The Role of Interferon Lambda in Hepatitis B infection

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Abstract

Hepatitis B infection in the Mediterranean countries has a broad range of seroprevalence: from middle (≥2%) to high frequency (≥7%). However, Iraq was regarded as intermediate endemicity with 3% of HBsAg seroprevalence in normal population. Chronic HBV infection pathogenesis contributed to the host innate and adaptive immune responses. Innate immunity induces an antiviral state against infected cells by producing interferons. IFNL plays pivotal roles in host–pathogen interactions and reduces replication of HBV through limiting the creation of HBV RNA enclosing capsids.

Sera of patients and controls was assessed for the levels of IgM to hepatitis B virus core antigen, IL-29, IL-28A and IL-28B using ELISA technique. In acute hepatitis B infected individuals, Anti-HBc IgM antibodies index unit mean were significantly higher than chronic with and without treatment, carrier patients and control. IL-29, IL-28A and IL28B levels in the sera of patient groups showed differences than control group, while these differences were not significant indicating the virus replication and persistence in the infected cell correlated with IFNL levels.

Keywords: Hepatitis B Virus, IFNL, Anti-HBC IgM, ELISA, aminotransferase.

Introduction

Hepatitis B infection is a liver disease caused by the HB virus possibly threatening the life. It is a most important international health problem. HBV can cause both acute, chronic illness and puts individuals at high danger of death from cirrhosis and cancer of the liver. Although the occurrence of chronic HB virus infection was Low (< 2%) within Iraq[16], still there are threats to the successful eradication of HBV worldwide.[13]

Hepatitis B virus is double stranded (ds)- DNA virus replicated via reverse transcription and considered as a member of the hepadnavirus group. The virion of HBV is a 42 nm particle containing 27 nm in diameter an electron-dense core which is nucleocapsid enclosed by an external envelope of the surface protein known as HBsAg inserted in membranous lipid obtained from the host. The surface antigen is created in excess via the infectedhepatocytes.[22]

HBV is transmitted by contact with contaminated blood, blood products and other body fluids.[9] Development and pathogenesis of chronic HBV infection contribute to the innate and adaptive immune responses of the individual with HBV-infection and regularly affect the efficiency of anti-HB virus drugs. Innate immunity is in charge for identification of viral RNA, viral proteins and host tissue injure. It stimulates an antiviral condition against infected cells through producing interferons.[7] IFNs are main cytokines establishing a complicated antiviral response. Three separate types of IFNs are currently recognized (type I, II and III) depending on their structural characteristics, receptor use and biological behaviors. While all IFNs are essential mediators of antiviral defense, their responsibilities in antiviral protection vary.[10]

Type III IFN includes IFN-λ1 (IL-29), -λ2 (IL-28A) and -λ3 (IL-28B). Interestingly, IFNL reveals activity at the interface of innate and adaptive immune response. Its family of innate cytokines is gradually more being ascribed as essential role in host–pathogen connections and reduces HB virus replication via limiting the formation of capsids containing RNA of HBV.[6] Once IFNλ is administered in HBV patients with suppressed viral replication rates, it can stimulate wide immune stimulatory characteristics and force activation of cytokine-manufacturing and cytotoxic Natural Killer cells, IFN-γ-producing HB virus-specific $CD4^+$ T and preservation of the antiviral and cytotoxic jobs of HB virus-specific $CD8^+$ T cells.[14] Due to the lack of local studies on these subjects, this study aimed to investigate the role of Interferon lambda (IL-29, IL-28Aand IL-28B) in the immune response of Iraqi patients with hepatitis B viral infection.

Material and Methods

Study Groups: A total of 89 individuals were included in this study, 74 individuals of them had hepatitis B infection (patients group); HBs Ag was detected in their sera by ELISA technique and confirmed by real time PCR analysis, these investigations with the diagnosis were made by the consultant medical staff in the Gastroenterology and Hepatology Teaching Hospital in Baghdad. 74 hepatitis B patients included 20 acute, 20 chronic with treatment, 20 chronic without treatment and 14 carriers. The patients were 51 males and 23 females with age range 9-70 years, while the other 15 individuals were the control group, 10 males and 5 females; their age range matched with patient group.

Specimens Collections: Specimens were collected by venipuncture; five ml of blood was dragged by using not reusable syringes. The blood sample was putted in disposable tubes, then left at room temperature (20°C) to form clots. After that sera were separated through centrifugation for five minutes at 3000 round per minute

(rpm). The separated serum was distributed into 4 aliquots in Eppendorf's tube and kept at -20ºC until assayed.

Biochemical Tests

Total Serum Bilirubin (TSB) determination: In order to determine TSB, BILTS Total Bilirub in Special Kit for quantitative TSB in human serum and plasma of adults was used by Cobas™ c 111 chemistry analyzer according to the manufacturer instructions.

Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) determination: Reflotron® GPT (ALT), GOT (AST) and ALP reagent strips were used for quantitative determination of ALT, AST and ALP respectively in blood, serum and plasma with Reflotron® Plus System by Roche Company according to the manufacturer instructions.

Detection of HBc IgM Antibodies: A solid phase, two-step incubation, antibody capture ELISA kit for qualitative determination of IgM-class antibodies to hepatitis B virus core antigen in human serum or plasma was used according to the manufacturer instructions.

Assessment of IL- 29, IL-28A and IL-28B serum levels: Sera of patients and controls were assessed for the level of IL-29 using commercially available kits (Boster, Austria) and for the level of IL-28A and IL-28B using commercially available kits (Elabscience, USA) by using of ELISA system and the procedure was done according to the kit instructions.

Statistical analysis: The results were analyzed statistically and the values were expressed as mean ± SD. The differences between means were assessed by ANOVA (Analysis of Variance) and correlation by correlations coefficient (r) using Microsoft office Excel 2007 software. All values were deemed significantly different when $P< 0.05$.

Results and Discussion

Anti-HBc IgM antibodies index unit mean ± SD were significantly higher ($P< 0.05$) in acute hepatitis B patients (3.9 ± 5.8) than chronic with and without treatment (0.37 ±0.25 and 0.45 ± 0.34) respectively, carrier patients (0.25 ± 0.21) and control (0.12 ± 0.1) as shown in table 1.

Anti-HBc IgM emerges in individuals with acute illness about the time of illness beginning and points out HB virus recent infection. This result resembles with Lavarini et al[11] study in Italy who found that 85% of patients with positive IgM anti-HBc test result confirmed that hepatitis was due to primary infection with hepatitis B virus. The concentrations levels of TSB, AST and ALT were higher than normal value in acute and chronic without treatment hepatitis B infected groups while there were insignificant differences ($P>0.05$) between study groups as shown in table 2. The mentioned characteristic of acute hepatitis caused by viral infection was the striking altitude in serum transaminase actions. The increase in aminotransferase, particularly ALT, through acute HB varies beginning from a mild/moderate raise of three - to ten -fold to a striking raise of >100-fold. [20] HBV infection may alter the serum levels of certain hepatic enzymes and compounds such as ALP, AST, ALT, TSB and albumin. The elevation of these enzymes and proteins above their upper reference limits is said to be abnormal except for serum albumin, which usually falls below its reference limit when it is abnormal.[1]

In table 3 and figure 1 the concentrations of IL-29, IL-28A and IL28B in the sera of patient groups showed differences than control group, while these differences were not significant. IL- 29, IL-28A and IL-28B highest levels were in control group than hepatitis B patient groups. IL-29 levels were elevated in chronic HB patients with treatment than carrier; acute groups and the minimum level with chronic without treatment group. IL-28A highest levels were in chronic HB patients with treatment than carrier, chronic without treatment groups and the minimum levels with acute group. While IL-28B highest level was in hepatitis B carrier patients which is slightly lower than control, acute, chronic with treatment groups and the minimum levels in chronic without treatment group.

The results of this study indicated that these ILs protected against HBV infection; its levels were up regulated in patients groups (lower than control) and might be the virus replication and persistence in the infected cell correlated with ILs levels. When ILs levels decrease, the virus can persist and cause chronic disease.

The result of this study differs from Dengming et al[3] results in part of IL-29, who found that among the HBV infected groups (immune tolerance, inactive HBsAg carriers, resolved HB), significant differences were detected in serum levels of 7 cytokines, together with IL-29 than control. The elevation of IL-29 in the inactive and resolved groups proposed that IL-29 plays a vital role in anti-HBV immunity, particularly evaluated with IFN-γ.

For IL-28A, increased expression of this interleukin was observed in HBV infected groups evaluated with controls and with Yu et al[21] results which demonstrated that IL-29 mRNA levels were significantly elevated in patients with chronic HB than those in healthy persons and showed that mRNA levels of IL-29 were much higher in human hepatoma HepG2.2.15 cells that hold an integrated HB virus genome than those in human hepatoma HepG2 cells with no HB virus genome. The differences between previous studies results and the results of this study may be from the small number of subjects in this study.

Results of this study were similar with Shi et al[17] who reported that serum IL-28B protein levels showed no significant variations existing among the inactive carrier and healthy control groups. On the other hand, the levels were significantly lower in chronic Hepatitis B, cirrhosis and

Hepatocellular carcinoma patients. When chronic Hepatitis B infected patients were applied according to HBeAg status, IL-28B protein levels were elevated in HBeAg-positive compared to negative patients.

Gron et al[8] examined whether patients chronically infected with HBV demonstrated enhanced levels of IFNλ1 (IL-29) in serum. They found that no variations were shown when comparing chronic HB virus patients with healthy persons or chronic HC virus patients. Serum IFNλ1 levels of HB virus chronically infected individuals in receipt of nucleot(s)ide analog and intensely infected HB virus individuals were also determined and demonstrated insignificant variations when compared to any of the abovementioned patient/healthy control groups, immune-tolerant and inactive carriers but no variations in IFN λ1 serum levels were detected.

The chronic HB virus patients were separated in three clinical groups; immune-active, HB virus triggers IL-29, Interlukin-8 and COX-2 expression and these 3 inflammatory cytokines organized each other in the order IL-29/IL-8/COX-2, which included positive regulation and negative feedback. HB virus not only damages the antiviral activity of IL-29 and favors the establishment of persistent HB viral infection but as well as stimulates the high appearance of proinflammatory factor COX-2 to preserve the inflammatory environment correlated with HB virus infection.[21]

During acute HB viral infection, deficient in IFN-I and IL-15 at the time of viremia might be an alternative of IFN-λ1, which was revealed to be created in the liver and to have antiviral potential against HB virus.[18] While IFN-λ1 was circulating at elevated levels compared to IFN-I, it was not stimulated higher than concentrations in healthy persons. [3]

IL-29 and IFN-I induce the same antiviral reactions in spite of their utilization of dissimilar receptors. IL-29 may have therapeutic assessment against chronic viral hepatitis in patients.[4] IL-28B reduces HB virus replication in hepatocyte cell lines and has been taking into consideration as a potential novel treatment for viral hepatitis.[15] The genetic polymorphisms near the *IL28B* gene are powerfully correlated with sustained viral response and spontaneous viral clearance chronic HBV infected individuals. Thus, genetic difference of IL-28B may avoid progression of HB virus infection by dropping viral load and liver inflammation.[12]

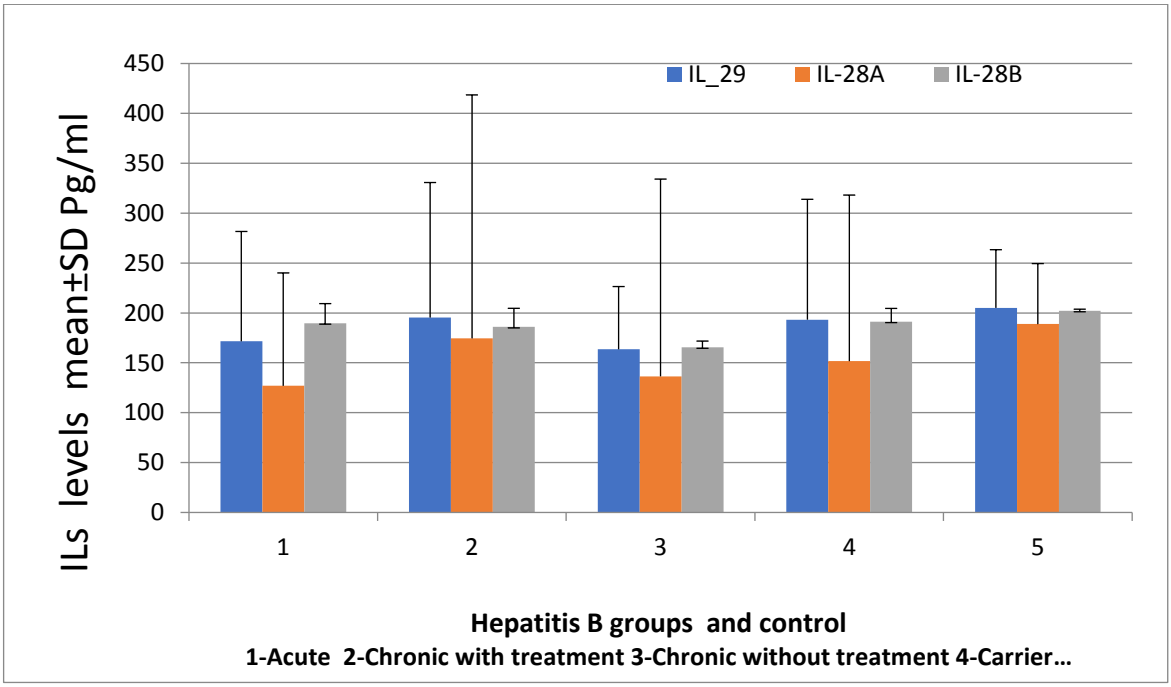

Figure 1: IL- 29, IL-28A and IL-28B levels mean in study groups.

Table 1
Anti-HBc IgM antibody index unit in Hepatitis B patients.

Specimen groups	Biochemical tests concentrations Mean ± SD			
	TSB mg/dl	AST U/L	ALT U/L	ALP U/L
Acute hepatitis B patients	2.09 ± 2.2	54.8±104.6	65.3± 64.0	55.2± 12.6
Chronic hepatitis B patients with treatment	0.69± 0.52	23.4 ±5.21	22.35± 12.0	59.18±20.23
Chronic hepatitis B patients with no treatment	1.23±2.57	50.69±117.1	66.1± 159.1	52.0±33.20
Carrier	0.65±0.25	20.61±8.1	22.0±11.0	50±21.0
Control	0.55± 0.15	15.0±4.9	22.92±11.4	43.65±22.0
P Value	0.129 NS	0.36NS	0.44 NS	0.38NS

Table 2
Biochemical tests in hepatitis B patients and controls group.

Specimen groups	Anti HBc IgM antibodies index unit Mean ± SD
Acute hepatitis B patients	3.9 ± 5.8
Chronic hepatitis B patients with treatment	0.37 ±0.25
Chronic hepatitis B patients with no treatment	0.45± 0.34
Carrier	0.25 ± 0.21
Control	0.12 ± 0.1
P value	< 0.05

Optimal values: TSB up to 1.4 mg/dl, ALT up to 41 U/L, AST up to 40 U/L and ALP 40-129 U/L.[2] NS: non significant.

Table 3
IL- 29, IL-28A and IL-28B levels mean in study groups.

Specimen groups	IL-29 levels Pg/ml	IL-28 A levels pg/ml	IL-28 B levels pg/ml
Acute hepatitis B patients	171.62 ± 76.90	126.93±113.12	189.77± 110.04
Chronic hepatitis B patients with treatment	195.38 ± 92.59	174.53± 243.95	185.95 ±135.33
Chronic hepatitis B patients with no treatment	163.59±82.91	136.51± 197.68	165.57 ±62.86
Carrier	193.27± 122.64	151.73±166.44	191.31 ±120.64
Control	205.04 ± 81.36	188.82±60.64	202.17 ±58.33
P Value	0.69 NS	0.8 NS	0.91NS

NS: Non-significant.

Conclusion

IFN-λ plays a significant role in immune response against HBV Iraqi patients and it is probable that these cytokines may in addition be helpful as a therapeutic agent for viral infections treatment especially HBV; resembles to the recombinant IFN-λ which is assessed first as a potential therapeutic stand by IFN-α for the treatment of HC virus infection.

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