

# Prevalence of Hepatitis B Virus Markers amongst Patients in Mosul-Iraq

<sup>1</sup>Ali A. Dawood, <sup>2</sup>Eman Y. Thanoon, <sup>3</sup>Fadya F. Mohammed

<sup>1</sup>Ph.D. Microbiology, Dept. of Medical Biology, College of Medicine, University of Mosul

<sup>2</sup>Ph.D. Microbiology, Lab. of Bacteriology, Ibn –Al-Atheer Hospital, Mosul

<sup>3</sup>B.Sc. Microbiology, Lab. of Virology, Ibn –Al-Atheer Hospital, Mosul

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**Abstract:** Hepatitis is a disease caused by several types of viruses that infect the liver cells, in particular, leading to morbidity and mortality. Hepatitis B can prevalent in all parts of the world which affects both sexes and all ages and global statistics show that three-quarters of the world are infected with this disease in the period of their lives. **Objectives:** the aim of this study is to compare HBV markers taken from patients in Mosul. **Methods:** We evaluate 152 patients presumably with HBV in acute and chronic cases whom have HBsAg positive using ELISA. One step multi HBV markers test device (Immunochromatography) was used in this study. **Results:** By using ELISA for detecting HBsAg, the numbers and percentages of individuals who gave positive and negative results were 116(76.31%), 36(23.68%) respectively. Results by using HBV markers were: positive and negative results for HBsAg were 116(76.31%), 36 (23.68%), positive results for anti-HBs were 16(10.52%), negative 136(89.47%) with the mean (1.89), positive results for HBeAg were 4(2.63%) and 148(97.36%) negative with the mean (1.9) while for anti-HBe were 86(56.57%) positive, 66(43.42%) negative with the mean (1.43), number and percentage of positive, negative, mean, and SE results for anti-HBc total were 118(77.63%), 34(22.36%), (1.22), and ( 0.048). **Conclusions:** Using ELISA for detection HBsAg with the other markers could be a verification and significant for detection disease. Nowadays, These methods are applied in the clinical practice.

**Keywords:** HBV, ELISA, HBsAg.

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## 1. INTRODUCTION

Hepatitis B virus is a serious global public health problem. The World Health Organization (WHO) has estimated that over 350 million people worldwide are chronically infected with HBV[3]. This infection can be transmitted through sexual intercourse, parenteral contact or vertical transmission (mother-to-child), and blood transfusion. Severely cases of HBV can lead to chronic liver disease, including cirrhosis and hepatocellular carcinoma [4]. Acute disease typically occurs in the infected adolescents or adult who have not been vaccinated. This acute presentation can be life threatening due to massive liver damage from the host immune reactions [14]. Chronic HBV(CHB) infection can be define as the presence of hepatitis B surface antigen (HBsAg) in the serum of an infected individuals for at least six months or as the presence of HBsAg in a patient who is negative for immunoglobulin M antibodies to the hepatitis B core antigen (anti-HBc) [8]. The prevalence of HBV infection varies in different parts of the world, with most of the disease burden occurring in Asia and Africa[14]. HBV is the smallest DNA virus with 3200 base pairs, which contains four overlapping genes encoding the viral envelope (S and pre- S), nucleocapsid (Precore and Core), polymerase with reverse transcriptase enzyme and X proteins[19]. Previously, HBV genotypes have been classified into eight genotypes (A-H) and because of genome diversity is a hallmark of HBV virus allowed its classification into 10 genotypes (A-J)[11,15].

## 2. MATERIALS AND METHODS

This study was done among populations who were infected(patients) or under suspicion infected persons with hepatitis B virus in Mosul city and its suburbia. A total of 152 serum samples were collected from patients with hepatitis B virus. The samples were collected from January 2014 to November 2014. A (152) of serum samples were enrolled in this study: 88

males (57.89 %), 64 females (42.11%) and their ages ranges between (1.5-72) year old. Percentage of single patients was (55%) and for married was (45%). A (114) of samples collected from Central Public Health Laboratory, in Mosul and (38) samples from Hemodialysis patients in Ibn– Sina Teaching Hospital. The serum was separated and stored in multiple marked clean tubes at (-20 ° C) for both ELISA and for HBV markers test.

**Bioelisa HBsAg Assay kit:** Bioelisa is a direct immunoenzymatic method for detecting HBsAg. A 100 µl of each sample was transferred to wells. After incubation at 37C, and washing step, a 100 µl of diluted conjugate was transferred into each well, then, a 100 µl of substrate-TMB solution was added to each well with more incubation. After adding 100 µl of stopping solution, reading the absorbance of each well in Beckman mode using a 620 - 630 nm.

**Kit for Multi –HBV Markers Test Device:** One step multi HBV test Device which detects 5 markers of HBV (HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc). This kit consists of 5 chromatographic strips. Each strip detects a certain HBV marker. A 60µl of serum sample was added to each well and simultaneously timing start. The results were recorded after 15 minutes. We used SPSS software version 16 for statistical analysis in this study.

### 3. RESULTS

The results for detecting HBsAg with ELISA kit, numbers and percentages of individuals who gave positive and negative results for HBsAg are 116(76.31%), 36(23.68%) respectively. HBV markers were used in this study are anti-HBs, HBeAg, anti-HBe, anti-HBc as well as HBsAg. Recently, these markers are usually used to identify HBV infection in labs. This study integrated all these markers numbers, percentages, mean, SD and SE are illustrated in Table (1).

Table (1): Numbers and percentages of the prevalence of HBV markers investigated in this study.

Marker	Positive No. (%)	Negative No. (%)	Mean±SD	SE
HBsAg	116(76.31%)	36(23.68%)	1.24±0.42	0.049
Anti-HBs	16(10.52%)	136(89.47%)	1.89±0.3	0.035
HBeAg	4(2.63%)	148(97.36%)	1.9±0.16	0.018
Anti-HBe	86(56.57%)	66(43.42%)	1.43±0.49	0.057
Anti-HBc	118(77.63%)	34(22.36%)	1.22±0.41	0.048
Total	152(100%)			

According to distribution of HBV markers with groups of infection cases and genders are showed in table (2).

Table (2): Distribution of HBV markers with numbers of cases infection groups, genders and percentages.

Case group	%	Male	Female	HBsAg	Anti-HBs	HBeAg	Anti-HBe	Anti-HBc
Inactive HBsAg Carrier	51.31	44	34	+	-	-	+	+
Chronic	15.86	16	8	+	-	-	-	+
Acute	2.62	2	2	+	-	+	-	+
Incubation	5.25	4	4	+	-	-	-	-
Past infection, recovery	6.56	6	4	-	-	-	+	+
Non-infected	7.88	8	4	-	-	-	-	-
Vaccinated	9.21	6	8	-	+	-	-	-
Unusual marker	1.31	2	0	+	+	-	+	+

#### 4. DISCUSSION

ELISA assay is a common technique used in the medical labs., which is currently used in the most public health institutions. HBsAg is the main diagnostic marker used for screening blood products in hospitals and health care facilities. This test is carried out for the patients who are intended for operations which includes detection HBV, HCV and HIV. Although, HBsAg is the surface envelop protein of HBV, this protein is not an infectious agent but existing of this protein in blood refers to presence of the virus in the body. HBsAg is the first virologic marker detectable in the serum within (1–12) weeks [6,9] usually between week (4) and week (10) in acute infection while in chronic HBV infection, the HBsAg persistence for six months [7,8,16]. In this study, the numbers and percentages of individuals who gave positive and negative results for HBsAg are 116(76.31%), 36(23.68%) respectively. The negative results do not interpret that these persons are not infected. Recently, most physicians reliant to diagnose HBV infection clinically and laboratorial by detecting HBsAg for the first inspection because HBsAg is the major marker can be easily determination. Sometimes, the number of HBsAg is diminish or insufficient to identify HBV virus under acute , chronic or occult infection therefore, other markers must be taken under observation.

Anti-HBs is the protective antibody. It is an immunoglobulin secreted by plasma cells against HBsAg for neutralizing antigen. Anti-HBs becomes detectable in serum after HBsAg disappears and remains detectable indefinitely thereafter. Anti-HBs can be easily detected after giving vaccination. Anti-HBs can exist in blood for a long time and gradually decreases with age. lately, in this study the number of patients who gave positive results for anti-HBs were 16(10.52%), negative 136(89.47%) with the mean (1.89) with significant  $P < 0.01$ . (2) out of (16) anti-HBs positive obtained positive for HBsAg while remaining (14) obtained negative for HBsAg. This will be explained when will be given a commentary types of infected cases through markers. HBeAg is a protein produced during active viral replication and may act as an immunogen or a tolerogen, leading to persistent infection [7]. HBeAg appears concurrently with or shortly after HBsAg. HBeAg can be detected after total degradation of HBcAg which residues short time in the serum. It can be detected on average (6) to (12) weeks after exposure [9,24]. HBeAg seroconversion is usually followed by normalization of serum transaminases to anti-HBe and improvement of liver histology. Thus, HBeAg seroconversion usually represents a transition from chronic hepatitis B to an inactive HBsAg carrier state [10]. Anti-HBe is not a protective antibody which produce after seroconversion of HBeAg. In this study, the numbers of patients who gave positive results for HBeAg were 4(2.63%) and 148(97.36%) negative with the mean (1.9) while for anti-HBe were 86(56.57%) positive, 66(43.42%) negative with the mean (1.43) with significant  $P < 0.01$ . Prevalence studies were done in Iraq showed as an example: HBeAg and anti-HBe among chronic groups were (36%, 58%) respectively but among the carrier group were (2%, 94%) respectively [1]. Salim [26](2012) recorded for HBeAg and anti-HBe positive (26% and 29%) while negative (26% and 23%) respectively. HBcAg is not found in serum therefore, there is no available commercially kit to detect it but it is available in hepatocytes. For this reason it can be available to detect the antibody of HBcAg (anti-HBc) only in serum and its presence may refer to the replication of HBV in hepatocyte [17]. Although IgM anti-HBc antibodies typically decline to undetectable levels within (6) months, the IgG class (IgG anti-HBc) persists indefinitely as a marker of past HBV infection [18,21]. The high level of IgM-specific anti-HBc is frequently detected at the onset of clinical illness, because such antibody is directed against the (27) nm internal core component of HBV. Anti-HBc is considered as the best serological marker of acute HBV infection as well as anti-HBc IgM marker of activity of the disease, which is usually increased in acute phase and falls to a low titer or undetectable level after 6 months, but may become detectable again during reactivation of infection, whereas anti-HBc total present in acute and chronic hepatitis B infection [5]. In this study, we used anti-HBc total for detection. IgG anti-HBc remains positive for life following exposure to HBV, also it persists for many years, although, unlike anti-HBs and anti-HBe are not protective antibody [25]. Recently, the number and percentage of positive, negative, mean, and SE results of anti-HBc total were 118(77.63%), 34(22.36%), (1.22), and (0.048) respectively. A study was done in Mosul showed that positive results for anti-HBc were 89 (49.2%), negative 6 (3.3%) and non-infection 86 (47.5%) [27]. This study concurs with other studies done in the world. In this study, we found that there is a correlation significant between HBeAg and anti-HBs while we didn't find correlations significant between other markers and variables.

Present study showed the vast majority of patients were inactive HBsAg carrier group(51.31%,  $P < 0.05$ ). Inactive carrier form is the largest group in chronic HBV infected patients which is around (300) million people are inactive carriers [20]. Chronic cases came next in percentage which were (15.86%) due to persistent of HBsAg and anti-HBc for several weeks.

Furthermore, the percentage of HBsAg negative through three cases each one of them can be considered separately as 14(9.21%) vaccinated persons, 10 (6.56%) recovery or past infected persons and 12(7.88%) non-infected persons. In fact some persons who are HBsAg positive will develop detectable anti-HBs; however, these persons are still considered infectious due to the presence of HBsAg [23]. 8 patients with only positive for HBsAg are considered as incubation period cases with or without presence of a symptomatic disease. 2 patients of interpreted unusual marker has positive result for all markers except HBeAg. Here, antigens and their antibodies presence in the same patient at the same time. Furthermore, unusual markers which existed in patients in a way that are not identified and can be readily classified as an acute or chronic cases. Repeat testing of the same sample or possibly of an additional sample is advisable when tests yield discordant or unusual results [13, 22].

## 5. CONCLUSION

Using ELISA for detection HBsAg with the other markers could be a verification and significant for detection disease. Seroprevalence of anti-HBc between genders and estimated a considerable in prevalence of HBeAg and between genders. In this study, we found that there is a correlation significant between HBeAg and anti-HBs while we didn't find correlations significant between other markers and variables.

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