Serological and Molecular Detection of Hepatitis B Virus Infection in Baghdad, Iraq

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ABSTRACT

Hepatitis B is infected liver and may cause epidemics in many parts of the world like Asia and Africa. Chronic liver disease is mainly caused by chronic hepatitis B infection, causing liver damage and fibrosis. The virus is transmitted by infected person blood or body fluids contact. About 400 blood samples were collected from Patients suspected infected with hepatitis B virus that attended the Middle East laboratory from Baghdad, Iraq during 2017. The age of patients ranged from 8 to 70 years old. Approximately 5ml of venous blood were collected and serum was separated. HBV markers, HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb were measured by ELISA and DNA was extracted for determination of viral load and genotypes, the viral load of HBV in serum specimens of the infected patients was detected by real time PCR using COBAS-TaqMan- HBV test using primers and probes targeting the highly conserved pre-core and core region. Total of 158 samples were found positive to HBV infection, fourteen of them gave viral load ≥ 10^7 and the rest 144 samples ranging from 2×10^2 to 2×10^4 IU/mL. The mean age of female patients was 37.17±6.4 in comparison with male age mean 33.3±9 year (ranging from 8 to 70 years old). Most patients were female 58.23%. The HBV infections rising markedly in female patients, and particularly among females in age group 26-34 (17.7%) while in male 22 (13.9%) followed by age group 35-43 (20.2%) of both genders. The resent study suggested that the history of HBV transmission was predictive for Operations (Opr), and Dentist (Dent) gain equal rates (32.91%) followed by blood transfusion (11.39%), Kidney dialysis (10.13%), and Caesarean delivery (7.59%). Hepatitis B virus infection by other means (Inf) and Cupping (Hijama) were the least. Most cases were positive for HBsAg (73.4%) and HBcAb (77.2%) with high percentage, while HBsAb and HBeAg were very low positive percentage (2.5, 6.3) respectively. About 73.4% of patients were found positive to HBsAg, HBcAb, and negative to HBsAb most patients (73.4%) were chronically infected with HBV and 17.7% are Susceptible. Most patients were chronically infected with HBV followed by more people susceptible for infection that needs to be considered for vaccination.

Keywords: HBV, ELISA, Seroprevalence, Transmission routs, Real Time PCR

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INTRODUCTION

Hepatitis B is infected liver and may cause epidemics in many parts of the world like Asia and Africa [1]. Hepatitis B virus, belongs to Hepadnaviridae family, 42nm in size consisting of double-stranded DNA. It is a complex and large virion having a diameter of 42nm and consists of a number of proteins. It is a positive sense single-stranded template that can be transcribed into a covalently closed circular DNA molecule. This DNA is called the pregenomic DNA and it is the template for the synthesis of viral messenger RNA. The viral genome contains the genes for the structural proteins (S, C, and X) and the viral enzymes. The viral RNA is translated into the viral envelope protein, which then is incorporated into the mature virion. The virion contains a nucleocapsid, the nucleocapsid is surrounded by a envelope and the envelope contains the viral surface proteins. The virus is transmitted by infected person blood or body fluids contact. About 400 blood samples were collected from Patients suspected infected with hepatitis B virus that attended the Middle East laboratory from Baghdad, Iraq during 2017. The age of patients ranged from 8 to 70 years old. Approximately 5ml of venous blood were collected and serum was separated. HBV markers, HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb were measured by ELISA and DNA was extracted for determination of viral load and genotypes, the viral load of HBV in serum specimens of the infected patients was detected by real time PCR using COBAS-TaqMan- HBV test using primers and probes targeting the highly conserved pre-core and core region. Total of 158 samples were found positive to HBV infection, fourteen of them gave viral load ≥ 10^7 and the rest 144 samples ranging from 2×10^2 to 2×10^4 IU/mL. The mean age of female patients was 37.17±6.4 in comparison with male age mean 33.3±9 year (ranging from 8 to 70 years old). Most patients were female 58.23%. The HBV infections rising markedly in female patients, and particularly among females in age group 26-34 (17.7%) while in male 22 (13.9%) followed by age group 35-43 (20.2%) of both genders. The resent study suggested that the history of HBV transmission was predictive for Operations (Opr), and Dentist (Dent) gain equal rates (32.91%) followed by blood transfusion (11.39%), Kidney dialysis (10.13%), and Caesarean delivery (7.59%). Hepatitis B virus infection by other means (Inf) and Cupping (Hijama) were the least. Most cases were positive for HBsAg (73.4%) and HBcAb (77.2%) with high percentage, while HBsAb and HBeAg were very low positive percentage (2.5, 6.3) respectively. About 73.4% of patients were found positive to HBsAg, HBcAb, and negative to HBsAb most patients (73.4%) were chronically infected with HBV and 17.7% are Susceptible. Most patients were chronically infected with HBV followed by more people susceptible for infection that needs to be considered for vaccination.

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DNA genome with core antigen (HBCag) surrounded by a surface antigen (HBsAg). Based on the antigenic epitopes presented on the viral envelope proteins it is divided into four major serotypes contain (adr, adw, ayw, ayr) serotypes, and according to the sequence variation it was divided into eight genotypes (A-H) [2,3]. Chronic liver disease is mainly caused by chronic hepatitis B infection, causing liver damage and fibrosis [4]. The virus is transmitted by infected person blood or body fluids contact. Chronic infection has been observed in 30% to 90% of children aged less than five years. Exposed adults become HBV chronic carriers in 2 to 5% of cases. Immune tolerant phase is the first phase of chronic hepatitis: It is HBeAg positive and high HBV replication. Immune Reactive Phase, This may occur after several years of immune tolerance in patients infected during adulthood. It is HBeAg positive and lower level of HBV replication [5,6]. Inactive HBV Carrier State where anti-HBe antibody positive and very low or undetectable serum HBV DNA. HBeAg-Negative Chronic Hepatitis B: HBeAg seroconversion and variable levels of HBV DNA [7]. Hepatitis B Surface Antigen (HBsAg) Negative Phase: Low HBV replication level with loss of HBsAg, low cirrhosis risk [8,9] about 240–400 million people with chronic HBV in China with annual mortality from HBV-related cirrhosis and hepatocellular carcinoma, exceeding 600,000 persons [10].

In Iraqi population HBV has a moderate endemicity with rate 3% [11]. However, there is an increased level of HBV infection among suspected outpatients with HBsAg positive [12]. The aim of current study is to determine markers of hepatitis B virus and the main transmission routes in hepatitis suspected patients in Baghdad province.

MATERIALS AND METHODS

About 400 blood samples were collected from patients suspected infected with hepatitis B virus that attended the Middle East laboratory from Baghdad, Iraq during 2017. Unrelated to the age and sex, they were questioned about dental visits, Operations, barber visits, blood transfusions, sexual multipartners tattoos, intravenous drug use, homosexual contact, living with an HBsAg-positive parent, Kidney dialysis, Caesarean delivery, and Cupping (Hijama). The age of patients ranged from 8 to 70 years old. Approximately 5ml of venous blood were collected and transferred into gel tubes, let to stand until coagulant formed then centrifuged at 3000rpm for 5 minutes. Serum was dispensed into seven Eppendorf tubes and stored at -20ºC until used.

Serological diagnosis

Hepatitis B markers, HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb were measured by ELISA kit (foresight, USA) and according to the manufacture instruction [13].

Molecular diagnosis

For determination of viral load and genotypes, high pure viral nucleic acid extraction kit (Roche, Germany), the viral load of HBV in serum specimens of the infected patients with HBV was detected by real time PCR using COBAS-TaqMan- HBV Test, v2.0 kit (Roche, Germany) using primers and probes targeting the highly conserved pre-core and core region detecting HBV genotypes A-H. Linear range 2x10^1 – 2x10^8IU/mL.

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Statistical analysis

Data were analysed using SPSS software program. Chi-square test was performed to explore the significance of different variables relationship; P value ≤ 0.05 was considered significant.

RESULTS AND DISCUSSION

In the recent study conducted in Baghdad province, out of 400 blood samples examined for hepatitis B markers serologically and molecular with quantitativereal-time PCR using the core region, 158 samples were found positive to HBV infection, fourteen of them gave viral load ≥ 10^7 and the rest 144 samples ranging from 2x10^2 to 2x10^4 IU/mL. The Ct value was considered significant >35 Ct. HBV is considered as variable DNA virus with high ability to induced mutations. The Roche kit, which is widely used for HBV DNA quantification around the world, uses one pair of primers and a single probe with good confident but kit targeting two region is more confidant and the probability of sensing low levels of DNA is greater [14].

The mean age of female patients was 37.17±6.4 in comparison with male age mean 33.3±9 year (ranging from 8 to 70 years old). Most patients were female 58.23% as shown in figure (1). Significant difference among age from 17 to 43 (P ≤ 0.05) and there is no biasedness towards the gender in HBV infection. The HBV infections rising markedly in female patients, and particularly among females in age group 26-34(17.7%) while in male 22(13.9%) followed by age group 35-43 (20.2%) of both genders (Table 1), which is contestant with Namibian study concluded that the HBV infection increase in female age group 15 - 39 years, and reaching the peak in the age group 30 - 34 years [15].

Iraq is one of the countries that have Gender equality, both genders has equal opportunities in education, employment and social life and therefore the high rate of female infection can be attributed to the rout of transmission due to frequent caesarean delivery and other gynecological operations. There is a variation from one community to another in the mode of transmission according to the health awareness, level of education, ethical precautions, preventive blood transfusion and sterilization in dental operations and social factors. Li et. al., (2012) concluded that the old age male patients who had history of surgical operations were related to high risk of chronic HBV infection in china. The main rout of HBV transmission were perinatal and sexual routs in HBV endemic area like Nigeria[17]. In Turkey, the risk factors for acquiring HBV infection represented by house hold and sexual contact with HBV positive individuals[18].
The resent study suggested that the history of HBV transmission was predictive for Operations (Opr), and Dentist (Dent) gain equal rates (32.91%) followed by blood transfusion (11.39%), Kidney dialysis (10.13%), and Caesareandelivery (7.59%). Hepatitis B virus infection by other means (Inf) and Cupping (Hijama) were the least. No patient declared multiple sexual relationships or homosexual activity therefore we put it under the other means (Inf) (Figure 2).
About 8.2% of general population of Duhok city in Kurdistan Region north of Iraq showed prior exposure to HBV infection and 1.14% were HBsAg carriers, and indicated that a previous history dental procedure was predictive for the transmission of HBV which is constant with our finding\textsuperscript{19}. Al-Rubayeet. al.(2016) study conducted Basra south of Iraq recruiting 69915 blood donors, about 0.2% of the donors were found positive for HBsAg and 2.1% were found positive for HBcAb. In 2018, estimation from the WHO reveal that about 325 million individual worldwide are suffering from chronic HBV or HCV infection, mostly from East Asia or sub-Saharan Africa\textsuperscript{21}.

Most cases were positive for HBsAg (73.4%) and HBcAb (77.2%) with high percentage, while HBsAb and HBeAg were very low positive percentage (2.5, 6.3) respectively as mentioned in Table (2). About 73.4% of patients were found positive to HBsAg, HBcAb, and negative to HBsAb most patients (73.4%) were chronically infected with HBV and 17.7% are Susceptible (Table 3).

The small research sample compared to the size of the Baghdad community, and limited only to subjects suspected of being hepatitis infected is likely to hide true relationship of the spread risk factor of hepatitis B virus and the rout of transmission in society.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
Test & HBsAg % & HBsAb % & HBeAg % & HBeAb % & HBcAb % \\
\hline
Positive & 73.4 & 2.5 & 6.3 & 62 & 77.2 \\
Negative & 26.6 & 97.5 & 93.7 & 38 & 22.8 \\
\hline
\end{tabular}
\caption{ELISA test results}
\end{table}
Table 3: Different identified phases of HBV infection [22]

<table>
<thead>
<tr>
<th>No. of patients (%)</th>
<th>Result</th>
<th>Interpretation</th>
</tr>
</thead>
</table>
| 116 (73.4)          | HBsAg  Positive  
HBcAb  Positive  
HBsAb Negative     | Active Chronic                   |
| 28 (17.7)           | HBsAg  Negative  
HBcAb  Negative  
HBsAb Negative     | Susceptible                      |
| 4 (2.5)             | HBsAg  Negative  
HBcAb  Positive  
HBsAb Positive      | Immune due natural infection     |
| 10 (6.3)            | HBsAg  Negative  
HBcAb  Positive  
HBsAb Negative      | Resolved infection or low level chronic infection (Occult) |

CONCLUSION

Most patients were female; however, there is no biasedness towards the gender in HBV infection with significant difference among age from 17 to 43. The HBV infections rising markedly in female patients, and particularly among females in age group 26-34 followed by age group 35-43 of both genders. The majority of infected patients had predictive history of HBV transmission either by Operations (Opr) or Dentist (Dent). Most patients were chronically infected with HBV followed by more people susceptible for infection that needs to be considered for vaccination.

REFERENCES


