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Hepatitis B Virus in Al- Diwaniya Governorate

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Abstract:

Infection with the hepatitis B virus (HBV) is a public health concern because it can lead to liver conditions like cirrhosis and hepatocellular cancer.

The basic immunology idea of an antigen attaching to its particular antibody is used in enzyme-linked immunosorbent assays, which enable the detection of extremely minute amounts of antigens like proteins, peptides, hormones, or antibodies in a fluid sample.

By using ELISA screening, 80 samples were positive for the HBV surface antigen (HBsAg). The study sample consisted of 59 males and 21 females, aged between 12 and 75. Results were 33 samples (41.25%) were positive for HBV-DNA. 47 (58.75%) of the samples tested negative for HBV-DNA using PCR. According to the current study, there is no discernible difference between males and females or between rural and urban areas in the incidence of HBV.

Introduction

Hepatitis B virus (HBV) infection is still a major global public health concern. According to statistical, 1 million people per year terminate because of acute and chronic HBV infections, and from (15 - 25)% of these persons will eventually progress liver cirrhosis and hepatocellular carcinoma (1).

There were 59 (73.75%) male patients and 21 (26.25%) female patients who tested positive for HBs Ag using the ELISA technique. There was no statistically significant difference between the two groups (p value = 0.066). To prevent bias in results caused by differences in gender distribution, statistical matching to the study group is essential.

Materials and Methods

Enzyme-Linked Immuno-Sorbent Assay (ELISA) Kit The contents of this ELISA kit (made by Fortress in the U.K.) for the detection of hepatitis B surface antigen (HBs Ag) in human serum.

The quantitative direct sandwich enzyme immunoassay techniques are used in this assay. Monoclonal antibodies (IgG antibodies to HBsAg) that are specific to HBs have been pre-coated on the micro-ELISA plate. This makes up the antibody in solid phase. The test sample is an antigen (HBsAg) that has been incubated in a well like this. If the reaction is affirmative, the labeled antibody binds to any solid-phase antibody HBs Ag complex that has already developed. When the enzyme substrate is incubated, the test well turns blue; when the reaction is stopped with sulphuric acid, it turns yellow. Without HBs Ag, the sample cannot precisely bind the designated antibody; instead, only a faint background color appears.

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Assay procedure

The strips were placed on a strip holder, and a sufficient number of wells were numbered, three of which served as negative controls (e.g., B1, C1, and D1), two of which served as positive controls (e.g., E and F), and one of which served as a blank (e.g., A), to which neither samples nor horseradish peroxidase-conjugate were added. With the exception of the Blank well, 50 µl of each of the following were added to each well: 50 l of Specimen, 50 µl of Negative Control, and 50 µl of Positive Control. Then, 50 l of HRP-Conjugate was added to each well. For 60 minutes, the plate was incubated at 37 °C with the plate cover on.

After the incubation period, the plate cover was taken off and thrown away then, 350 µl of diluted wash buffer was applied five times to each well to wash it. The micro well was given a 30 - 60-second soak each time. The dish was placed upside-down on blotting paper and taped to eliminate any leftovers after the last washing cycle. Each well, including the blank, received 50 µl of Chromogen A solution and 50 µl of Chromogen B solution, which were then gently mixed by tapping the plate. the plate was covered with a plate cover and left unlit for 15 minutes at 37 °C. In positive control and HBs Ag positive sample wells, an enzymatic reaction between the chromogen solution and the HRP-Conjugate results in the blue hue. It was taken off the plate cover. Each well received fifty l of Stop Solution, which was gently mixed in. The optical density (OD value) of each well was read at 450 nm to detect when HBs Ag positive sample wells and positive controls become yellow. The following equation was used to determine the cutoff value (COV) for HBs Ag: The mean absorbance value for three negative controls serves as the cut-off value (C.O.), which is equal to NC x 2.1 NC. S: each specimen's unique optical density (OD) Samples that have absorbance greater than or equal to the cut-off value are considered to have positive results (S/C.O.1). Samples that give negative results (S/C.O. 1) have absorbances below the cut-off value.

The primer for the detection of the virus were supplied by “Bioneer. Korea” with the design described by Naito (5). (Table 2) The PCR of amplicon for HBV:

	Primer	**Sequence (5'-3') & Amplicon size**
HBV -PCR	P1 (universal, sense)	“TCA CCA TAT TCT TGG GAA CAA GA” (1063bp)
	S1-2 (universal, anti-sense)	“CGA ACC ACT GAA CAA ATG GC”

Five millimeters of venous blood were collected from each of the 80 patients. The serum has been collected in Eppendorf tube then stored at -20c to be used for DNA extraction and PCR technique.

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Extraction of DNA

Genomic viral DNA was extracted from utilizing "gSYAN Genomic DNA Mini Kit" (Geneaid, USA).

Detection of HBV

PCR master mix was prepared for direct detection of HBV by using (AccuPower® PCR PreMix Kit). The resultant mixture was 20 µl. which is constituted of P1gene primer(10pmol) 1µl, S1-2 gene primer(10pmol) 1µl, PCR water 13µl and DNA template (DNA extraction) 5µl.

After that, these PCR master mix components that mentioned in placed in standard AccuPower PCR PreMix Kit that contains all other components which needed to PCR reaction such as (Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, $MgCl_2$, stabilizer, and tracking dye). Then, all the PCR tubes transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes. the Thermocycler program was as follow: Primary denaturation step by incubation at 94 ^oC for 5 minutes, proceeded for 40 cycles. Each cycle includes the following: 1- denaturation at 94 ^oC for 30 seconds. 2- annealing at 55 ^oC for 1 minute. 3- elongation at 72 ^oC for 1.5 minute. 4- Extension at 72 ^oC for 5 minutes.

Results and Discussion:

HBV distribution

In this study (80) patients, having HBV infection, confirmed were through detected for HBs Ag by ELISA technique to diagnose their infection with Hepatitis B virus. Recorded results of these patients (100%) were positive for HBs Ag as shown (Table 3).

Table 3: ELISA screen test for HBs Ag among 80 patients.

Gender	No. of samples	No. of positive samples
Males	**59**	**59**
Females	**21**	**21**
Total	**80**	**80**

According to Table 4, there were 59 (73.75%) male patients and 21 (26.25%) female patients who tested positive for HBs Ag using the ELISA technique. There was no statistically significant difference between the two groups (p value = 0.066). To prevent bias in results caused by differences in gender distribution, statistical matching to the study group is essential.

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Table 4: Elisa Screen Test for HBs Ag (positive) among (80) patients based on gender.

Hospital or center name	Total	Positive cases and percentage	
		Females	Males
Teaching Hospital of AL Diwaniya	19	8 (42.11)	11 (57.89)
Al Shamiyah General Hospital	6	1 (16.66)	5 (83.33)
Afak General Hospital	6	0 (0)	6 (100)
Al Hamzah General Hospital	10	2 (20)	8 (80)
Central Blood Bank	13	0 (0)	13 (100)
Haemodialysis	6	2 (33.33)	4 (66.66)
Public Health Laboratory of Al-Diwaniya	20	8 (40)	12 (60)
Total	80	21 (26.25)	59 (73.75)

X2 = 11.825*, P value = 0.066

***non-significant differences at p≤ 0.05**

The present research confirmed the results of a previous study by (16), which discovered that there was a high level of males compared to females but that this variation was not statistically significant. Moreover, according to another research, males were more likely than females to have HBs Ag (0.7%), but this variation was statistically insignificant (p=0.07). Despite the fact that there were far less women donors than there were men, the men to women ratio for HBs Ag positivity was 1.2, which is consistent with the results (3).

Males donate blood more frequently than females, according to a study by (4) that found the male to be hepatitis-infected (44.20%) and the female to be hepatitis-infected (16.6%). Males are more likely than females to be chronic hepatitis virus carriers in the majority of human societies. In response to infection, females are more likely than males to produce anti-hepatitis. Males are more likely to have diseases that have higher carrier frequencies (5).

According to documented data, there were 50 patients (62.5%) in rural areas and 30 patients (37.5%) in urban areas who tested positive for HBs Ag using the ELISA technique. However, there was no statistically significant difference between the two groups of patients (p value = 0.755), as shown in Table 5.

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Table 5: ELISA Screen Test for HBs Ag (positive) among (80) patients based on location.

Hospital or center name	Total	Positive cases and percentage	
		Urban	Rural
Teaching Hospital of AL Diwaniya	19	6 (31.57)	13(68.42)
Al Shamiyah General Hospital	6	3 (50)	3 (50)
Afak General Hospital	6	2 (33.33)	4 (66.66)
Al Hamzah General Hospital	10	4 (40)	6 (60)
Central Blood Bank	13	5 (38.46)	8 (61.53)
Haemodialysis	6	4 (66.66)	2 (33.33)
Public Health Laboratory of Al-Diwaniya	20	6 (30)	14 (70)
Total	80	30 (37.5)	50 (62.5)

X2 = 3.418*, P value = 0.755

non-significant differences at p≤ 0.05

The current findings are consistent with a prior study by (8), which found that there was no discernible difference between HBV donors in urban and rural locations in terms of their positive (P=0.755).

According to a study done by (11), metropolitan regions have a higher prevalence of the HBs Ag virus than rural areas do.

This study showed that (80) serologically positive samples which were tested by PCR technique for HBV- DNA. Thirty-three samples (41.25%) were positive for HBV- DNA, while forty-seven (58.75%) were negative (Table 6) (Figure 1**).**

Table 6: **Distribution of the detection of HBV DNA by PCR technique among (33) patients.**

Gender	No. of sample	No. of positive sample	%
Male	59	24	40.67

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Female	21	9	42.86
Total	80	33	41.25

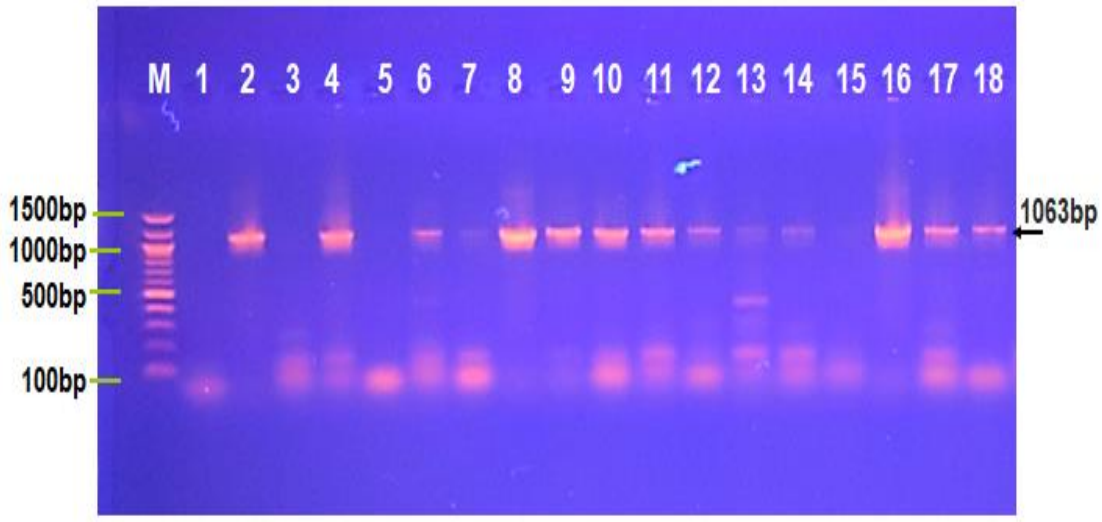

Figure (1): "Gel electrophoresis" of PCR assay product study for HBV from serum samples. M "Marker ladder 1500-100bp". Lane (1-18) positive HBV at 1063bp size.

The disparity between the screening ELISA test and PCR results does not refute the existence of HBV infection in these 47 patients. In order to validate the presence of an HBV infection, HBs Ag is currently considered to be the primary HBV serological sign (6, 7). Since the chronic HBV disease course is known to naturally progress through four phases, some of which are non-replicative (8 and 9) and some of which are HBs Ag positive, this may be explained by the occurrence of the so-called "non-replicative phase" in the disease. Furthermore, it is understood that patients in the non-replicative phase frequently undergo seroconversion from HBe Ag positive to HBe Ab positive. HBV DNA is merely undetectable in blood HBV DNA levels at this period, indicating an immune response to the infection (9, 10). different investigations conducted in different parts of Iraq concur with this finding. Similar findings (12 and 2) were made. The current investigation found no appreciable differences between patients who were male and female.

In this study it was found that (21, 63.63%) patients were from the rural and (12, 36.36%) patients were from the center of the city (Table 7).

Table 7: Distribution of HBV DNA by PCR technique based on location

Hospital or center name	Total	Positive cases and percentage	
		Urban	Rural
Teaching Hospital of AL Diwaniya	9	3 (33.33)	6 (66.66)

Al Shamiyah General Hospital	2	1 (50)	1 (50)
Afak General Hospital	3	1 (33.33)	2 (66.66)
Al Hamzah General Hospital	3	2 (66.66)	1 (33.33)
Central Blood Bank	4	1 (25)	3 (75)
Haemodialysis	1	1 (100)	0(0)
Public Health Laboratory of Al-Diwaniya	11	3 (27.27)	8 (72.72)
Total	33	12(36.36)	21 (63.63)

X^2= 3.765, P value = 0.708

Its values were p. value (0.708) with no locationally significant differences. Numerous studies conducted in Vietnam have indicated that the prevalence of HBV is higher in rural areas than it is in urban areas (13). Accordingly, the vast majority of our patients in the Dohuk research were from rural areas. And (14) conducted a study. They discovered that participants from rural areas were more likely to have HBV infection than those from urban areas (p> 0.05).In a different study (15), it was discovered that rural residents had a considerably greater frequency of HBV infection. This can be brought on by a dearth of immunization services and educational initiatives. Any educational program should focus on the regions where the virus is most common.

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