work was obtained from each one prior the collection of samples.

Results:

The results of bacterial culture for samples taken from hepatitis patients gave positive results at percentage 64 (45.7%) for all samples blood and urine taken from viral hepatitis patients. These results agreed with finding obtained by other [10]who found that (64 %) of total samples were positive for bacterial growth in patients admitted to hospital, half of which were serious.

Regarding blood culture, 20 cases were positive bacterial culture. The predominant bacterium of Gram-positive was *Staphylococcus haemolyticus*, and for Gram-negative was *Pseudomonas aeroginosa* (Table 1).

Table (1) Distribution of Gram-positive and Gram- negative bacteria in blood isolates

Bacterial isolates from blood	No. of isolates
Staph. haemolyticus	1 (5%)
Kocuriakristinae	1 (5%)
Pseudomonas aeruginosa	17 (85%)
Pseudomonas luteala	1 (5%)
Total	20 (100%)

Bacterial cultures were detected in forty four cases for urine culture Also, *Staphylococcus haemolyticus* was the predominant Gram positive bacterial type in urine samples of viral hepatitis patients and *E.coli* was the predominant type for Gram negative bacteria (Table 2).

Table (2) Distribution of Gram-positive and Gram- negative bacteria in urine isolates

Bacterial isolates from urine	No. of isolates
Staph. haemolyticus	14 (58.3%)
Staph. epidermidis	1 (4.2%)
Staph. saprophiticus	3 (12.5%)
Rothiadentocariosa	2 (8.3%)
Lactobacillus spp.	4 (16.7%)
Escherichia coli	10 (50%)
KlebsiellaPneumoniae	6 (30%)
Proteus mirabilis	1 (5%)
Citrobacterfarmeri	1 (5%)
Enterobacter cloacae	2 (10%)
Total	44 (100%)

The current results show no significant differences ($p < 0.05$) between patients with viral hepatitis and controls in level of serum IFN-γ and in complement component (C3and C4) levels (Table 3).

Table (3) level of IFN-γand Concentration of complement C3and C4 in study groups

Study groups	Mean± SD	Rang	P Value
IFN-γ			
Patient	572.2 ± 286.8	2.3- 994.2	0.051*
Control	343.9 ± 273.4	40.2- 880.2	
C3			
Patient	151.7 ± 56.1	82- 248	0.193*
Control	128.8 ± 25	90- 169	
C4			
Patient	18.9± 8.1	9- 34.4	0.149*
Control	24.6 ± 9.9	8- 36	

* p value < 0.05 was significant

Discussion

These results obtained in this work agreed with finding obtained byother[10] who found that (64 %) of total samples were positive for bacterial growth in patients admitted to hospital, half of which were serious.

Bacterial infection may be responsible for up to 20 % of deaths in the few cases which prognoses to fulminant hepatic failure or subacute hepatic necrosis[11].

Also, the results for this study are similar to that mentioned by other[12] who indicated that the positive culture for both blood and urine samples in hepatitis patients, as a results from, pneumonia, blood stream infection and urinary tract infection.

Infection of the urinary tract, bacteremia and pneumonia are the most common types of infection of patients with liver disease. Often recognition of infection is made more difficult by the absence of the normal clinical feature of infection that is fever, rigors, hypotension, and leukocytosis[13].

Despite the widespread use of broad spectrum antibiotics, bacterial infection is responsible for up to a quarter of the death of patients with liver disease. The occurrence of bacterial infection in patients with different types of liver disease is difficult to ascertain from published reports[6].

The predominant bacterium was *Staphylococcus haemolyticus*, and *Pseudomonas aeroginosa*.

The results in this study agree with other[14] who reported that *S. haemolyticus* related to hepatitis patients bacteremia and can be isolated from hospitalized patients as a major Gram-positive bacteria.

The case of *Kocuriakristinae*isolated from bacteremia in patients with hepatitis. Infections related to *Micrococcus spp.* are uncommon but are recognized, especially in immunocompromised patients with underlying disease[15].

In this study a case of bacteremia in patient due to *Pseudomonas luteola* (formerly Ve-1 and *Chryseomonas luteola*). The patients had bacteremia and were immunocompromised (hepatitis patient)[16].

Also, the results of the current study were similar to that introduced by other[17] who indicated that *P. aeroginosa* is the major pathogen implicated in blood stream in hepatitis patients. These bacteria are a very important pathogen associated with different types of infections especially immuncompromised patients.

This result serves as evidence that these bacteria can be isolated from immuncompromised patients such as hepatitis patients.

Urinary tract infections (UTIs) are caused by a variety of bacterial species, coagulase negative *staphylococci* are among the dominant organisms colonizing the urethra and periurethra in males and females[18] and the *E.coli* is the most common etiological agent of UTI in hospital and community patients[19].

The predominance of *E.coli* is clear from the high percentage in urine specimens and several studies in past have shown that *E.coli* is the most common etiological agent of UTI in hospital and community patients. This agreed with other[19] who found that the most commonly isolated organisms from urine specimens in hepatitis patients were *E.coli*.

In this study two cases of *Rothia dentocariosa* isolated from urine in hepatitis patients. Patients were immunocompromised[20].

The most important cause for increasing the host susceptibility to bacterial infections is the lowering of immune response caused by hepatitis viruses which attack directly the immune cells or downregulate the immune response. The IFN-γ is produced mainly by TH-1 lymphocytes and has important role in the host defense against infection by viral and microbial pathogens[21]. It is able to induce bactericidal activity of macrophages and stimulates the expression of MHC system, and inhibits microbial proliferation[22]. Interferon-γ plays a direct role against intracellular bacterial infections[23].

The decreased level in the concentration of C3 reflects an abnormal immunological functions in complement system as the C3 is the key complement for all the three pathways of complement activation; classical, alternative, and lectin pathways required for immune responses against bacterial infections[24].

Patients with severe liver disease (e.g., alcoholic cirrhosis or viral hepatitis), who have lost significant liver function and therefore cannot synthesize sufficient complement proteins, are predisposed to infections caused by pyogenic bacteria[7]. Complement activation is one of the earliest responses to infection including viral hepatitis and its level has been shown to be reduced in viral hepatitis diseases[25]. The results obtained in this work agreed with a study carried out in Babylon Province, Iraq that indicated that the unimmunoresponsivness to bacterial antigens is a problem that increased the susceptibility to bacterial infections[26].

In current study, the patients exhibit no significant increase in levels of serum IFN-γ and complement components (C3, C4) as compared to healthy. These results indicate unresponsive to bacterial antigens, depending on the facts that under antigenic stimulation as in case of hepatitis patients, the immune components begin to activate and release their mediators involving complement components (C3, C4) and IFN-γ.

Conclusion:

Bacterial infections were detected in viral hepatitis patients as a result of a state of immunosuppressive state.

Acknowledgment :

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