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Serological Evaluation Of Hepatitis B Virus And Correlation Of Infection To Viral Load

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Abstract
Background*: Chronic hepatitis is a viral disease of serious complications which lead to increasing the rate of mortality.*
Aim*: Serological diagnosis of Hepatitis B surface antigen (HBsAg), antibody to Hepatitis B surface antigen (anti-HBs), Hepatitis B core Ab (anti-HBc) IgM, Hepatitis B core Ab (anti-HBc) IgG, Hepatitis B e antigen (HBeAg), and Hepatitis B e antibody (anti-HBe). The optimum serological works up in correlation to viral load and state of chronicity.*
Materials and methods*: The current study was carried out on 217 Iraqi patients with hepatitis B residing in Al-Qadisiyah province during February (2020) to April (2021). Information about age and gender of each participant were obtained and blood samples were with draw from all participants.*
Results*: The most frequently detected markers were by far hepatitis B surface antigen (HBsAg) which was seen in 90.3 % of all patients and there was no significant difference between males and females. Hepatitis B core antigen (HBcAg) was seen in 2 patients while Hepatitis B core antibody (HBcAb) was seen in the majority of patients (86.6%). A significant difference in the core markers between males and females was not reported. Hepatitis B e antigen (HBcAg) was seen in 5 patients, 4 males and a single female, while Hepatitis B e antibody (HBcAb) was seen in the majority of patients (82.0%). The range of viral load was extremely wide from as low as 0.01 to as high as 469000000 and the median and the inter-quartile range were 38.5 and 1450.05, respectively and there was no significant difference in viral load level between males and females.*
Conclusion*: Hepatitis B virus serological workup is a complicated investigation aiming at not only diagnosis of infection but also for characterizations of the infection as an acute one, chronic one or cure state and the identification of viral load can provide a picture about functional recovery and plan of treatment.*
Keywords*: Chronic hepatitis, HBcAb, HBcAg, Al-Qadisiyah, Iraq*

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I. Introduction

Hepatitis is inflammation of the liver tissue that is attributed to a wide range of causative agents; however, viral etiology is one of the most frequent and very well known causative agents associated with both clinically acute and chronic viral hepatitis (1-3). Viruses that cause liver inflammation can be hepatotropic (4) and non-hepatotropic (5). Non-hepatotropic viruses are responsible for systemic viral illnesses that are accompanied by liver involvement such as Epstein-Bar virus, Cytomegalovirus and Herpes simplex virus (5), while hepatotropic viruses cause predominantly liver inflammation with significant liver damage and a variety of clinical and biochemical abnormalities (4). Hepatotropic viruses include hepatitis A, B, C, D and E viruses which vary substantially with respect to rout of entry, incubation period, proportion of acute presentation, ability to cause continuous chronic inflammatory state, association with liver cirrhosis and predisposing to hepatocellular carcinoma (6-10). From clinical point of view most episodes of viral hepatitis are acute and self liming, nevertheless, hepatitis B, C and E viruses have the potential to causes chronic states (11).

Chronic hepatitis can lead to serious complications such as cirrhosis and carcinoma (12). The rate of mortality in association with viral hepatitis and its associated complications is estimated to be around1.4 million per year globally and most of these are attributed to hepatitis B and C viruses (13).

With respect to Hepatitis B virus, it belongs to *Hepadnaviridae* virus family and it is a DNA enveloped small virus. It has the ability to cause chronic liver inflammation, liver cirrhosis and malignant liver neoplasm. Hepatitis B viral infection in human beings is known to be dated back to at least 500 years (14), but the discovery of the virus was established in 1966 (15) and its identification using electron microscopy was done by Dane et al at 1970 (16). Since that time, health institutes and substantial medical research have been directed toward establishing an efficient antiviral agent and immunization program aiming at limiting virus spread among population and reducing its clinical sequelae in already infected individuals (17-21). Despite these efforts, hepatitis B virus is still a major public health threat. Actually, worldwide there are about 257 million

people suffering chronic viral hepatitis B and about 887,000 die annually because of its associated complications (22-23).

The transmission of hepatitis B virus is through exposure of mucosal surfacesto infected body fluids or via dermal inoculation. There is very rare possibility of oral-fecal transmission. The typical incubation period of HBV infection is between 30 and 180 days. Recovery is common; however, a small proportion of infected individuals can progress to a chronic state, in which the serological detection of HBsAg remains for more than six months (24). For purpose of diagnosis and evaluation the following serological tests are often indicated: Hepatitis B surface antigen (HBsAg), antibody to Hepatitis B surface antigen (anti-HBs), Hepatitis B core Ab (anti-HBc) IgM, Hepatitis B core Ab (anti-HBc) IgG, Hepatitis B e antigen (HBeAg), and Hepatitis B e antibody (anti-HBe), (25).

In the current study, a sample of Iraqi patients with Hepatitis B viral infection were evaluated for these serological markers in order to figure out, the optimum serological work up in correlation to viral load and state of chronicity.

II. Materials and methods

The current study was carried out on 217 Iraqi patients with hepatitis B viral infection residing in Al-Qadisiyah province, mid-Euphrates region of Iraq. The study extended from February 2020 through April 2021. Information about age and gender of each participant were obtained and blood samples were with draw from all participants in order to perform the following serological tests: HBSag, HBcAb, HBcAg, HBeAb and HBeAg.

The approval of the study was obtained from the ethical approval committee of the medial college in the province and formal agreement was issued by local health directorate. Verbal consent was obtained from all patients after thorough illustration of the goals and the techniques of the present research project.

Data were transferred into an SPSS spread sheet (statistical package for social science) the software belongs to IBM, Chicago, USA (version 16). Qualitative data were presented in the form of counts and proportions, whereas, quantitative data were presented in the form of range, median and inter-quartile range (IQR). Mann Whitney U test was used to compare mean Ranks between any two groups. Chi-square test was used to evaluate differences in proportions. The level of significance was considered at $p \leq 0.05$ (26).

III. Results

The present study included a total of 217 patients with confirmed hepatitis B virus infection, 114 (52.5 %) males and 103 (47.5 %) females with a male to female ratio of 1.12. The mean age of all patients was 44.89 ±17.66 years and it ranged from 5-86 years. The mean age of male patients was 47.11 ±17.46 years and that of female patients was 42.44 ±17.64 years. The age range of males was 10-86 years and that of female patients was 5 -85 years (Table 1).

The objective of the current study was to evaluate the positive detection rate of various hepatitis B virus serological markers and to correlate their positivity with the clinical situation of patients. The positive detection rates of these serological markers are shown in table 2 and a comparison was made between males and females. The most frequently detected markers was by far hepatitis B surface antigen (HBsAg) which was seen in 196 (90.3%) of all patients and there was no significant difference between males and females ($p = 0.163$).

Hepatitis B core antigen (HBcAg) was seen in 2 patients while Hepatitis B core antibody (HBcAb) was seen in the majority of patients, 188 (86.6 %). A significant difference in the core markers between males and females was not reported ($p > 0.05$).

Hepatitis B e antigen (HBcAg) was seen in 5 patients, 4 males and a single female, while Hepatitis B e antibody (HBcAb) was seen in the majority of patients, 178 (82.0%). A significant difference in the e markers between males and females was not reported ($p > 0.05$).

Viral load in patients with hepatitis B virus infection is shown in table 3. The range of viral load was extremely wide from as low as 0.01 to as high as 469000000 and the median and the inter-quartile range were 38.5 and 1450.05, respectively and there was no significant difference in viral load level between males and females ($p = 0.153$).

Table 1: Comparison of mean age between males and females

Characteristic	**Total** ***n*= 217**	**Male** ***n* = 114**	**Female** ***n* = 103**	***p***
Age (years)				
Mean ±SD	44.89±17.66	47.11±17.46	42.44±17.64	0.051 I NS
Range	5 -86	10 -86	5 -85	

n: number of cases; SD: standard deviation; I: independent samples *t*-test; NS: not significant at $p > 0.05$

Table 2: The rate of positive detection of hepatitis B virus serum antigens and antibodies

Characteristic	**Total** ***n*** **= 217**		**Male** ***n*** **= 114**		**Female** ***n*** **= 103**		***p***
	n	%	*n*	%	*n*	%	
HBSag	196	90.3	106	93.0	90	87.4	0.163
HBcAg	2	0.9	1	0.9	1	1.0	1.000Y
HBcAb	188	86.6	101	88.6	87	84.5	0.372
HBeAg	5	2.3	4	3.5	1	1.0	0.429 Y
HBeAb	178	82.0	96	84.2	82	79.6	0.378

HBSag: Hepatitis B surface antigen; HBcAg: Hepatitis B core antigen; HBcAb: Hepatitis B core antibody; HBeAg: Hepatitis B e antigen; HBeAg: Hepatitis B e antibody

Table 3: Viral load in patients with hepatitis B virus infection

Viral Load HBSag	**Total** ***n*** **= 217**	**Male** ***n*** **= 114**	**Female** ***n*** **= 103**	***p***
Median (IQR)	38.5 (1450.05)	29.80 (1056.70)	55.80 (1923.72)	0.153 M NS
Range	0.01 -469000000	0.01 -16500000	0.01 -469000000	

IQR: inter-quartile range; M: Mann Whitney U test; NS: not significant at $p > 0.05$

IV. Discussion

Worldwide, the majority of the short term and long term liver disorders are attributed to HBV (27). In one previous study, 72.4% of the patients with liver disease were infected with chronic hepatitis B virus (28) and in another previous report; chronic hepatitis B virus was responsible for 63.4% of cases of liver disorders (29). On the basis of HBsAg prevalence rate the endemicity of HBV is classified into three groups. A prevalence rate of 8% is found in highly endemic regions, a rate ranging from 2 % to 7 % is found in regions with intermediate endemicity and a rate of 0.5 up to 2 % is seen in areas with low endemicity (30).

In our study, the proportion of males was higher than males and this finding is similar to that reported by Prabina *et al* (28). In our study, anti-hepatitis B core antibody was detected in 86.6 % and the majority of them were also positive for hepatitis B surface antigen and the appearance of anti-hepatitis B core antibody usually follow the appearance of HBsAg within a short period (31) and in tow previous reports the prevalence rate of anti-HBc antibody was reported in almost all infected patients (28, 31, 32). In our study, HBsAg was reported in a higher frequency in males than in females; however, the difference was statistically insignificant. In the study of Prabina *et al* (28) the frequency of males with positive HBsAg was higher than that of females and this finding was also reported by several previous authors (33-35). The HBeAg in our study was seen in a minority of patients (2.3 % out of total) and the presence of such antigen indicates high contagious state as it indicates higher replicative potential. In previous reports however, the rate of seropositivity to HBeAg is higher than 50 % (28). In our study, none of patients was positive for anti-HB antibody and this was similar to the findings of previous authors (28, 36). Indeed, seronegativity to this anti-body is an indication of difficulty of recovery (36).

The recovery from acute hepatitis is when HBsAg disappears and anti-HBsAg antibodies and anti-HBc immunoglobulin G (IgG) appear in addition to normalization of liver enzymes levels (37, 38). However, it has been reported that in spite of disappearance of markers of the infection, cDNA remains in the nucleus of the hepatocyte as a minichromosome or an episome from which RNA and this DNA are produced to initiate replication viral (39). The identification of hepatitis B e antigen and antibody is essential since it provides guidelines for treating chronic infections (40). In our study, Hepatitis B e antigen was detected in a minority of patients whereas, anti-hepatitis e antibody was discovered in the majority of patients.

Viral load in our study was highly variable and this is related to the state of the patient whether acutely infected or chronically infected (40). The identification of viral load is essential for both functional recovery characterization and for deciding when to treat and how to treat (40). The problem of Hepatitis B viral infection in our community is common and therefore identifying risk factors in order to avoid infection will limit the rate of viral transmission in the community.

V. Conclusion

Hepatitis B virus serological workup is a complicated investigation aiming at not only diagnosis of infection but also for characterizations of the infection as an acute one, chronic one or cure state and the identification of viral load can provide a picture about functional recovery and plan of treatment.

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