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Genotyping of occult hepatitis B virus infection in Egyptian hemodialysis patients without hepatitis C virus infection

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KEYWORDS
Occult HBV infection (OBI);
Real time-PCR;
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Summary
Background: Occult hepatitis B viral infection is the presence of hepatitis B viral nucleic acids in the serum and/or liver in the absence of hepatitis B surface antigen.
Aim: The study aimed to determine the prevalence of occult hepatitis B virus infection among hepatitis C virus-negative hemodialysis patients and to identify their genotypes.
Methods: of 144 patients on maintenance hemodialysis, 50 hepatitis B surface antigen and hepatitis C virus nucleic acid-negative patients were selected according to strict inclusion criteria to avoid the effect of confounding variables. The following investigations were done: serum AST and ALT; HBsAg; HBcAb; HCV-Ab; HCV-RNA; and HBV-DNA.
Results: Positive hepatitis B viral nucleic acid was confirmed in 12/144 (8.3%) hemodialysis patients and 12/50 (24%) in our study group (occult infection). Mean hemodialysis periods for negative patients and occult hepatitis B virus patients were 27.3 ± 18.8 and 38.4 ± 8.14 months, respectively, and this

Abbreviations: HBV, hepatitis B virus; OBI, occult HBV infection; HBsAg, hepatitis B surface antigen; PCR, polymerase chain reaction; ESRD, end-stage renal disease.

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difference was significant (p-value = 0.02). Mean alanine transaminase levels were 20.27 ± 5.5 IU/L and 25.3 ± 9.6 in negative patients and occult infection patients, respectively. This difference was non-significant. Aspartate transaminase levels were 21.4 ± 10.2 IU/L and 27.3 ± 4.6 IU/L, respectively, in negative patients and infected patients; this difference was significant (p-value = 0.03). Half (6/12) of the positive samples belonged to genotype 'B', 33.3% (4/12) to 'C', and 16.6% (2/12) to genotype 'D'.

Conclusion: OBI is likely among hemodialysis patients even without HCV coinfection (24%). Genotype D cannot be the only genotype distributed in Upper Egypt, as the current study reported relatively new results that 50% of the patients with occult B carry genotype B, 33.3% carry genotype C and only 16.6% carry genotype D.

Background

Hepatitis B virus (HBV) infection is a global health concern with multiple clinical presentations, e.g., occult HBV infection (OBI) [1]. OBI is defined as the presence of a low amount of HBV DNA (less than 10^4 copies/mL) in patients with negative hepatitis B surface antigen (HBsAg) serum/plasma [2]. The risk of infection transmission from OBI through blood transfusion could be avoided by utilization of highly sensitive polymerase chain reaction (PCR)-based techniques, which can identify HBV DNA in HBsAg-negative individuals [3].

OBI is highly prevalent among patients with end-stage renal disease (ESRD) on maintenance hemodialysis because of frequent blood transfusions [4]. ESRD is a serious problem globally; however, the prevalence is higher in developing countries, especially in the Middle East [5].

The exact cause of OBI is not understood. Several studies have suggested that OBI could be caused by mutant viruses that cannot be detected by current HBsAg assays. Other studies have suggested that OBI could be due to a failure of viral replication, gene expression, or virus release [6]. HBV has been classified into ten genotypes (labeled A–J) [7]; some genotypes are associated with different clinical outcomes. Genotypes C and D generally tend to be related to more severe prognosis (particularly hepatocellular carcinoma) than genotypes A and B [8]. Real-time PCR using genotype-specific primers is a very important technique because fluorescent probes allow for the highly sensitive detection and quantification of HBV DNA, while melting curve analysis allows the determination of genotypes, which aids in the prediction of therapeutic outcomes [9].

Different HBV genotypes have different geographic distributions. The HBV genotype D is common in Mediterranean area data [10]. Screening of OBI in hemodialysis patients in Egypt is limited [11], and little information exists about the relation between the HBV genotypes and OBI in Egypt [12]. Therefore, we investigated the prevalence of OBI occurrence without HCV-coinfection among Egyptian hemodialysis patients, and we identified their different genotypes using a highly sensitive and specific quantitative PCR assay.

Materials and methods

Patients

The study included all patients (144) on maintenance hemodialysis (more than six months) recruited from Minia University Hospital (MUH) at the time of the study. Serum samples were routinely collected for the detection of HCV antibodies and Hepatitis B surface Antigen (HBsAg) before the start of dialysis. Exclusion criteria for our study has included: 1 – acute or chronic HBV infection, as determined by positive Hepatitis B core antibodies (anti HBc) and HBsAg, respectively; 2 – HBV vaccination history (to exclude vaccine-induced immunity); and 3 – HCV RNA and HCV antibodies, to exclude coinfection with HCV and other liver diseases.

A full history was collected from the selected group of patients (50/144 patients) with emphasis on their histories of blood transfusions, liver diseases and durations of hemodialysis. Clinical examinations were conducted with emphasis on signs of chronic liver disease, e.g., jaundice,