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Detection Subtype (AYW) of Hepatitis B Virus amongst Patients in Mosul-Iraq

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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 detect subtype of hepatitis B virus among patients in Mosul. Methods: We evaluate 21 patients presumably with HBV in acute and chronic cases whom have HBs Ag positive. Subtype of hepatitis B was determinated using enzyme immunoassay EIA. Results: A 20 (95.2%) out of 21 of serum samples showed the subtype (ayw) while the last one showed negative result. Conclusion: The standardized EIA assay is a rapid and accurate method for detection and differentiation of subtypes of hepatitis B virus. This method can be applied in the clinical practice.**

Keywords: **AYW, HBs Ag positive, HBV in acute, Detection Subtype.**

1. INTRODUCTION

The World Health Organization (WHO) has estimated that over 350 million people worldwide are chronically infected with HBV(3). Hepatitis B virus can be transmitted through sexual intercourse, parenteral contact or vertical transmission (mother-to-child), blood transfusion. Sever of HBV can lead to chronic liver disease, including cirrhosis and hepatocellular carcinoma (4). Acute hepatitis typically occurs in the infected adolescents or adult who have not been vaccinated (8). Chronic HBV (CHB) infection can be define as the presence of hepatitis B surface antigen (HBsAg) in serum of an infected individuals for at least six months or as the presence of HBsAg in a patient who is negative for IgM antibodies to the hepatitis B core antigen (anti-HBc)(6). The prevalence of HBV infection varies in different parts of the world, with most of the disease burden occurring in Asia and Africa(8). HBV is the smallest DNA virus with 3200 base pairs, which contains four overlapping genes encoding the viral envelope (S and pre S), necleocapsid (Precore and Core), polymerase with reverse transcriptase enzyme and X proteins (11). The clinical relevance of such genotype is yet unclear. However, because the HBV-induced disease is the resultant of virus-host interaction, the disease characteristics may be influenced by the genotypes of the virus. Interaction between hepatocyte genome and HBV genome may also vary according to the prevalent HBV genotype (1). 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)(9,7). The major classification of HBV subtype is classified into 4 subtypes or serotypes (adr, adw, ayr, and ayw) (5,14). The molecular basis for this classification was variation at few sites in the S region. The a determinant (amino acid 124-148) is the major antigenic determinant common and confers protection against all serotypes (1,8),while the d/y and w/r variations depend on Lys/Arg substitutions at residue 122 and 160 respectively (2). If the amino acid at position 122 is Arg (122R) then the subtype is y, and if it is Lys (122K) then the subtype is d. Similarly, 160R defines the r subtype and 160K defines the w subtype of HBV subtype is classified into 4 subtypes or serotypes (adr, adw, ayr, and ayw) (5,15). The molecular basis for this classification was variation at few sites in the S region. The a determinant (amino acid 124-148) is the major antigenic determinant common and confers protection against all serotypes (1,8),while the d/y and w/r variations depend on Lys/Arg substitutions at residue 122 and 160 respectively (2). If the amino acid at position 122 is Arg (122R) then the subtype is y, and if it is Lys (122K) then the subtype is d. Similarly, 160R defines the r subtype and

160K defines the w subtype. The four possible combinations define the major subtypes and additional amino acids contribute to immunogenicity. These subtypes can be further classified into 9 serotypes (: adw2, adw4q-, adrq+, adrq-, ayw1, ayw2, ayw3, ayw4 and ayr according to sequence analysis. Epidemiologic studies found that the prevalence of these serotypes varies in different parts of the world. To date, there has been very little data on the clinical significance of HBV serotypes (5,17). While the ability to detect HBsAg was of obvious importance for the safety of the blood supply, serotyping was useful for epidemiological studies, including studies of nosocomial and iatrogenic infections and intra-familial transmission (10). The genetic differences between HBV serotypes are not constricted the S gene. They may found all over the HBV genome, with the highest variations within the Pre-S region. There is no stringent correlation between the phenotypic HBsAg markers and sequence variation outside the S gene, gene, but such a correlation between genetic and phenotypic markers is not required for epidemiological studies(16). These subtypes have been widely employed in clinical, virological and epidemiological studies (14).

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 21 serum samples were collected from patients with hepatitis B virus. The serum was separated and stored in multiple marked clean tubes at(-20º C) for both ELISA and EIA assays. Detect HBsAg by ELISA: Bioelisa is a direct immunoenzymatic method used in this study. A 100 µl of each sample and controls are transferred to the corresponding well and incubate for 60 minutes at 37°C. Washing steps were done then, a 100 µl of diluted conjugate was transferred into each well of the microplate, except the blank well an incubated for 30 minutes at 37°C.More washing steps were done and a 100 µl of substrate-TMB solution was added to each well, including the blank. Microplate was incubated for 30 minutes at (20°C) then a 100 µl of stopping solution was added and the result read at 450 nm. After a second incubation and subsequent washing, an enzyme substrate containing a chromogen is added. The substrate will develop a blue colour if the sample is positive for HBsAg. The blue colour changes to yellow after blocking the reaction with sulphuric acid. The intensity of the colour is proportional to the amount of HBsAg in the test specimens.

Determination of Subtype: Enzyme Immuno Assay EIA based hepatitis B surface antigen subtyping kits with monoclonal antibodies were used in this study came from (Institute of Immunology Co., Japan). This kit has been developed as a reagent for research purposes to detect respective subtypic determinants d, y, w, and r in hepatitis B surface antigen (HBsAg) positive samples for identification of HBsAg subtypes such as adw, adr, ayw and ayr. Detection of subtypic determinants is based on the solid-phase sandwich EIA. A 96 wells of the microplate are coated with monoclonal antibody against the common determinant a of HBsAg. HBsAg in samples dispensed to wells is caught on the solid phase and their subtypic determinant, d, y, w, or r, is detected by peroxidase-labeled monoclonal antibody against corresponding determinant. According to the manufacturer's instruction, the 1st reaction started by adding 50µl of serum (positive result for HBsAg) and +ve control to wells, incubated for 3 hrs at 37ºC and washing step 5 times. After taped the plate, a 50µl of enzyme labeled against determinant d, y, r or w w. For each type of determinant, the labeled monoclonal antibody was added. Second reaction started with incubation for 2 hrs at 37ºC and 3 times washing step then, a 100µl of enzyme substrate was added in all wells and incubated in dark place at 15 ~30ºC for 30 min. Finally, a 100µl of reaction stopper was added to all wells.

Statistical analysis: All wells were measured by absorbance reader (Beckman Coulter) under (main wavelength 450 nm and sub wavelength 620 nm). The positive and negative determination was measured by: The absorbance of the Negative Control + 0.2.

Negative: Absorbance of samples < Cut-off value
Positive: Absorbance of samples ≥ Cut-off value.

3. RESULTS

The first ELISA assay has shown that all 20 (95.2%) samples outcame positive for HBsAg. The second test for detection subtype using EIA test had shown that 20 (95.2%) of HBsAg related to subtype (ayw) while the last one got negative result for any determinant detection because of negative result for HBsAg. The EIA reader table below shows the cut-off value for 21 sample, blank, positive and negative controls:

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Table 1: EIA Beckman coulter reader shows the cut-off value.NC: Negative control, PC: Positive control, S: Sample, (d,y,r,w): Determinants of HBsAg

BECKMAN COULTER
AD340 S
Quick Mode:

Date : 2/24/14 12:21

Date of Evaluation : 2/24/14 12:21
Page : 1

Legend OD
Status

Test-Information
Filter : 450 nm / 620 nm

	d	y	r	w		d	y	r	w	d	y	r	w	
Blank	0.020 OK	0.017 OK	0.018 OK	0.01 OK	S6	0.974 OK	3.608 OK	0.043 OK	0.702 OK	0.089 OK	4.000 Overflow	0.030 OK	3.902 OK	S14
NC	0.020 OK	0.061 OK	0.332 OK	0.02 OK	S7	0.230 OK	4.000 Overflow	0.042 OK	2.225 OK	0.042 OK	4.000 Overflow	0.039 OK	1.595 OK	S15
PC	2.133 OK	2.190 OK	1.972 OK	1.70 OK	S8	0.043 OK	4.000 Overflow	0.042 OK	3.559 OK	0.037 OK	4.000 Overflow	0.178 OK	1.764 OK	S16
S1	0.070 OK	4.000 Overflow	0.029 OK	2.54 OK	S9	0.043 OK	4.000 Overflow	0.031 OK	1.359 OK	0.031 OK	4.000 Overflow	0.157 OK	0.742 OK	S17
S2	0.030 OK	4.000 Overflow	0.052 OK	0.33 OK	S10	0.051 OK	4.000 Overflow	0.037 OK	2.543 OK	0.172 OK	4.000 Overflow	0.043 OK	2.969 OK	S18
S3	0.022 OK	4.000 Overflow	0.035 OK	3.36 OK	S11	0.047 OK	4.000 Overflow	0.031 OK	1.104 OK	0.028 OK	0.022 OK	0.387 OK	2.695 OK	S19
S4	0.043 OK	4.000 Overflow	0.030 OK	2.98 OK	S12	0.025 OK	4.000 Overflow	0.029 OK	2.773 OK	0.022 OK	4.000 Overflow	0.053 OK	0.989 OK	S20
S5	0.034 OK	0.997 OK	0.029 OK	0.09 OK	S13	0.027 OK	4.000 Overflow	0.034 OK	3.714 OK	0.025 OK	0.125 OK	0.024 OK	0.045 OK	S21

4. DISCUSSION

Serotyping of HBV was used for epidemiological purposes, or to predict disease progression (3). Studying serotypes is important for several reasons, first, apart from its apparent value in elucidating the specificity of sero diagnosis of HBV. Second, it provides an opportunity to use previous information on the world-wide distribution on HBs Ag subtypes as background information for directing future research with regard to HBV molecular epidemiology based on S gene sequencing (13). It has been estimated that the ayw serotype occurs in all genotypes except in genotype C while the serotype adw has been associated with all genotypes except D and E (1). According to subtype studies by institute of immunology showed that the anti-r and anti-y is higher while that of anti-d and anti-w is lower. Therefore, determinants d and w in samples with low HBsAg titers may not be efficiently determinant. In this study, we found that only ayw subtype is the predominant serotype in Mosul city and its suburbia. However, the genotype D and subtype ayw are the main types in Iran, Turkey and Saudi Arabia, it has been showed 2% of total cases is ayr subtype in Iran which is related to the genotype C (12). Sample number 6 and 19 have shown detection d and r determinants respectively as a positive result but they have outcome negative result because the cut-off value of sample 6 showed over than other determinants while in sample 19 gave mismatch between determinants y and r. The highest proportion of injuries for hepatitis B recorded in the 15-45 year age group (94% for both), and this corresponds with the numbers and percentages of registered globally and which refers to the increased incidence of the disease with the nimber injuries for hepatitis B recorded in the 15-45 year age group (94% for both), and this corresponds with the numbers and percentages of registered globally and which refers to the increased incidence of the disease with the numbers and percentages of registered globally and which refers to the increased incidence of the disease with age as a result of constant exposure to the disease. Also in this study, we have demonstrated that there is no correlation between type of sex and type subtype of HBV which is related to genotype D. Moreover, this study confirmed the correlation between increase numbers of positive HBsAg with the number of population so that the infected individuals hepatitis B virus in Mosul city is higher than in its suburbia.

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5. CONCLUSION

This study has registered for the first time in Iraq for detecting the subtype of HBV and the standardized EIA assay is a rapid and accurate method for detection and differentiation of subtype of hepatitis B virus. This method can be applied in the clinical practice.

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