Hepatitis B virus genotype among Acute and chronic hepatitis B virus infection in Ninavah Governorate/ Iraq

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Abstract

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Back ground:

There are eight genotypes of Hepatitis B virus (A-H) and subgenotypes are recognized

Methods: A total of 100 patients with Hepatitis B surface Antigen positive, were enrolled in this study, they were classified into 25 patients with acute hepatitis, 75 patients with chronic hepatitis, we used Elisa technique to detect HBSAg (plasmatic U SA) HBV genotypes were determined using INNO- LipA methodology which based one the reversed hybridization principle.

Results:

This study showed that genotype D constituted 100% of the total infections among Aucte and chronic hepatitis.

Conclusion:

we found that genotype D was main and frequent HBV genotype in Ninavah Governorate north Iraq.

Introduction:

There are eight genotypes of hepatitis B virus (HBV) designated A to H based on greater than 8% nucleotide variation over the entire genome()

.The eight genotypes of HBV show a distinct geographical distribution, The prevalent genotypes in Asia are genotypes B and C () studies of the Asian patients revealed that compared to genotype B, genotype C is associated with higher prevalence of hepatitis B e antigen (HBeAg), higher serum level of HBV DNA, higher histological activities, more sever acute exacerbation, alower rate of spontaneous HBeAg seroconversion, and a higher rate of cirrhosis and hepatocellular carcinoma (). Hepatitis B virus genotyping may evolve from a research tool into being an essential clinical diagnostic test, very much as HCV genotyping did, one hurdle in the introduction of HBV genotyping to clinical practice is the lack of simple, rapid, and accurate test (). Currently, HBV genotypes can be determined by several methods, including direct sequencing ().Restriction fragment length polymorphism analysis () line- probe assay ().Ligase chain reaction assay and enzyme-linked immunosorbent assay for genotype-specific epitopes () Direct sequencing is the most accurate and permits detection of the common as well as uncommon mutations but is also the most expensive and tedious development of rapid, simple and standardized assays that can detect all know genotypes can accelerate progress in research on the clinical significance of HBV genotypes.

Aims of study:

To investigate the frequency of HBV genotypes in Iraqi patients by line probe assay.

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Patients and Methods:

Between March 2011 until January 2012, a total of 100 patients with hepatitis B surface antigen (HBsAg) positivity were enrolled in this study, those patient were classified into: 25 patients with acute hepatitis (AH) diagnosed by HBsAg, HBC-IgM, 75 patients with chronic active hepatitis (CAH) characterized by presence of HBsAg with increased alanine aminotransferase (ALT) level for more than 6 months. HBV was diagnosed depending on clinical data, liver function tests done by (Chemwel l, Uk), HBV serum markers done by ELISA technique (plasmatic Uk) and HBV DNA by real time PCR (one step RT- PCR using qtower, Germany).

- DNA extraction
- HBV DNA extraction test were performed using instant virus DNA kit (analytic jena, Germany).200 µl lysis solution TLS,
- 200µl of serum and 25µl proteinase k had been add, mix vigorously by pulsed vortexing for 10 second, incubate at 50C for 15 minutes.
- 400µl binding solution TBS had been add to the lysed sample, mix by vortexing several times.
- Apply the sample to the spin filter located in a 2.0µl receiver tube. Close the cap and centrifuge at 10.000xg for 1 minute. Discard the receiver tube with the filtrate, place the spin filter into a new 2.0 ml receiver tube.
- Open the spin filter and add 500 µl washing solution HS, close the cap and centrifuge at 10,000xg for I minute, discard the receiver tube with filtrate. Place the spin filter in to a new 2.0ml reciver tube.
- Open the spin filter and add 650µl washing solution MS, close the cap and centrifuge at 10.000xg for 1 minute. Discard the receiver tube with the filtrate. Place the spin filter in to a new 20.ml receiver tube.
- Centrifuge at max speed for 2 minutes to remove all traces of ethanol. Discard the 2.0 ml receiver tube.
- Place the spin filter into a 1.5 ml elution tube. And open the cap of the spin filter and add 100 µl pre-heat elution buffer (50C).
- Incubate at room temperature for 2 minutes. Centrifuge at 6,000xg for 1 minute.

Real time RCR tests.

- Quantitative of HBV DNA.

HBV DNA quantitation test were preformed using HBV DNA (primer design kit) and used real times PCR detection (9 tower for analytic gene company, Germany).

- Prepare a reaction mix according to the table below

Component	Volume
2x precision TM Master Mix	5 µl
pathogen primes probe Mix	0.5 µl
Eternal extraction control primer/probe Mix	0.5 µl
RNAse/DNA free water	1.5 µl
Final volume	7.5 µl

- Pipette 7.5 µl of this mix into each well.
- Pipette 2.5 µl of DNA template and standard in to each well and negative control for negative control weels use 2.5 µl of RNA se/DNA free water.
- Preparation of standard curve dilution series.
- Pipette 900 µl of RNA se/DNAse free water into 5 tube and lable 2-6.
- Pipette 100 µl of positive control template in to tube 2. Vortex thoroughly.
- Chang pipette tip and pipette 100 µl from tube 2 into tube 3.
- Vortex thoroughly
- Repeat steps 4 and 5 to complete the dilution series.

Amplification protocol

	Step	Time	Tempreture
	Enzyme activation	10 mints	95 °C
50 cycles	Denaturation	5 sc	95°C
	Data Collection	25 sc	60°C

Interpretation results according the instruction manual.

Lip A amplification:

Hepatitis B virus genotyping was performed for all PCR- positive samples by a reverse hybridization line probe assay (INNO- Lip A HBV Genotyping assay, Innogenetics Nv, Ghent, Belgium), The extracted DNA was amplified by nested PCR according to the instructions of the manufacturer (Innogenetics) for amplification of the HBSAg region to provide a biotinylated product, The HBV genomic region amplified extends from nucleotides 456 to 798 for the nested inner primers. These procedures in brief includes initial denaturation of the biotinylated PCR products [0]

Which were then incubated with a test strip for hybridization of the denatured amplicon to genotype specific probes immobilized as a parallel lines on each strip,following hybridization, the strips were stringently washed and incubated with streptavidin conjugated to allow color development the biotinylated DNA bound to the strip.

Statistical analysis:

Analysis of data was carried out with the aid of SPSS package version 10.0 software (Chicago, Illions, USA). Parameters were compared using the chi- square test. P values less than 0.05 were considered statistically significant.

Results:

A total of 100 patients with a meanage 39.5± 11.75 years, including 30% females and 70% males, were enrolled in this study. Genotype detection by hybridization of the PCR products to the kit membrane strips was performed as a describe above. A representation of membrane strip with all the immobilized control and genotype specific oligonucleotide band is shown in figure 1. For all genotypes except genotype G, several reactive bands can indicate a specific genotype. Interpretation of the test strips was relatively straight forward. However, in certain cases faint bands appeared, and these made interpretation of the genotype unclear, 1 red marker line, 2 control lines, and 14 parallel probe lines. The conjugate control line is a control for the color development reaction and the amplification control line contains universal HBV probes to check for the presence of amplified HB genomic material.

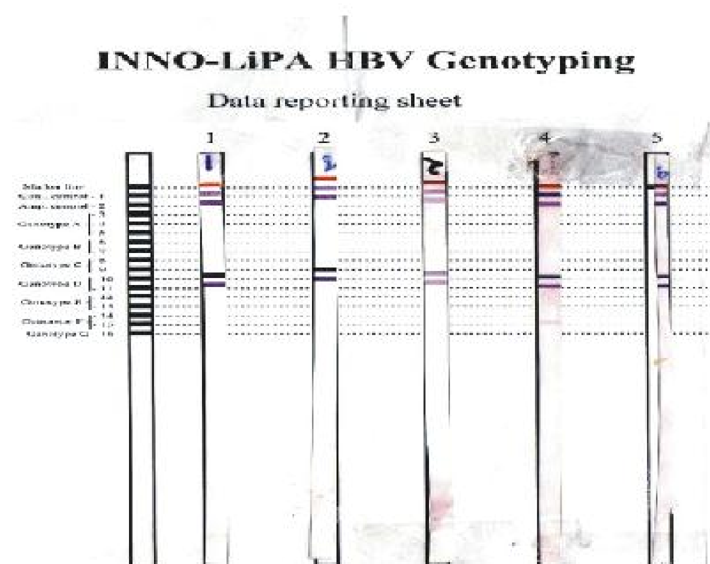

Figure 1. Bands representing oligonucleotide probes specific for HBV genotype in the studied group

Distribution of HBV genotypes:

This study showed (figure 1 and table 1) HBV infection in hepatitis patients are attributed predominantly to viral genotype D Constituted 100% of the total infection No HBV genotypes A, B, C, E or G were found in our study.

Table(1): HBV genotype among acute and chronic Hepatitis B virus infection

Patients	Number	HBV genotype								
		A	B	C	D	E	F	G	H	Mixed genotype
Acute Hepatitis B virus	2s	0	0	0	25(100%)	0	0	0	0	0
Chronic Hepatitis B virus	75	0	0	0	75(100%)	0	0	0	0	0

Discussion:

In this study, we focused on studying of HBV genotype. According to obtained results of our research genotype D were detected as the dominant genotype in our region (Ninavah Governorate north of Iraq).HBV has been classified into 8 genotype (A-H), that show a distinctive geographical distribution [0], Because of HBV is an etiologic agent of acute and chronic disease through the world [0]. And also its genotypes might influence mutation patterns in precore and core promoter region, severity and activity of liver disease, pattern of serological reactivity replication of the virus, prognosis and response to antiviral treatment [0]. Detection of HBV genotype is very important to clarify the pathogenesis, rout of infection and virulence of the virus, in our country data about HBV are very limited, and because in some cases, differences in the distribution of the genotypes can be found within a single country[11]

. HBV genotype should be determined in different district of each country. There is no information for this in Ninavah Governorate. Thus in presented study, we focused on detection of HBV genotypes in Ninavah Governorate, that located in north Iraq.In our study obtained results for genotype D are similar to other reports from Iran and Other contries of Midle East area. For example, in Iran genotype D was the only detected HBV genotype in 109 patients that were used for genotype by Inno- Lip A methodology[14].Also in other study by using type- specific primers method, done in shiraz province (in south west Iran) revealed HBV genotype D was the only detectable genotype in different in different clinical form of HBV infections in patients with acute, inactive HBsAg or chronic hepatitis [0] There is also other study, that carried out by Amini*etal*., reveald that all 26 chronic hepatitis B Iranian patients were HBV genotype D carriers [0]. In study was jone in Saudi Arabia, by nested PCR- mediated amplification of the target sequence and hybridization with sequence- specific oligonucleotides method, showed that 57 patients of 70 were genotype D [0]. There is also a study in Egypt showed HBV- D genotype was dominant in this region[0]. In addition one study by phylogenetic analysis that genotype D was predominant genotype [0]

Also similar study in India determined genotype D as the predominant genotypes of HB. In one study in Pakistan, genotype A, B and C.Reported as the predominant genotype and genotype D was rare [0]. In Turkey, a study showed all study patients were infected by genotype D[0]

.Recommendation:

This study can open the way for doing other studies such as understanding of antiviral drugs effects on the

patients affected with HBV. Otherwise in another research, we can study and detect the HBV subgenotypes and serotypes, and by this we can help to survey main and effective antiviral drug to cure the affected patients.

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