

Investigation of Hepatitis B virus, a species of *Hepadnaviridae* in Misan province

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Abstract:

Hepatitis B virus (HBV) is recognised as a serious and public health concern globally. The virus infects human in different ages and sex, and many countries and states have reported HBV infection. In Iraq, the researches revealed that the HBV is existed in all governorates as well as health-authorized establishments declared that many pateints infected with HBV. In this research, recent serological method that is Enzyme Linked immunosorbent Assay (ELISA) was facilitated for investigation of HBV infection in Misan governorate. Up to 149 blood specimens were taken from the patients in order to HBV diagnosis using HBSAG ELISA TEST KIT. The specimens were red by ELISA apparatus (BIOtek) and statically analysed by SPSS software in order to evaluate the incidence of HVB. The results showed that there are positive cases in both males and females^[1] with different ages. There are 17 (68.0%) samples were positive in males and 8 (32.0%) positive samples in females, the total samples were 149 samples. Further statistical analysis was used to calculate the incidence rate of HBV, which confirmed the infection rates were statistically significant. Therefore, the data of this present research support the previous finding, which confirmed the existence of the HBV in patients in Misan governorate and the infection rate were low similar with other cities.

Keywords: HBV, liver infection, ELISA, Misan city

Introduction

Hepadnaviridae is a family of DNA viruses that infect both humans and animals. Hepatitis B virus (HBV), is a species of this family, which is infect human, particularly the liver ^{1,2}. HB viruses distribute globally ³ and cause acute and chronic liver infection in human, in advanced causes HBV infection leads to liver failure and death due to cirrhosis and hepatocellular carcinoma ^{1,4,5}. HBV transmits in different ways including horizontal and vertical transmission such via blood transfusion, contaminated body fluids with virus ^{4,6}, as well as the virus can transmits to the fetus from his mother ⁷. In addition, the sexual transmission was reported ⁸⁻¹⁰ and the HBV infections were confirmed in health workers and drug takers ¹¹⁻¹³.

Pervious researches revealed that there is a high percent in morbidity and mortality rates of HBV infection globally. Many countries have recorded HVB infection including north and south America, Europe, Africa and Asia ^{3,14-16}. In addition, other researches stated that there are two billion of HBV infections in humans worldwide including around 292 million causes

are chronic infection¹⁷. In Iraq, previous studies reported^[17] that Hepatitis B virus (HBV) were identified in many Iraqi provinces^{18–21}. For instance, in 2022, there are 1,279 cases of hepatitis caused by HBV were reported in Iraq provinces, many of these cases are pregnant women. Some Iraqi provinces show high incidence rate including Al-Anbar, Al- Dewaniya and Babylon, while others show low incidence rate including Al-Muthanna, Thi-Qar and Misan²¹. Dispute vaccination and treatment availability that were described for prevention and recovery, HBV virus still consider a health threat around the globe²².

There are different techniques have been proposed to diagnose HBV including serological approaches. These diagnostic techniques are able to detect antibodies and virus-specific antigen in the blood, as these antibodies and antigens present as a result of virus infection. To illustrate, Enzyme Linked immunosorbent Assay (ELISA), which has been used widely in laboratories for diagnosis HBV using blood samples from patients^{23–25}. So, the major objective of this study is investigation of HBV in suspected patients in Misan city using ELISA technique in order to evaluate the incidence rate of HBV infection and for update pervious data related to HBV in Misan.

2. Material and methods

2.1 Elisa

The blood specimens were taken from patients attended Ministry of Health/ Central Health laboratory in Misan city from October 2023- to February 2024. During this period, 149 blood samples were accumulated, 101 male ages between (11 – 71) years and 48 female ages between (18-45) years. The present of HBV in the blood were investigated serologically by ELISA apparatus (BioTek) using HBSAG ELISA TEST KIT (Hightopbio) according to manufacture's protocol. In brief, the experiment was done in isolated condition to avoid any expected contamination, and then the sera were prepared from the patient's blood. Firstly, the reagents were reached at room temperature for 15 minutes, and the washing buffer was diluted at the rate of 1:40 with dH₂O. Secondly, three wells of each positive and negative control were provided on each plate with one well as a blank control. Forward, 20 µL of biotin in the corresponding well and 100 µL sample were mixed in this well. Afterwards, 100 µL of negative and 100 µL of positive controls were added in the controls wells. Next, the wells were sealed, shacked and incubated at 37 °C for one hour. After, the wells were washed five times with washing buffer for 20 seconds, then the plate over onto blotting paper to remove any remainders. Afterwards, 100 µL of conjugate was added and mixed gently, also the plate was sealed and incubated at 37 °C for 30 minutes and then washed five times with buffer for 20 seconds. Subsequently, 50 µL of each substrate A and substrate B were added and incubated at 37 °C for 30 minutes. At least, 50 ul of stopping solution were added to each well and mixed gently to be ready to read the result by ELISA.

2. Data analysis

The data were statistically analysed using SPSS V 28.0 software, as well as Microsoft excel software was utilised to generate tables and graphs.

3. Results

The goal of this research is for utilising serological technique, which is ELISA technique to

evaluate the incidence of Hepatitis B virus in Misan governorate. It can be seen from the data in Table 3.1 that there is a present of HBV in the serum of the patients. The finding revealed that 17 (16.8%) males were infected, 84 (83.2%) were negative of total 101 males cases ages between (11-17) years. Also, there are 8 (16.7%) females are infected, 40 (83.3%) were negative of 48 females cases in total ages between (18-65) years. It is apparent from the table below that the infection rate is similar between both males and females in 101 tested blood specimens, which that confirmed by statistical analysis, Chi-Square test, carried out by SPSS indicating that there are non statistical significant in infection between males and females as p value > 0.05 .

Table 3.1: Incidence of HBV for patients attended Central Health laboratory in Misan from October 2023 to February 2024.

Sex	Age (Year)	Variables	Cases		Total
			Positive	Negative	
Male	11-71	Count	17	84	101
		% within sex	16.8%	83.2%	100.0%
		% within infected	68.0%	67.7%	67.8%
		% of Total	11.4%	56.4%	67.8%
Female	18-65	Count	8	40	48
		% within sex	16.7%	83.3%	100.0%
		% within Infected	32.0%	32.3%	32.2%
		% of Total	5.4%	26.8%	32.2%
Total		Count	25	124	149
		% within Sex	16.8%	83.2%	100.0%
		% within Infected	100.0%	100.0%	100.0%
		% of Total	16.8%	83.2%	100.0%

*Chi-Square= 0.001, p value > 0.05 .

The present research was designed to determine the incidence rate of HBV infection in Misan city. These finding did not detect any evidence of increasing the numbers of HBV infection in Misan province, which is similar to previous data that were reported by other researchers^{21,26}. The results agreed with the other findings of previous studies, in which showed that the incidence rate of HBV in Iraqi governorates including Misan were low^{18,26}. Additionally, the results shown that there are non-differences in the infection ratios between males and females as shown in Figures 3.1. Therefore, this study supports other data of other studies about HBV infection in Iraq.

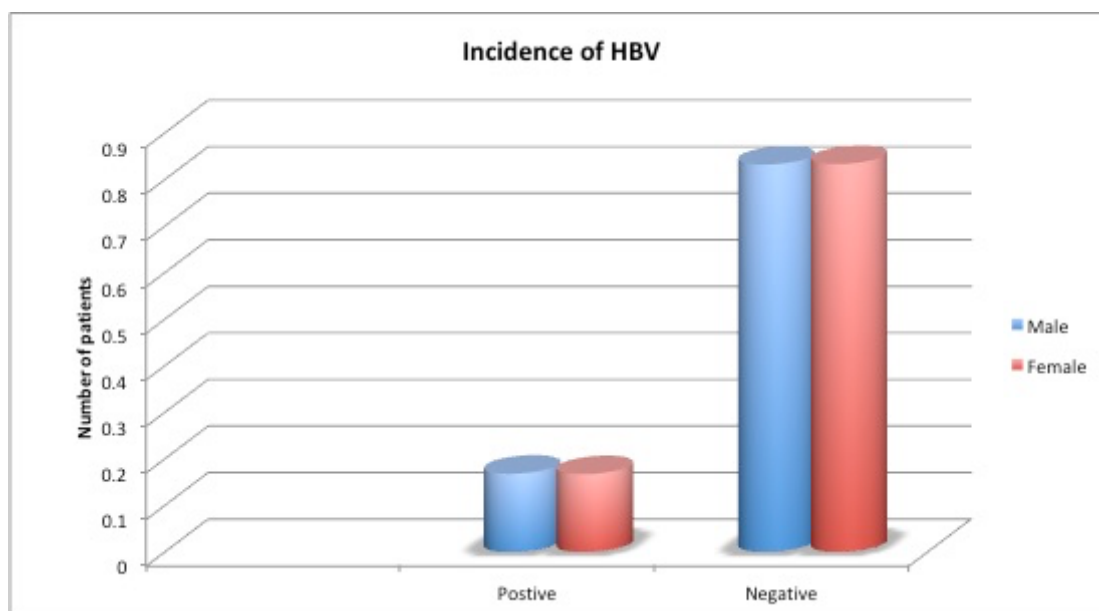


Figure 3.1: Statistical diagram of incidence of HBV infection. HBV was investigated by ELISA using blood specimens of males and females from October 2023 to February 2024.

4. Conclusion

The current study was designed to investigate HBV using serological experiment, particularly, diagnosis of HBV in blood of patients who attended the Central Health laboratory in Misan province. The findings of this investigation show that there is an incidence of HBV virus in this region. The results of this research express the infections rate were non statistical significant between males and females. In general, it seems that HBV is an endemic virus in Misan and other Iraqi governorates, as the reports mentioned that there are past HBV infection in these provinces ²¹, The current study has confirmed the pervious findings of Kadhemi et al., 2019 that found HBV infection in Misan. So, the Iraqi Ministry of Health and other health organizations in the country should aware the citizens in the media and social media about this virus and how to avoid to acquire infection. Further experimental investigations are needed for evaluating the incidence rate of HBV and test the existence of HBV in Misan governorate for better dealing with this virus.

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