Molecular and Serological Detection of Hepatitis B Virus (HBV) and its Relation to ABO Blood Groups in Thi-Qar Province/South Iraq

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Abstract— Hepatitis B Virus (HBV) infections are a significant public health problem all over the world. Among infectious disease, Hepatitis B virus is of great concern because of their prolonged viraemia and carrier or latent state. The present study aimed to determine the frequency of HBV among blood donors of Thi-Qar province and to evaluate the association of ABO blood type to HBV infections. The anti-HBc and HBs Ag technique was implemented to detect the presence of HBV, and the real-time PCR used to determinant HPV DNA in blood samples. The tube method used to detect blood group. This study was conducted on 200 serum samples from different age groups. The results showed that 3.5% of positive anti-HBc individuals, gave positive results for HBsAg by ELISA III technique and 5.5% of cases gave positive results for HBV DNA by real-time PCR. The ABO finding showed that the highest percentage of HBV infections were among patients of blood 97 (48.5%), followed by blood group B 68(34%) and blood group A 42(21%), while the lowest percentage was among patients of blood group AB 3(1.5%). The most important points concluded from the current study, that is the low infection percentage of HBV in Thi-Qar province (5.5%). And the ABO blood type may have an important role in HBV infection.

Keywords— HBV, ELISA, real-time PCR, ABO, transfusion transmitted infection

I. INTRODUCTION
Hepatitis B virus (HBV) is the highest frequency detected reason for chronic liver disease all over the world. HBV is categorized as a DNA virus that is spread in essence through blood transfusion and sexual contact (Keefe, 2006; Sabri, 2018). Liver disease due to HBV has turned into a large trouble generally (Kumar et al., 2004). It is expected that overall 2 billion persons have been infected with HBV and in excess of 350 million have chronic liver disease (WHO, 2018). The superficial of erythrocytes has diverse polysaccharides and proteins called blood group antigens. Incompletely 700 red blood cell antigens are found and part of them that are recognized by each other qualified into 33 blood assembly by the International Society of Blood Transfusion (TBTC), such as ABO and Rh groups are the most imperious (Garratty et al., 2002; Anonymous, 2012). Numerous studies have been prepared to decide association between contagious diseases and blood groups. Some blood groups can act as a receptor and ligand for microorganisms. “The possible pathogenesis for this defenselessness is that the same number of creatures that may bond to polysaccharide on cells and solvent blood gather antigens may obstruct this authoritative” (Gerald and Douglas, 2000; Marion and Christine, 2014). The present study was carried out to determine the frequency of HBV among blood donors of Thi-Qar province and to assess the association of blood group type to the positivity for HBV infections.

II. SUBJECTS AND METHODS
A. Patients:
A total of 200 (positive anti-HBc) individuals referred to the central blood bank at Thi-Qar province. These patients were diagnosed at virology and the venereal lab of central blood bank. The study population included males only, from different age groups.

B. Serological test:
Serological markers of HBc antibody (anti-HBc) and HBs-Ag (anti-HBs) were tested by using commercial enzyme linked immunosorbent assay (ELISA) kit (BioElisa, Spain) according to the instructions of the manufacturer (Gerald and Douglas, 2000).

C. Molecular test:
DNA extraction: Viral DNA was extracted from blood samples (Frozen Blood) by using DNA extraction kit (Geneaid/USA), and done according to company instructions.
D. Estimation the Concentration and Purity of viral DNA

The estimation of DNA concentration (ng/µl) and its purity by reading the absorbance at (260/280 nm) was done by utilizing Nanodrop spectrophotometer (THERMO/USA).

E. Detection the HBV by AccuPower® HBV Quantitative PCR Kit

The AccuPower® HBV Quantitative PCR Kit (Bioneer/Korea) was used for detection of presence of HBV in blood samples.

F. ABO blood group detection

The blood group type was determined by using the tube method, one drop of anti-A was added in clean, labeled test tube, drop of anti-B was added into another clean, labeled test tube. One drop of 2-5% tested RBCs suspension was added to each tube, the content of the tubes was mixed gently and centrifuged for 15-30 seconds, the RBCs bottoms was re-suspended and examined for agglutination (Marion and Christine, 2014).

III. RESULTS

A. Finding of anti-HBsAg

The present study revealed that out of 200 positive anti-HBc individuals, from different regions of Thi-Qar province, only 7 (3.5%) cases of those, gave positive results for HBsAg by ELISA III technique as shown in fig. (1).

B. Real-time PCR Detection of HBV

The real-time PCR results showed, that out of 200 positive anti-HBc individuals, 11 (5.5%) cases gave positive results for HBV, as viewed in fig. (2).

C. Comparison between HBsAg and Real-time PCR HBV DNA results

There is a difference in HBsAg by ELISA III technique results and the real-time PCR results, where HBsAg results were 7 (3.5%), while the real-time PCR results 11 (5.5%), out of out of 200 positive anti-HBc individuals, gave positive results of HBV infection, as viewed in fig. (3).

Fig. 3: Comparison between HBsAg and HBV Real-time PCR results.

D. Association Between Positivity of anti-HBc infection and Blood Group

The current study showed that the highest percentage of HBV infections were among patients of blood O 97 (48.5%), followed by blood group B 68(34%) and blood group A 42(21%), while the lowest percentage was among patients of blood group AB 3(1.5%), as shown in table (1).

Table (1): Percentage of anti-HBc infections, according to Blood Group.

<table>
<thead>
<tr>
<th>Blood group</th>
<th>A</th>
<th>B</th>
<th>AB</th>
<th>O</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of infections</td>
<td>42</td>
<td>68</td>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>Percentage %</td>
<td>21%</td>
<td>34%</td>
<td>1.5%</td>
<td>48.5%</td>
</tr>
</tbody>
</table>

IV. DISCUSSION

Hepatitis B is potentially fatal liver infections, which caused by the HBV viruses. It's considered as a major international health problem. Its can effect a chronic infection and makes people at high-risk of death from cirrhosis and liver cancer (Babu et al., 2015). The HBsAg was the predominant markers in patients with chronic Hepatitis B virus. The present study showed that out of 200 positive anti-HBc individuals, only 7 (3.5%) cases were positive results for HBsAg, This mode (when the anti-HBc is positive and the HBsAg is negative) can be explained through immune due to natural infection or through (HBsAg) negative phase: low viral load of HBV may still arise in the liver after the loss of HBsAg, and the HBsAg loss is related with progress in outcome with a lowered risk of cirrhosis, Decompensation, and Hepatocellular carcinoma, and may be to (False-positive anti-HBc), thus susceptible (Al-Joudi et al., 2014; Babu et al., 2015).
The observing of HBV DNA in blood samples, has become a significant tool to detect individuals with high viral replication, to observe patients on therapy, and to predict whether antiviral therapy is effective. The study results revealed that out of 200 positive anti-HBc individuals, 11 (5.5%) cases were positive results for HBV by using the real-time PCR technique. This shows that 94.5% of positive anti-HBc has been cured during the acute infection or that due to false-positive anti-HBc, and that 5.5% of positive anti-HBc become ongoing infection (Mendy et al., 2006; Samiee et al., 2019).

The difference in HBsAg results and the real-time PCR results may be due that the low level of virus in chronic infection can be detected by the real-time PCR technique, and cant distengeshid by HBsAg immunological assay.

Numerous studies have tried to explain the association between transmissions of infection with ABO blood groups. There are facts that people with O blood group and positive Rh are more susceptible to blood borne infections (Sathe et al., 1973). Several studies have attempted to know the relationship of blood groups with HBV infection. The current study showed that the highest percentage of HBV infections were among patients of blood O, followed by blood group B and blood group A, while the lowest percentage was among patients of blood group AB. This finding agreement with Aljooani et al. (2012), Mohammadeli and Pourfathollah, (2014), and with Babu et al., (2015), who showed a significant association between blood group of donors and hepatitis B virus infections. And disagreement with Anwer et al., (2011) and Pourhassan, (2014), who showed no significant association between blood group of and HBV virus infections.

V. CONCLUSION

The most important points concluded from the current study, that is the low infection percentage of HBV in Thi-Qar province (5.5%). And the ABO blood type may have an important role in HBV infection.

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REFERENCES


