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# Molecular identification of genotypes of Hepatitis B virus in Borno State, Nigeria

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**Hepatitis is a significant disease of the liver caused by different genotypes of hepatitis B virus. Neonates delivered of HBsAg and HBeAg positive mothers are most susceptible. The sequelae of infection and prognosis vary with the age of patient infected and the genotype (A-J) of the virus involved. These genotypes have 7.5 to 8% difference in their genomic sequences. This study was aimed at identifying the genotypes among hepatitis B surface antigen positive individuals. Four hundred and ten blood samples were randomly collected in a hospital-based cross sectional study. Sera samples were obtained for qualitative identification of the serological markers of hepatitis B virus (HBsAg, Anti-HBs, HBeAg, Anti-HBe and Anti-HBc) using rapid chromatographic immunoassays test kit (Qingdao Hightop Biotech Co., China). However, 34 presumptive hepatitis B surface antigen positive samples were assayed by polymerase chain reaction (PCR) using universal outer primer for identification of all HBV genotypes. Nested polymerase chain reaction assay was performed to determine genotype composition of the positive samples. The outcome of the molecular analysis of the 34 positive samples showed that 9 (26.5%) were positive comprising only 2 genotypes (B and E) which presented in mixed infections. Hepatitis B virus genotypes B and E were identified. The identification of genotype E corroborates reports of its existence in the West African region. However, the identification of genotype B in West Africa region is novel.**

**Key words:** Hepatitis B virus (HBV), Nested polymerase chain reaction (PCR), genotype, Borno, Nigeria.

## INTRODUCTION

Hepatitis is a disease of public health importance all over the world. Munshi et al. (2017) reported that a third of world population suffer from either acute or chronic hepatitis. According to Schweitzer et al. (2015) and WHO (2017), a quarter of a billion people in the world suffer from chronic hepatitis which results in approximately 887,000 deaths annually.

Hepatitis B virus (HBV) belongs to the genus Orthohepadnavirus of the family Hepadnaviridae and is a significant aetiology of viral hepatitis (Chang, 2014). The predilection site in human body is the hepatocyte. Upon attachment and entry into the nucleus, the deoxynucleic acid (DNA) molecule of the virion is changed into a covalently closed circular DNA (cccDNA), which serves as a template for initiation of replication. The classification of HBV into ten genotypes denoted A - J, is

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based on a difference of 7.5-8% in their DNA sequences (Kramvis, 2014; Pourkarim et al., 2014). Studies have suggested that the severity of liver diseases and impact of treatment with antiviral drugs depends on the HBV genotypes implicated in each case (Kramvis, 2014; Sunbul, 2014; Kramvis, 2016). The geographical distribution HBV genotypes have been reported to vary from region to region. Genotypes A, B and C have been reported to be prevalent in North America/Africa, Southeast Asia, and the Far East respectively. Genotype D has a global spread. Genotypes E and F are predominant in the West African region and Brazil respectively; genotype G in the USA and France while genotype H is mostly detected in North and Central America. Genotypes I and J are reported to be found mostly in the Vietnam and Japan respectively (Tran et al., 2008; Yu et al., 2010; Ranjbar et al., 2011; Compri et al., 2012; Jia-Horng, 2011).

Neonates delivered of HBsAg and HBeAg positive mothers are most susceptible. The sequelae of infection and prognosis vary with the age of patient infected and the genotype (A-J) of the virus involved. In Nigeria, molecular analysis is hardly conducted routinely during diagnosis of hepatitis not least in North Eastern Nigeria. This may be due to high cost of molecular analysis or lack of awareness, hence the paucity of information on the circulating genotypes of hepatitis B virus in Borno State. Therefore, this research was aimed at identifying the HBV genotypes of HBsAg positive individuals in Borno state.

## MATERIALS AND METHODS

### Patients

Four hundred and ten sera samples were collected at random from patients in a cross sectional study for qualitative detection of HBsAg, Anti-HBs, HBeAg, Anti-HBe and Anti-HBc using rapid chromatographic immunoassays test kits from Qingdao Hightop Biotech Co., China. The proportion of male participants (248:60.5%) was higher than females' (162:39.5%).

### Sample collection and preliminary analysis

These were carried out according to method previously described by Oyinloye et al. (2019). Positive samples (34) were stored frozen and transported to DNA Lab, Kaduna State, Nigeria for molecular analysis.

### Extraction of DNA molecule

DNA was extracted from two hundred microlitre (200 µl) of serum using a commercial DNA extraction mini kit (QIAGEN, Germany).

### Molecular detection of HBV and nested PCR assay

Both conventional and nested PCR protocols used to identify hepatitis B genotypes present were according to Naito et al. (2001).

## RESULTS

### HBV PCR amplicons

Molecular analysis of the 34 hepatitis B surface-antigen positives showed 9 (26.5%) positive and 25 (73.5%) negative for HBV. The percentage of infected male (7/9:77.8%) was higher than female (2/9: 22.2%) (Figures 1 to 3).

### HBV genotypes detection

Unusual results where all 9 positive samples (1063 bp) showed mixed infection of 2 genotypes (B+E) out of 6 genotypes which can be identified were obtained. No single genotype was detected in any individual patient (Figures 4 and 5).

## DISCUSSION

The hazards associated with hepatitis caused by different genotypes of hepatitis B virus are increasingly being highlighted in the recent years both in the developing and developed worlds and this has led to development of advanced techniques in the diagnosis of the disease and treatment regimen. These achievements have yet to overtly impact positively on the routine diagnosis of hepatitis in Nigeria especially in the North East region. This might have occasioned endemic morbidity and perhaps high mortality rate since cases are either not reported or are underreported. Yet, HBV genotyping technique has proven to be significant in the determination of the pathogenesis and resolution of viral hepatitis and it has also helped to shed more light on the evolution and geographic distribution of the virus (Komatsu et al., 2015).

The method employed in this study was according to Naito et al. (2001) who developed a multiplex nested PCR method for the detection of genotypes A-F. In the first round, universal outer primers were used. For each sample, two second round PCR reactions was performed using universal inner primers plus genotype-specific primers. The first reaction is to determine genotypes A-C, while the second determines genotypes D-F. The products of the second round were then run on an agarose gel giving genotype-specific band sizes. Universal outer primers (used in the first round) and inner primers (used in the second round) were designed according to the conserved nucleotide sequences between the pre-S1 and S gene. In contrast, genotype-specific primers were designed according to the nucleotide sequences in the region between the pre-S1 and S gene that were conserved within an HBV genotype but had poor homology with other genotypes.

All the positive samples in this study showed mixed infections with genotypes B and E. The detection of

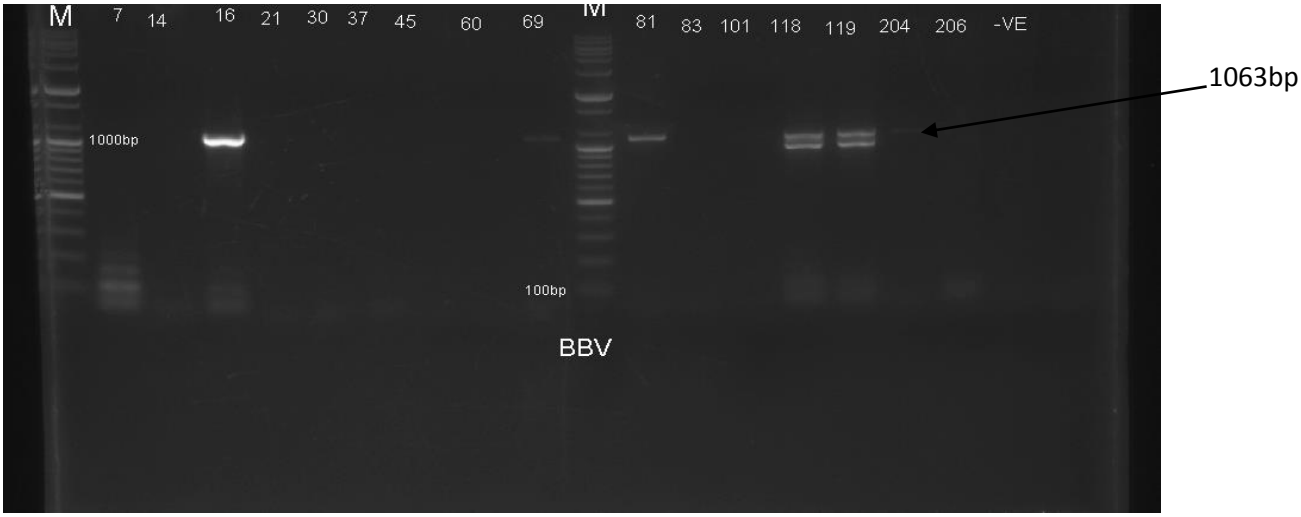


**Figure 1.** HBV PCR amplicons (1063 bp) identified by using universal outer primer. Positive Lanes denoted by sample Nos.: 16, 69, 81,101, 118, 119 and 204. M: 100 bp DNA ladder; -VE: Negative control.

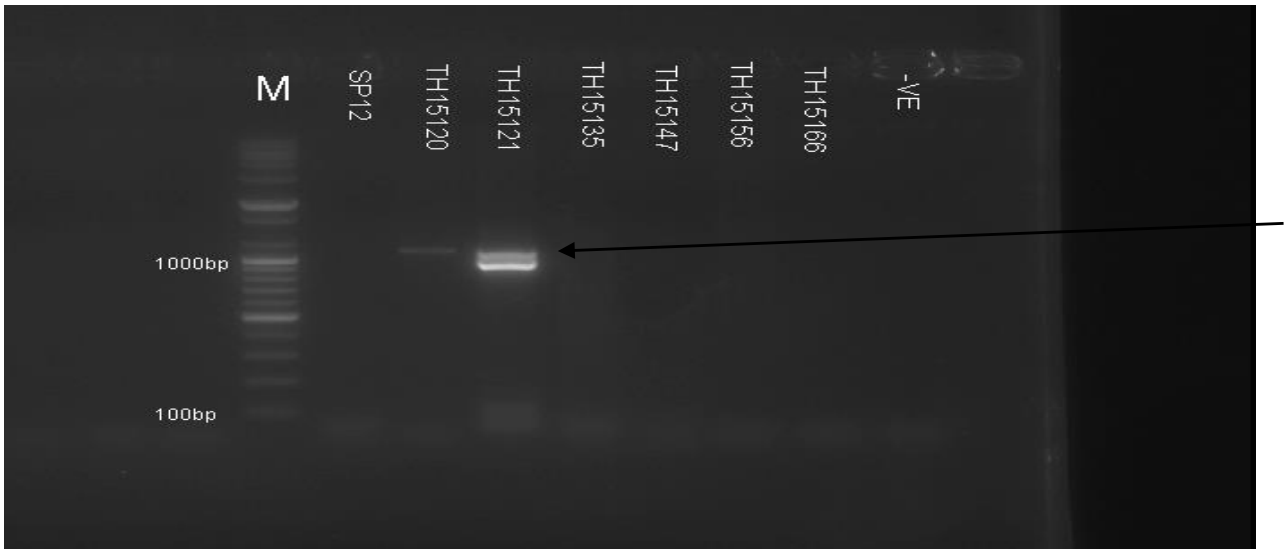


**Figure 2.** HBV PCR amplicons (1063 bp) identified by using universal outer primer. Positive Lanes denoted by sample Nos.: TH 15120 and TH15121. M: 100bp DNA ladder; -VE: Negative control.

genotype E in other parts of the world is traceable to emigrants from West Africa since it is limited only to that region of Africa (Kramvis, 2016). Therefore, the identification of genotype E in the study area corroborates reports of previous studies which confine the genotype to the West African sub-region (Dongdem et al., 2016); although Guirgis et al. (2010) had reported scarcity of information which describes its impact and the sub-genotypes on prognosis. However, the result presented in this study also highlights the possible clinical implication of disease progression and sequelae of infection with genotype E hepatitis B virus (Velkov et al., 2018).

Previous studies have reported that genotype E is transmissible through sexual route and by mother-to-child-transmission (Shi et al., 2012; Velkov et al., 2018). This means that either unprotected intercourse or having multiple sexual partners could have facilitated the transmission of this genotype in the study area, therefore since the HBV status of most pregnant women are hardly determined before or during pregnancy (especially in rural areas), this genotype could be transmitted from HBeAg positive mothers to their newborn leading to the sequelae (hepatocellular carcinoma and cirrhosis) of infection in infants.

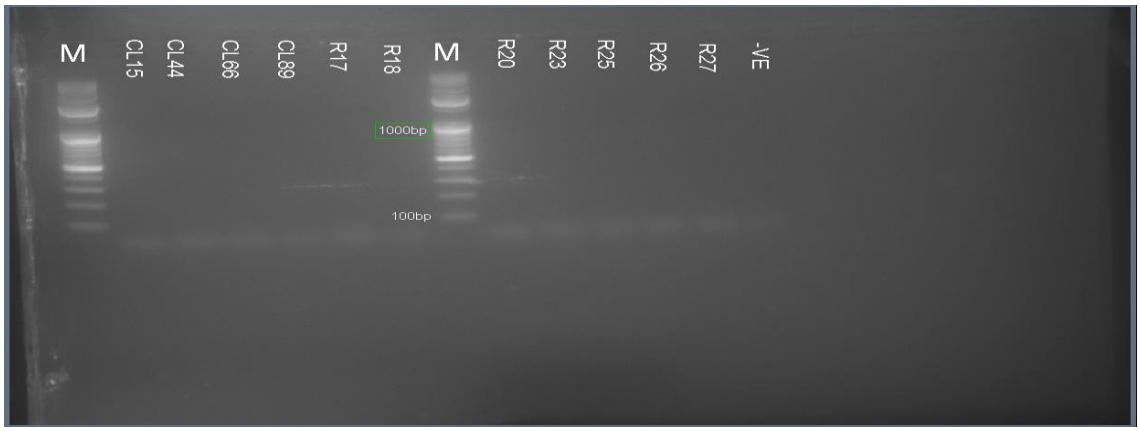


**Figure 3.** HBV PCR amplicons (1063 bp) identified by using universal outer primer. Positive Lanes denoted by sample No.: Nil. M: 100 bp DNA ladder; -VE: Negative control.

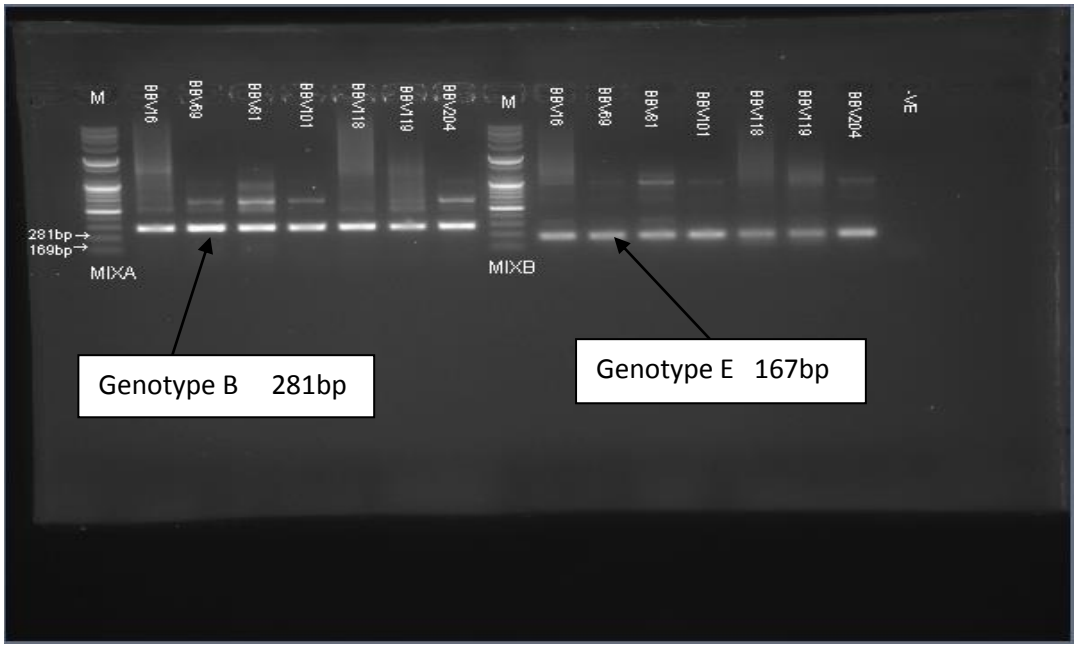


**Figure 4.** Nested PCR amplicons showing mixed infection with 2 genotypes: B+E were identified in sample No. 16, 69, 81, 101, 118, 119 and 204.

In this study, HBV genotype B was detected. This is contrary to the report by Al-Baqlani et al. (2014). The detection of genotype B is attributable to increased rate of human migrations which has occasioned refugee crisis and expectedly leading to a change in the pattern of HBV genotypes and sub-genotypes distribution. Alternatively, business and education trips among Nigerians (Borno state inhabitants inclusive) to and from Asian countries could be responsible for the identification of genotype B in this study. With respect to response to treatment with antiviral drugs, it has been reported that patients infected with HBV genotypes B show marked reduction in viral load with higher anti-HBe sero-conversion when compared to other HBV genotypes (Liu and Kao, 2013; Lin and Kao, 2015). Also, comparism between infection with genotype B and that with genotype C shows that the former experience has higher rate of HBeAg sero-conversion (Kramvis, 2016). The implication of this is that the patients infected with HBV genotype B, especially those advanced in age, in this study are less likely to experience a debilitating infection and therefore the possibility of a resolution after treatment is high.

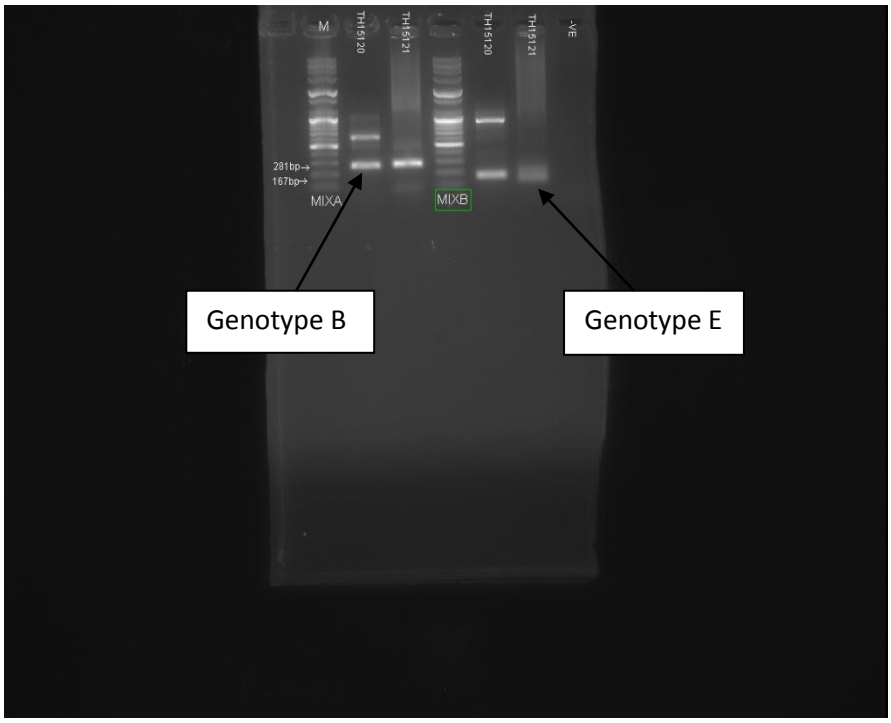


**Figure 5.** Nested PCR amplicons showing mixed infection with 2 genotypes: B+E were identified in sample No.: TH 15120 and TH15121.

However, those infected at a younger age have been found to develop hepatocellular carcinoma (Shi et al., 2012; Zhang and Ding, 2015).

## Conclusion

The identification, in Borno state, of genotype B otherwise known to be predominantly found in Southeast Asia, China, and Japan clearly indicates the transmissibility potentials of different genotypes from one geographic region to another and can change the course and eventually the outcome of infection with hepatitis B virus. Also, genotype E detection corroborates reports that it is confined to West Africa region. Since this genotype is spread sexually and especially vertically, it is necessary to evolve measures to mitigate its transmission to developing fetus by HBeAg positive mothers due to possibility of progression to hepatocellular carcinoma and cirrhosis later in life. Put together, the evolution of a clinically severe genotype B/E recombinant strain should be envisaged and proactive measures/policies to circumvent the consequence(s) of an epidemic should be established.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGMENTS

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## REFERENCES

Al-Baqlani SA, Sy BT, Ratsch, BA, Al-Naamani K, Al-Awaidy S, Al-Busaidy S, Pauli G (2014). Molecular Epidemiology and Genotyping of Hepatitis B Virus of HBsAg-Positive Patients in Oman. PLos ONE 9(5):e97759.

Chang MH (2014). Prevention of hepatitis B virus infection and liver cancer. Recent Results in Cancer Research 193:75-95.

Compri AP, Miura I, Porta G, Lemos MF, Saraceni CP, Moreira RC (2012). Hepatitis B virus infection in children, adolescents, and their relatives: genotype distribution and precore and core gene mutations. Review of the Society of Brazilian Medicine in Tropics 45:301-304.

Dongdem AZ, Dzodzomenyo M, Asmah RH, Nyarko KM, Nortey P, Agyei A, Adjei DN, Kenu E, Adjei AA (2016). Hepatitis B virus genotypes among chronic hepatitis B patients reporting at Korle-Bu

teaching hospital, Accra, Ghana. The Pan African Medical Journal 25(1):5.
Guirgis BSS, Abbas RO, Azzazy HME (2010). Hepatitis B virus genotyping: current methods and clinical implications. International Journal of Infectious Diseases 14(11):941-953.
Jia-Horng K (2011). Molecular Epidemiology of Hepatitis B Virus. Korean Journal of Internal Medicine 26(3):255-261.
Komatsu H, Inui A, Fujisawa T, Takano T, Tajiri H, Murakami J, Suzuki M (2015). Transmission route and genotype of chronic hepatitis B virus infection in children in Japan between 1976 and 2010: A retrospective, multicenter study. Hepatology Research 45:629-637.
Kramvis A (2014). Genotypes and genetic variability of hepatitis B virus. Intervirology 57:141-150.
Kramvis A (2016). The clinical implications of hepatitis B virus genotypes and HBeAg in pediatrics. Review of Medical Virology 26:285-303.
Liu CJ, Kao JH (2013). Global perspective on the natural history of chronic hepatitis B: Role of hepatitis B virus genotypes A to J. Seminars in Liver Disease 33:97-102.
Lin CL, Kao JH (2015). Hepatitis B virus genotypes and variants. Cold Spring Harbor Perspectives in Medicine 5(5):a021436.
Munshi SU, Tran TTT, VoTNT, Tabassum S, Sultana N, Nguyen TH, Jahan M (2017). Molecular characterization of hepatitis B virus in Bangladesh reveals a highly recombinant population. PLOS https://doi.org/10.1371/journal.pone.0188944
Naito H, Hayashi S, Abe K (2001). Rapid and specific genotyping system for hepatitis B virus corresponding to six major genotypes by PCR using type-specific primers**.** Journal of Clinical Microbiology 39(1):362-364.
Oyinloye SO, Bukbuk DN, Sanda FA (2019). Surveillance of Hepatitis B Biomarkers in Borno State, Nigeria. Journal of Advances in Medicine and Medical Research *28*(10):1-8.
Pourkarim MR, Amini-Bavil-Olyaee S, Kurbanov F, Van-Ranst M, Tacke F (2014). Molecular identification of hepatitis B virus genotypes/subgenotypes: Revised classification hurdles and updated resolutions. World Journal of Gastroenterology 20:7152-7168.
Ranjbar R, Davari A, Izadi M, Jonaidi N, Alavian SM (2011). HIV/HBV co-infections: epidemiology, natural history, and treatment: a review article. Iran Red Crescent Medical Journal 13:855-862.
Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ (2015). Estimations of worldwide prevalence of chronic hepatitis B virus infection: A systematic review of data published between 1965 and 2013. Lancet 386:1546-1555.
Shi W, Zhu C, Zheng W, Carr MJ, Higgins DG, Zhang Z (2012). Subgenotype reclassification of genotype B hepatitis B virus. BMC Gastroenterology 12(116).
Sunbul M (2014). Hepatitis B virus genotypes: Global distribution and clinical importance. World Journal of Gastroenterology 20:5427-5434.
Tran TT, Trinh TN, Abe K (2008). New complex recombinant genotype of hepatitis B virus identified in Vietnam. Journal of Virology 82:5657-5663.
Velkov S, Ott JJ, Protzer U, Michler T (2018). The Global Hepatitis B Virus Genotype Distribution Approximated from Available Genotyping Data. Genes 9(10):495.
World Health Organization Global Hepatitis Report (WHO) (2017). World Health Organization; Geneva, Switzerland: 2017.
Yu H, Yuan Q, Ge SX, Wang HY, Zhang YL, Chen QR, Zhang J, Chen PJ, Xia NS (2010). Molecular and phylogenetic analyses suggest an additional hepatitis B virus genotype "I". PLoS ONE 5:e9297.
Zhang X, Ding HG (2015). Key role of hepatitis B virus mutation in chronic hepatitis B development to hepatocellular carcinoma. World Journal of Hepatology 7(9):1282-12866.