Prevalence of HBV Genotypes in Egypt among Hepatitis Patients

Iman A.El Aziz Khaled [*1], Ola M. Mahmoud[1], Abeya F.Saleh[1], and Emad A. Baioumi[2]

[1]Haematology &Blood Bank, [2]Tropical Medicine, Theodor Bilharz Research Institute (TBRI).Cairo, Egypt
[*]iman_khaled@yahoo.com

Abstract: Phylogenetic analysis has led to the classification of hepatitis B virus into eight genotypes, designated A to H. The genotypes have differences in biological properties and show heterogeneity in their global distribution. These attributes of the genotypes may account not only for differences in the prevalence of hepatitis B virus mutants in various geographic regions, but also makes them responsible for differences in the clinical outcome and response to antiviral treatment in different population groups. Africa is one of the highly endemic regions of HBV with five genotypes (A-E) identified. Almost all patients in the Mediterranean area are infected with genotype D. However, there is little information of genotype distribution in Egypt. A total of 140 Egyptian patients with hepatitis B surface antigen (HBsAg) positive were enrolled in this study. Of the 140 patients, only100 patients were HBV DNA positive and only these were included in the study. They were classified in to 20 patients with acute hepatitis (AH), 75 patients with chronic active hepatitis (CAH) and 5 patients with hepatocellular carcinoma (HCC)]. HBV genotypes were determined using INNO-LiPA methodology which is based on the reversed hybridization principle. Results: This study showed that genotype D constituted 87% of the total infections (75% CAH, 7% AH & 5% HCC). The other 13% showed mixed infections of D/F. Conclusion: These findings show that the most prevalent genotype in Egypt is genotype D especially in CAH and HCC patients while the mixed type D/F is mostly encountered in AH. [Journal of American Science. 2010;6(11):185-190]. (ISSN: 1545-1003).

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1. Introduction

It is estimated that 350 million individuals are chronically infected with hepatitis B virus (HBV) and that more than 1 million die from cirrhosis and hepatocellular carcinoma (HCC) each year [1-3]. Approximately 5-10% of infected adults and 80-90% of children become chronic carriers of HBV [4]. HBV has been classified into eight genotypes (A-H) based on the sequence divergence of > 8% in the entire genome, which consists of about 3200 base pairs [5-7]. Different HBV genotypes have distinct geographical distributions. Genotype A is found mainly in Northwest Europe, the United States, India, and Sub-Saharan Africa. Genotypes B and C prevail in East Asia, while genotype D is common in the Mediterranean countries. Genotype E is only found in Africa and genotype F is found mainly in Central and South America. The distribution of HBV genotypes G and H still needs to be determined [8]. Africa is one of the highly endemic regions of HBV with five genotypes (A-E) identified: genotype A in Kenya [9], genotype D in Tunisia [10], genotype (A-D) in South Africa [11] and genotype E in Nigeria [12]. Apart from these reports, however, there is little information of genotype distribution in Egypt despite the importance of HBV infection in this region of Africa. According to Egyptian studies [13, 14], the prevalence of HBsAg in Egypt is of intermediate endemicity (2–8%). Nearly 2-3 million Egyptians are chronic carriers of HBV. Structural and functional differences between genotypes can influence the severity, course and likelihood of complications and hepatitis Be antigen (HBeAg) seroconversion. In addition, HBV genotypes may be associated with differences in response to antiviral therapy. Some studies indicate that HBV genotypes respond differently to interferon in patients with chronic hepatitis B [15].

Several technologies have been developed for genotyping of HBV. Including direct sequencing [16], restriction fragment length polymorphism analysis [17], line probe assay [18], PCR using type specific primers [19], colorimetric point mutation assay [20], ligase chain reaction assay [21] and enzyme linked immunosorbent assay for genotype-specific epitopes [22]. Our aim in this study was to use the line probe assay (INNO-LiPA HBV Genotyping assay; Innogenetics N.V., Ghent, Belgium) to detect the most prevalent genotypes of HBV among Egyptian hepatitis patients

2. Patients and Methods

The study was approved by the ethical committee of Theodor Bilharz Research Institute (TBRI) (No 52) and informed consents were obtained from patients participating in this study. A total of 140 patients with hepatitis B surface antigen (HBsAg) positivity were enrolled in this

study. Of the 140 patients, only 100 patients were HBV DNA positive and those were included in our study and classified into: 20 patients with active hepatitis (AH) diagnosed by HBsAg and 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, and 5 patients with hepatocellular carcinoma (HCC) diagnosed by ultrasonography. HBV was diagnosed depending on clinical data, liver function tests done by (Hitachi 902), HBV serum markers done by ELISA technique (Abbott AxSYM® HBsAg Assay) and HBV DNA by real time PCR (Two step RT-PCR using Applied Biosystem). Patients were excluded if they were co-infected with hepatitis C virus (HCV) or human immunodeficiency virus (HIV).

DNA Extraction

The QIAamp DNA extraction kit (QIAGEN GmbH) was used for DNA extraction from serum samples according to the manual. The extracted DNA was used for amplification in the LiPA procedures. LiPA analysis was performed within approximately 5 days following DNA extraction. If DNA extracts were not used immediately, they were stored at -20°C.

LiPA amplification and detection

HBV genotyping was performed for all PCR-positive samples by a reverse hybridization line probe assay (INNO-LiPA HBV Genotyping assay; Innogenetics NV, Ghent, Belgium [available for research use only, not for use in diagnostic procedures]) [23]. 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 415 to 824 for the outer primers and nucleotides 456 to 798 for the nested inner primers (the numbering is based on the sequence with GenBank accession number AY128092). These procedures in brief includes initial denaturation of the biotinylated PCR products [24], which were then incubated with a test strip for hybridization of the denatured amplicon to genotype-specific probes immobilized as parallel lines on each strip. Following hybridization, the strips were stringently washed and incubated with a streptavidin conjugate to allow color development from 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.

3. Results

A total of 100 patients with a mean age of 37.17 ± 11.75 years, including 25% females and 75% males, were enrolled in this HBV genotype study. Genotype detection by hybridization of the PCR products to the kit membrane strips was performed as described above. A representation of the membrane strip with all the immobilized control and genotype-specific oligonucleotide bands 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 straightforward; however, in certain cases faint bands appeared, and these made interpretation of the genotype unclear. The INNO-LiPA HBV genotyping strip contains 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 HBV genomic material.

Distribution of HBV genotypes

This study showed that HBV infections in hepatitis patients are attributed predominantly to viral genotype D constituted 87% of the total infections. In addition, there was a relatively high prevalence of mixed infections (D/F) represented 13% among the studied group. No HBV genotypes A, B, C, E or G were found in our study (Figure 1,2). The Association between liver disease and the prevalence of HBV genotypes was as following: Genotype D was found significantly more often in patients with CAH and HCC than in patients with AH [75/75(100%), (5/5(100%) v (7/20 (35%)]. Mixed infection (D/F) was only found in AH group [13/20 (65%)] (Figure 3).

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

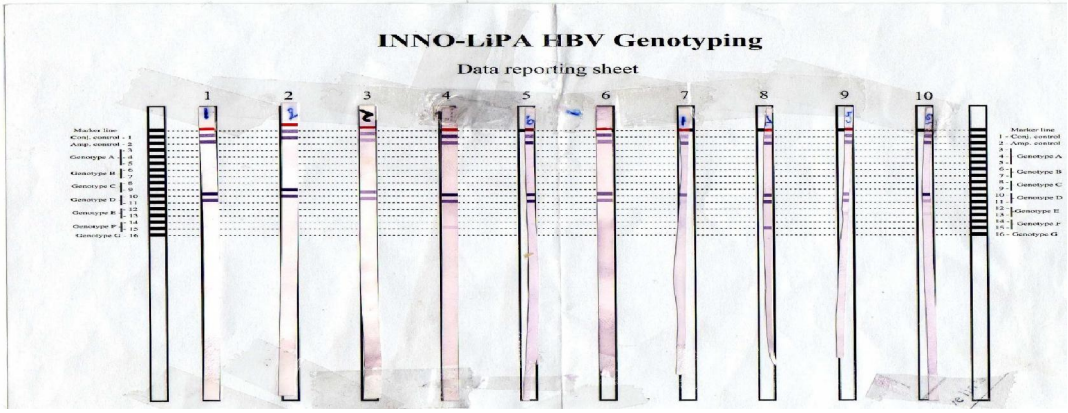
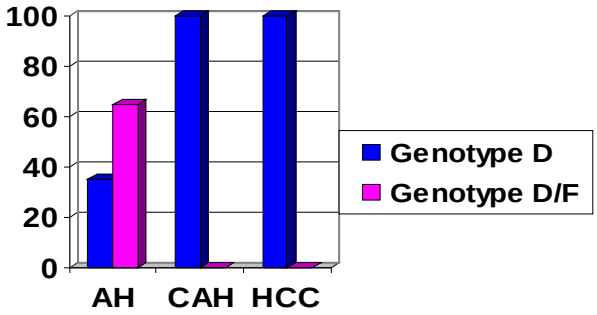


Figure 2. HBV genotype distribution in the studied group.

Figure 3. The Association between liver disease and the prevalence of HBV genotypes.



4. Discussion

Hepatitis B virus (HBV) infection is a global health problem with a continuously increasing burden in developing countries like Egypt. A greater demand for genotyping of patient strains of HBV is growing as specific clinical associations with each genotype becomes increasingly apparent [25-27]. Presently, based on an intergroup divergence of 8% or more in the complete nucleotide sequence, HBV can be classified into eight genotypes A-H, and different HBV genotypes are dominant in various parts of the world [8].Thus, it is imperative to collect more information on HBV genotypes from all over

the world to reach a decision concerning their clinical utility [28].

The most important finding in our results was the predominance of the genotype D as the predominant HBV genotype in the studied subjects (87%) followed by mixed genotype (D/F) that constituted 13%. These findings conform with other studies done in Egypt. Saudy et al (2003) studied the genotypes of HBV isolated from 100 serum samples of Egyptian carriers by sequencing and found that HBV genotype D was the most prevalent in Egypt [29], but did not detect mixed infection. Discrepancy may be correlated to the difference in sensitivity between the two methods used. In other words, sequence analysis provides information only on the majority strain, while LiPA appears to overcome this limitation by its sensitive detection of mixed genotypes [30]. On the other hand, Naito et al (2001) examined 2 serum samples positive for HBV DNA by primer specific to be of genotype D but they didn't find other genotypes as they only examined 2 serum samples [19]. A third study was done on 70 pediatric cancer patients suffering from hepatitis and were diagnosed as HBV infection. In this study, genotype D was reported as the predominant HBV genotype in the study subjects [31]. This study also concurs with previous studies, indicating that HBV genotype D prevails in the Mediterranean area, near and Middle East [32, 33, 34]. A similar study performed in Syria showed that 97% of the studied patients were of genotype D, and 72% were HBeAg negative [35]. Moreover, study in Turkey revealed that all 44 patients studied had genotype D [34]. Another study in Yemen demonstrated that genotype D was the dominant genotype in a settled population, while genotype A was found only in communities with continuing African links [36]. In addition, two studies in Iran revealed genotype D was the most prevalent HBV genotype [37, 38].

The clinical impact of HBV genotype D has been studied less extensively. However, initial studies have found that it may be associated with lower rates of sustained remission and HBsAg clearance and more severe liver disease compared with genotype A [39]. Emerging evidence suggests that patients with genotype D infection may develop fulminant hepatitis with high frequency [40]. A study from Syria and India indicated that genotype D is more often associated with HBeAg-negative chronic hepatitis B (CHB), more severe diseases and may predict the occurrence of HCC in young patients [35, 41, 42]. Several studies have reported lower response rates to interferon and pegylated interferon-α therapy in patients with genotypes C and D than in those infected with genotypes B and A [43,44]. Evidence suggests that the emergence of lamivudine resistance develop later and less frequent in patients with genotype D infection than in those with genotype A infection [45-47].

In this study, we reported a prevalence of mixed genotype infections D/F at an incidence of 65% in patients with AH. The existence of HBV genotype F in acute forms of liver disease suggests an association of genotype F with more severe and acute forms of liver disease. Mixed infection with two different HBV genotypes has been known since typing was done serologically [48, 49]. Mixed infection was accompanied by acute exacerbation of the chronic disease [31], and may be provoked by population migration [36, 50].

We therefore suggest that HBV genotyping become a routine exercise in clinical medicine and molecular epidemiology. As genotypes have different biological and epidemiological behavior, their detection and monitoring is more than just academic but also medically significant. Continued efforts for understanding HBV genotypes through international co-operation will reveal further virological differences of the genotypes and their clinical relevance Furthermore, efforts to prevent mixed infections (super-infection or co-infections) in patients with chronic hepatitis B should not be overlooked, especially in areas endemic for HBV infection. Since a small number of subjects were employed in our investigation, we propose that large scale studies be conducted to substantiate our findings. Such studies could also provide more insight into the association between co-infection and disease exacerbations as well as shed light on the molecular, virological and host mechanisms underlying the pathogenesis of HBV-related disease

Corresponding author
Iman A.El Aziz Khaled
Haematology &Blood Bank, Theodor Bilharz Research Institute (TBRI).Cairo, Egypt
iman_khaled@yahoo.com

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