ORIGINAL ARTICLE

Life Science Journal of Pakistan
http://www.lifesciencejournal.pk

Evaluation of IL-35 and IL-36 in patients suffered from Hepatitis B virus in Suwayrah, Wasit Governorate, Iraq

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ABSTRACT

Background: Hepatitis B virus (HBV) infects the human liver and causes both acute and chronic liver diseases. With 1.5 million people get that virus annually. IL-35 and IL-36 are novel cytokines and can be good indicators of liver disease.
Objective: This study aimed to identify the infection rate of HBV and its relationship with serum IL-35 and IL-36 levels by ELISA kits.
Materials and methods: This research was performed from June to December 2022 at Suwayrah General Hospital. One hundred blood samples as five milliliters were collected from patients under sterile conditions and left for ten minutes to allow spontaneous clotting at room temperature before centrifugation (6000 rpm) to separate the sera. Serum samples were stored at –20°C for subsequent determination of HBV, IL-35, and IL-36 levels.
Results: The results showed that HBV was noticed in 16 males (55%) and in 13 females (45%). The age period that showed high susceptibility to HBV was (21-40 years) 19 (65.5%) with a significant difference ($P < 0.000$). The level of IL-35 in HBV patients was (170±6 pg/ml), while in healthy persons was (320±9 pg/ml) with a significant decrease ($P < 0.001$). The level of IL-36 was (235±9 pg/ml) among patients with HBV compared to (120±15 pg/ml) among healthy with a significant increase ($P < 0.05$).
Conclusions: The hazard of HBV threatens both males and females. The age period of the individuals is significantly related to their susceptibility to viral infection. Both IL-35 and IL-36 are good indicators of liver disease such as HBV.

Keywords*:* Liver diseases, HBV, Hepatic inflammation, IL-35 and IL-36

Life Sci J Pak 2023; 5(02):03-08 DOI:- https://www.doi.org/10.5281/zenodo.10338240
(Received March 2023 – Accepted September 2023 – Published December 2023)

Introduction

Hepatitis B or (serum hepatitis) is one of the main inflammatory diseases of the liver in human beings. It is considered an epidemic in many regions in Asia and Africa, while endemic in China (1). HBV is a species of *orthheapdna- virus* genus as a member of the *Hepadnaviridae* (2). its double-stranded DNA genome with A virion 42 nm in size, HBV has a core antigen (HBcAg) encircled by a shell encompassing surface antigen (HBsAg).
Based on different serological reactivity of HBVsAg, HBV is divided into four distinct serotypes (adr, adw, ayr, ayw) and eight genotypes (A-H), basing on genomic nucleotide sequence variations (3). In general, one of the main sources of chronic liver disease that leads to liver damage and hepatic fibrosis is infection with chronic hepatitis B (4). Routes of transmission for HBV are direct contact with blood, transfusion of blood or its products, intravenous injections, and unsafe sexual intercourse (5). The initiation of liver cirrhosis from HBV infection is related to several factors for example age, gender, alcohol consumption, and co-infection with the hepatitis C virus (6). Absence of the routine serological investigations leads to raising the probability of HBV's transmission from donors to recipients in developing countries (7).

Interleukin 35(IL-35) is a recently recognized cytokine of the IL-12 family, it contains two heterodimeric subunits, IL-12 α chain p35 and IL-27 β chain Epstein-Barr virus-induced gene 3 (EBI3) (8). The main sources that produce this interleukin are $CD4^+CD25^+$ regulatory T cells (Tregs). IL-35 acts as an inhibitory mediator in autoimmune and infectious diseases (9). Host antiviral activity against many viral diseases will increase by the production of IL-35 (10). Another interleukin that has pro-inflammatory activity is termed Interleukin (IL)-36, which belonged to the

family of IL-1 cytokines. IL-36 shows an important effect in both innate and adaptive immune responses (11). In many illnesses like cancer; inflammatory bowel disease; rheumatoid arthritis and systemic lupus erythematosus, the activity of IL-36 as a pro-inflammatory cytokine appears increase clearly (12). In addition, IL-36 participates in infectious diseases, especially bacterial and viral infections through two main strategies, first through clearance of pathogens by stimulating immune responses, and second by increasing inflammatory responses, which lead to excessive immune infiltration and tissue damage (13).

Materials and methods

Patients

This research was performed on patients from June to December 2022. They presented with rise in temperature, weakness, dark urine, and pale skin and eyes were included in this study, which were checked at Suwayrah General Hospital.

Blood samples

One hundred blood specimens as (5 ml) were collected from each patient under sterile conditions. At room temperature, these samples were placed for 10 minutes to get spontaneous clotting followed by centrifugation at (6000 rpm) to gain the sera (14).

ELISA kits

Detection of HBV was performed by hepatitis B-Ab ELISA kit (Abnova/Taiwan). Measurement levels of IL-35 and IL-36 in patients with the virus was accomplished through comparing their levels in parallel amounts of samples from non-infected persons by Human Interleukin 35 (IL-35) and Human Interleukin 36 by ELISA Kit (Mybiosource, USA). The two types of the kits were done according to the manufacturer's instructions.

Statistical Analysis

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) program version 21.

Results

Of the 100 individuals included in this study, 29 persons were positive for HBV infection. In general, both genders were infected with HBV 16 males (55%) and 13 females (45%) as in Figure 1.

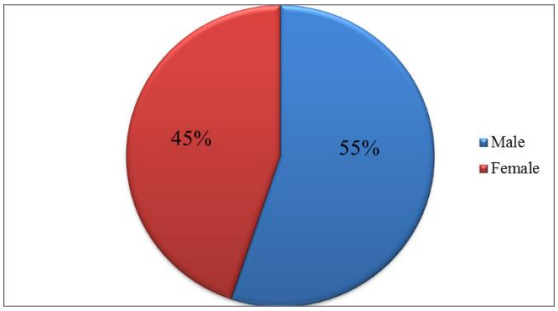

Figure 1: Genders distribution of patients with positive HBV infection.

These patients with positive HBV infection were divided according to their age ≤20 years 1(3.5%); 21-40 years 19 (65.5%) and 41-60 years 9(31%). According to their gender, ≤20 years 1 female (3%) and 0 male (0%); 21-40 years 10 female (34.5%) and 9 male (31%); 41-60 years 2 female (7%) and 7 male (24%) as showed in Table 1.

Table 1: Distribution of patients with HBV according to the gender in each age group.

Age / Gender	≤20 years	21-40 years	41-60 years	Total
Male	0(0%)	9(31%)	7(24%)	16(55%)
Female	1(3.5%)	10(34.5%)	2(7%)	13(45%)
Total	1(3.5%)	19(65.5%)	9(31%)	29(100%)

Both interleukins (IL-35 and IL-36) levels was measured and then compared their levels in patients with healthy persons similar to them in age periods and gender. Evaluation of IL-35 in this study found that it was (170±6 pg/ml) among patients with HBV, while in healthy persons was (320±9 pg/ml) with a significant decrease ($p < 0.001$) as in Fig (2). Also, IL-36 level in the presented research was (235±9 pg/ml) among those with HBV compared to (120±15 pg/ml) in healthy persons was significantly increasing ($P < 0.05$) as in Fig (3).

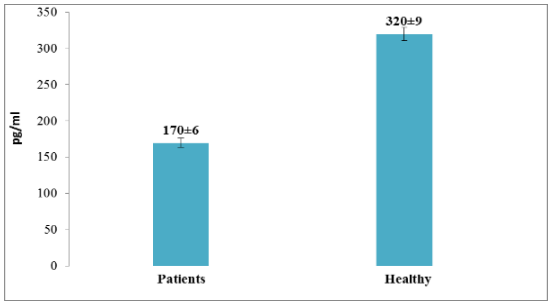

Figure 2: Comparison of IL-35 levels among healthy individuals and patients.

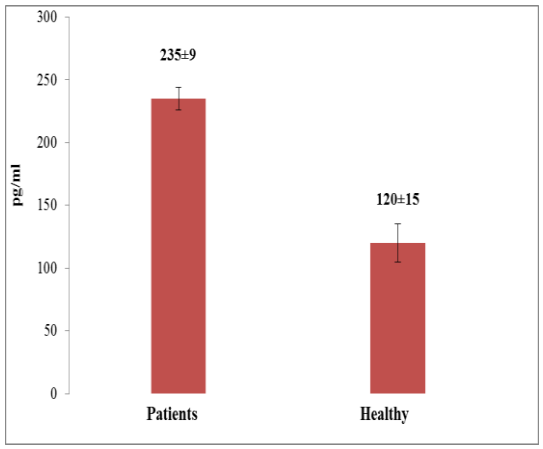

Figure 3: levels of IL-36 levels among healthy individuals and patients.

Discussion

Incidence of HBV in our study occurred in both genders males and females with no significant difference (P=0.72), this agrees with the results of Alheany and Abdulla (15) and Al-Kaif *et al*, (14). This may return to routes in which a person can get HBV infection such as cupping, sexual contact, surgical operations, and tattoo (16) found in both gender males and females in a comparable percentage.

The incidence of HBV positive cases was highly noticed in the age period 21-40 years with a significant difference than the other periods of age (P =0.000). This study was paralleled with studies of Al-Rubaye *et al*. (17) and Hussein *et al.* (18). The explanation of this finding referred that the risk of acquiring Hepatitis viruses in persons at age (21 to 40) years is high than other periods of age, because the high sexual activity of them at this period beside ignorance the sexual safety in some situations lead to increase the possibility of viral spread from one person to another by sexual contact. In addition, infection with HBV (19) arises further often in young adults during skin penetration and drug injections (20).

The level of IL-35 in the present research was decreased in HBV positive cases than in negative with a significant difference ($p < 0.001$), this result agreed with those of Cheng *et al*, (21) and Mohsen *et al*, (22). So, a mechanism of immune response including IL-35 is noticed in the progression of HBV infection. In general, this point out that liver damage by HBV is affected by the host immune response which is achieved by some of cytokines (23). The family of IL-12 which involves IL-35 plays a significant cause in increasing the susceptibility to numerous infections such as HBV (24). The scientists suggested a conceivable action of IL-35 in infection with HBV due to a recent description of it as one of IL-12 family which made it not well-investigated (25). On other hand, IL-35 shows an consistent function in controlling the persistence of the virus and inhibiting inflammatory responses and has an immunosuppressive role in viral hepatitis(26). Patients with HBV showed an expected abnormal level of IL-35 in their blood with an unclear mechanism leading to a decrease in the level of it. Thus, supplementary researches are surely essential to focus on IL-35's role in the immunopathogenesis of HBV infection, particularly those based on both IL-35 subunits' gene expression.

In our study, the level of IL-36 was significantly increasing in patients than healthy persons (P<0.05), this agreed with those of Gong *et al*, (27) and Wang *et al*, (28). In the liver, the main source of IL-36 is the infiltrated neutrophils, but not the hepatocytes. IL-36 has an important role in the activation of inflammatory responses via exciting both innate and adaptive immunity. Also, some studies establish the precious action of it in the treatment of tumors and prognosis (29). Several clinical conditions such as HBV infection, alpha-fetoprotein (AFP), liver cirrhosis, and metastasis have a significant relationship with IL-36 level (30). Even with its existing in hepatocytes, function of IL-36 is still indistinct in the liver (31). The proportion between IL-36 and DNA of HBV may explain the possible role of this interleukin as an antivirus throughout the chronic infection (27).

Conclusion

In the presented study, there was a relation between the age of the patients and HBV infection while gender didn't make a difference. Both cytokines were good indicators of infection with HBV. IL-35 level was lower in HBV patients than in healthy persons, and on the contrary, there was an elevation in the level of IL-36 in patients in correlation with the control. Further studies in these cytokines especially in the genetic field are recommended in the future to reach a complete understanding of their roles in immunity and treatment.

Acknowledgment

I offer my thanks and appreciation to the staff of the hospital for their support and help.

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