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Detection of Hepatitis B virus by serological test and PCR in Iraqi patients

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Abstract:

Hepatitis is a systematic disease with primary inflammation of the liver by any type of hepatitis viruses. The most common causes of viral hepatitis are five (Hepatitis A, B, C, D and E). Hepatitis B surface antigen (HBsAg): A protein on the surface of hepatitis B virus. It can be detected at high levels in serum during acute or chronic hepatitis B virus infection**.** A case control study was conducted on 110 HBV Iraqi patients with different stages of HBV disease. The cases and samples were obtained from Medical city hospital, Al-yarmouk teaching hospital, Al-Kendi teaching hospital and Central public health laboratory in Baghdad, Iraq. In addition, 40 healthy were enrolled in this study. Detect the human HBsAg by ELISA and Extract and amplified HBV DNA by using PCR technique. The results show that mean age 41.66 ± 2.42 and a range 16-70 years, 28 patient (70%) male and 12 patient (30%) Female. Serological findings show3 patients (7.5%) have high liver function enzymes of the final 40 specimen that used in this study while 37patient (92.5%) have normal levels of liver function enzymes, 40 patient (100%) were positive for HBsAg and 31 patient (77.5%) were positive for HBV Ab, while 9 patients (22.5%) were Negative for HBV Ab. The molecular results show 15 sample (37.5%) of 40 sample were have positive DNA bands. The current study showed that the infection in the male patients higher than females and the age distribution of infection higher positivity rates in the middle-aged individuals.

Key words: Hepatitis B virus, ELISA, PCR.

Introduction

Hepatitis B virus (HBV) infection is a serious worldwide health issue and the most severe form of viral hepatitis, causing cirrhosis and liver cancer when left uncontrolled. According to the World Health Organization, document that was issued on June 24, 2022, over 296 million people have chronic HBV infection in 2019, with 1.5 million new infections every year. During an infection, antibody responses are crucial in the removal of HBV particles and infected hepatocytes. Individuals infected with hepatitis B surface antigen (HBsAg) may naturally eliminate it and produce hepatitis B surface antibodies (anti-HBs); nevertheless, others may develop cirrhosis and hepatocellular carcinoma (HCC) (Tong *et al*., 2018; Hatazawa *et al*., 2018). The primary glycoprotein of the viral envelope, surface antigen (HBsAg), is used to provide a serological diagnosis of HBV infection. HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc immunoglobulin M and immunoglobulin G are all serological indicators for HBV infection. Various serological markers enable for the identification of HBV-infected patients, the research of chronic hepatitis B progression, and the monitoring of antiviral therapy (Kao, 2008). The existence of anti-HBs is thought to be a sign of immunity against HBV infection. The lack of HBsAg, on the other hand, is a criteria for ruling out infections. As a result, the HBsAg test is

commonly used to screen blood and organ donors (Perrillo, 2021; Dutch *et al*., 2022). In routine clinical practice, it is generally accepted that there won't be a simultaneous positive for both HBsAg and anti-HBs because general theory holds that the antibody to HBsAg (anti-HBs) can neutralize HBsAg in serological markers of HBV infection (Wang *et al*., 2017; Anastasiou *et al*., 2018; Yip and Wang, 2019), But previous studies have noted that chronic HBV carriers also have HBsAg and anti-HBs present at the same time (Lee *et al*., 2020; Zhu *et al*., 2020). Although there are various causes for the simultaneous occurrence of HBsAg and anti-HBs in the sera of patients with chronic hepatitis, the effect of this cohabitation for patients is mainly unclear. Furthermore, opinions differ, and the mechanism behind this serological pattern remains unclear.

Materials and Methods

Patients and samples

The present research was carried out at Baghdad, Iraq. From October 2022 to April 2023, A total of 110 Sera were collected from individuals infected with HBV at a various stages of disease. All of the Samples belonged to (Medical city hospital, Al-Yarmouk teaching hospital, Al-Kendi teaching hospital and Central Public Health Laboratory) in Baghdad, Iraq. In addition, collect 40 specimen of non-infected individuals as negative control.

Detection of human HBsAg by ELISA

A qualitative sandwich ELISA kit was used to measure the Liver function enzymes (ALT and AST) and HBsAg rendering to the instructions providing by producer My BioSource, USA. The ALT, AST and HBsAg were measured for all individuals. The HBV-Ab was measured using

Primers used in this study

The sequences of the primers used in this study were designed using the site https://www.ncbi.nlm.nih.gov/tools/primer-blast/primertool. The forward primer for S-1 gene ACACACTCTATGGAAGGCGG; and the reverse primers was CTGTATGATGTGATCTTGTGGC. According to the producer Macrogen, Inc., Seoul, Korea.

Nucleic Acid (HBV DNA) extraction

Blood samples were used to extract and purify DNA according to the manufacturer's instructions using Relia-Prep™ DNA extraction kit, Promega, USA. The collected abscess samples from patients were allowed to thaw and were mixed by using vortex. In 1.5ml micro-centrifuge tube, dispensed 20µl of proteinase K (PK) solution. Added 200µl of blood samples to the tube containing the proteinase K solution and briefly mixed them. To the same tube, 200µl of cell lysis buffer was added, capped the tube and mixed it by using vortex for at least 1 sec. then incubate the mixture at 56°C for 10min. place a Relia-Prep™ binding column in an empty collection tube while the blood sample was incubating. Remove the tube from the heating block, added 250µl of binding buffer, capped it and vortex it for 10sec. the tube's content transferred to the Relia-Prep™ binding column, capped and placed in micro-centrifuge. Centrifuged at full speed for 1 min. the binding column samples were transferred to a new collecting tube. Then added 500µl of column wash solution to the column and centrifuged it at maximum speed for 3min. the flow through was removed, and the process was repeated 3 times. Finally, 50µl of nuclease-free water was added to the column, and the samples were centrifuged at maximum speed for 1min.

HBV PCR detection

All primers were diluted according to the manufacturer and the reaction mixture was described according to the following Table1.

Table1. PCR mixture preparation of the two SNPs.

Component	20 µl (Final volume)
Luna script PCR Master mix	10
Forward primer (1:10)	0.5
Reverse primer (1:10)	0.5
Nuclease-free	4
DNA Sample Volume	5

The following parameters were used for the molecular detection of HBV DNA: initial activation at 95℃ for 10min, 40 cycles at 95℃ for 15sec, 60℃ for 1min for both Annealing /Extension. The size of the PCR products was determined by the migration pattern of a 100bp DNA ladder, and the final product was identified by electrophoresis on 1.5% agarose gel (Figure1).

Results

The research included only 40 specimen were used from the 110 specimen that was collected from patients that infected with HBV because some patients have baseline HBV-DNA levels below the lower limit for detection or due to the denaturation of the genetic material as a result of storage losses. The results show that mean age 41.66 ± 2.42 and a range 16-70 years, 28 patient (70%) male and 12 patient (30%) Female. The results showed 3 patients (7.5%) have elevated levels of the liver enzymes (ALT and AST), while 37patient (92.5%) have normal levels of the liver function enzymes. Also the serological findings for HBsAg shows that 40 (100%) were positive while the HBV-Ab shows that just 31(77.5%) were positive.

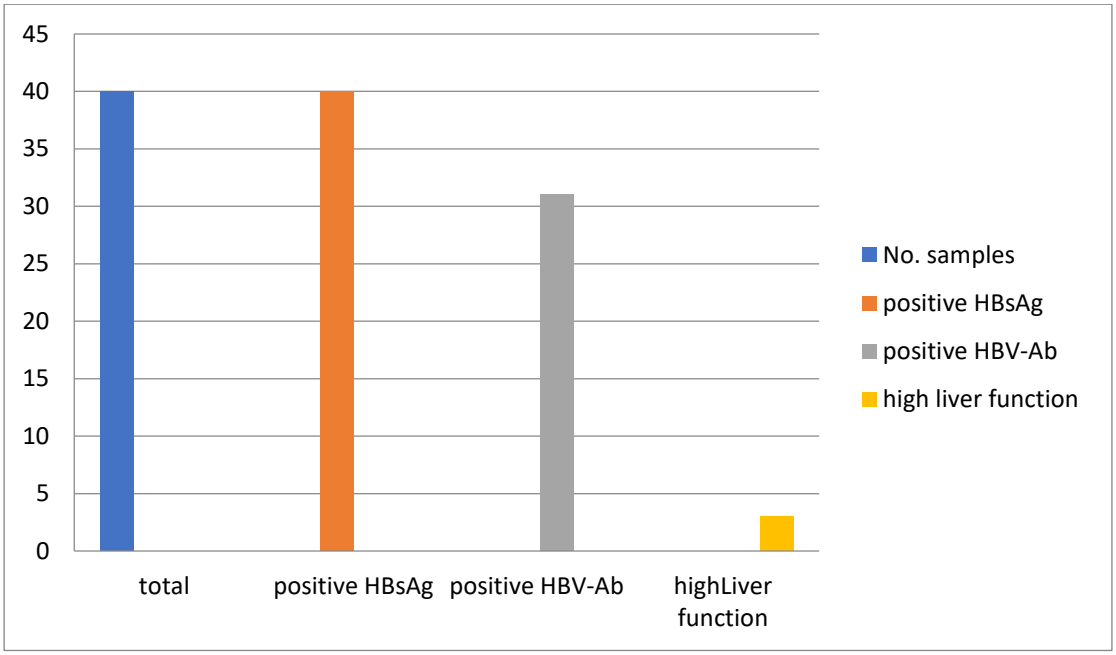

Figure1: shows the total positive HBsAg, positive HBV-Ab and high level of liver function.

by using PCR, Hepatitis B virus DNA was found in 15 (37.5%) of the tested 40 samples. All of the 15 patient tested positive for HBsAg. The final product were identified by using 1.5% agarose gel electrophoresis, and the size of the PCR product was assessed using the migration pattern of a 100bp DNA ladder (figure2).

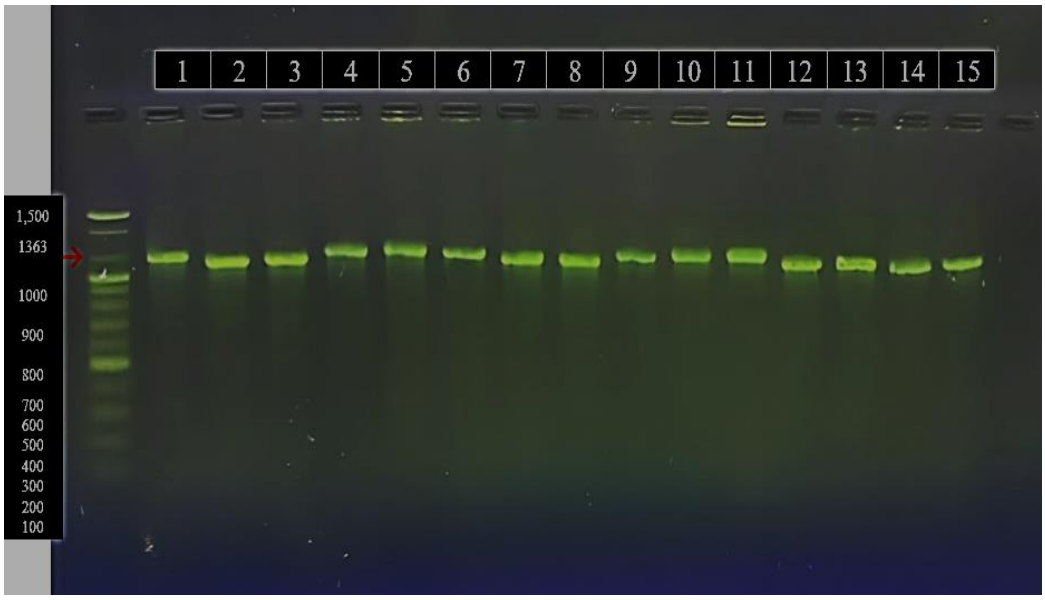

Figure2: Electrophoresis pattern of PCR products of HBV, at the detection step.

Discussion

In this study the results of the distribution age these findings corroborated those of Tarky *et al*., (2013) who discovered that HBsAg prevalence increased with aging, peaking at 2.4% in the fifth decade of life. HBsAg prevalence was lowest in the first decade of life (0.9%). The amount of HBsAg in the third decade doubles the amount of the HBsAg in the first decade. The findings of the HBsAg amount agreed with several other studies conducted in Iraq including those by Ahmed, (2013) and Abid *et al*., (2015) those found that the majority of chronic hepatitis B patients were located in the third and fourth decades of life, between the ages of (21-40) with a percentage of 51.3% and 5%, respectively. The age group between 30-39 years old that the greatest incidence of acute hepatitis B in 2013, whereas children and adolescents under the age of 19 had the lowest rates. The patient's distribution according to type of gender agreed with an Iraqi study by Abbas *et al*., (2023) examined 345 individual and found that the males were more likely to be infected with HBV than females. While Juon *et al*., (2023) who tested 291 blood sample and recorded that 54% of patients were male and 46% were female. Furthermore, the results of liver functions in this study agreed with other study by Pan *et al*., (2022) found that patients without increased HBsAg showed better baseline liver function in blood tests.

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