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Research paper

Occult hepatitis B virus infection in Greek patients with congenital bleeding disorders

Agoritsa Varaklioti [a,b], Anna Kouramba [a], Panagiota Ioannidou [a], Olga Katsarou [a,*]

[a] Blood Center and National Reference Center for Congenital Bleeding Disorders, Laiko General Hospital, Agiou Thoma 17, 11527 Athens, Greece
[b] Faculty of Social Sciences, Department of Health Management, Hellenic Open University, Patras, Greece

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ABSTRACT

Occult Hepatitis B Infection (OBI) is a form of chronic HBV infection characterized by low level HBV DNA, without detectable HBV surface antigen (HBsAg). OBI is frequently associated with the presence of anti-HBc and in some cases also with anti-HBs. Patients, who formerly received non-inactivated factor concentrates, can potentially be considered at high risk for OBI, especially since these patients usually are HIV or HCV co-infected. This study aimed to assess the prevalence of occult HBV infection in Greek patients with hereditary bleeding disorders. The study sample comprised of 114 patients from a single haemophilia center. All patients were screened for HBV serum markers and individually tested for HBV DNA using a qualitative PCR. Presence of HBV DNA was further confirmed by quantification of viral load with an ultrasensitive in-house real time PCR. 88 and 21 patients with haemophilia A and B, respectively, 4 patients with von Willebrand Disease and 1 patient with severe factor VII deficiency were screened for the presence of OBI. Anti-HBc were detected in 53 (46.5%) subjects; 18 of them were anti-HBs(−) and 35 anti-HBs(+). Anti-HBe were present in 26 subjects. Two out of 114 patients were HBsAg(+). Of the remaining 112 HBsAg(−) patients tested, two (1.8%) were found HBsAg(−), HBV DNA(+), anti-HBc(+) and anti-HBs(−) and were identified as potential OBI cases. Both cases exhibited very low DNA levels; 38.2 IU/mL in patient A and 14.2 IU/mL in patient B. Both patients were HBeAg(−), but patient A had HBe antibodies. Patient B was also HIV/HCV co-infected. In conclusion, two cases of OBI with low HBV viraemia were identified among patients with congenital bleeding disorders. Although the incidence in our sample is moderately low (1.8%), close monitoring of these infections is of great clinical significance, especially in patients with co-infections and concomitant immunosuppression.

1. Introduction

The most common definition of occult hepatitis B infection (OBI) used in clinical practice refers to the long-lasting persistence of HBV viral genomes in liver tissue and/or serum of individuals in the absence of detectable hepatitis B surface antigen (HBsAg), with or without anti-HBc or anti-HBs outside the pre-seroconversion window period. OBI can be classified into 2 groups, seropositive OBI (anti-HBc and/or anti-HBs positive) and seronegative OBI (anti-HBc and anti-HBs negative), on the basis of the HBV antibody profile (Kwak and Kim, 2014; Raimondo et al., 2008; Raimondo et al., 2007). Most OBI cases are seropositive, but >20% of patients with OBI are negative for all serum markers of HBV infection. Serum HBV DNA can be either detectable or undetectable, and when detectable, HBV DNA levels are usually very low (<200 IU mL^{-1}) (Raimondo et al., 2007).

The prevalence of OBI varies according to the different endemicity of HBV infection, cohort characteristics, and sensitivity and specificity of the methods used for detection. Greece is a country with intermediate endemicity with respect to HBsAg seropositivity (range between 3%–5%) and to previous HBV infection markers (range between 17% and 25%) (Raptopoulou et al., 2009; Zervou et al., 2001). A few reports have identified occult HBV infection cases among several patient groups in Greece; haemodialysis patients, HCV-infected patients, patients with non-viral hepatic diseases, or patients with autoimmune liver diseases (Georgiadou et al., 2009; Georgiadou et al., 2004; Mina et al., 2010; Siagris et al., 2006).

Patients with hereditary bleeding disorders, receive coagulation factor concentrates as replacement therapy, placing them at high risk of infection with parenterally transmissible viruses. In fact, numerous patients, who received frequent infusions of plasma-derived factor VIII or factor IX concentrates, acquired HCV infection (Makris et al., 1990). Between 1978 and 1986, two thirds of the HCV infected patients in USA were also infected with HIV. HCV seroprevalence rates were extremely high (close to 100%) among those who received infusions of plasma-derived factor VIII or IX concentrates frequently, while two

* Corresponding author at: Blood Center and National Reference Center of Congenital Bleeding Disorders, Laiko General Hospital, Agiou Thoma 17, 11527 Athens, Greece.
E-mail address: btchemoph@laiko.gr (O. Katsarou).

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thirds of the HCV-infected patients in the Unites states were also infected with HIV (Goedert et al., 2002). As safety measures, anti-HBV vaccination was introduced worldwide during early 1980s while virally inactivated concentrates were phased in during mid 1980s and completely by 1987 (Goedert et al., 2002). Nevertheless a patient, who in earlier times received non-inactivated factor concentrates, can potentially be considered at high risk for occult HBV infection.

So far, only a few reports have addressed the prevalence and significance of OBI cases among haemophilia patients (Arababadi et al., 2012; Borhany et al., 2011; Windyga et al., 2006; Windyga et al., 2013). In Greece several studies have reported the status of serological markers of HIV, HCV and HBV infections among haemophilia patients (Delladetsima et al., 2002; Yannitsiotis et al., 1977). Anti-HBV vaccination started in 1983 for the newly diagnosed hemophiliacs and for those without any HBV serological markers. In contrast available data regarding occult HBV infection in Greek haemophilia patients are missing. Therefore, we conducted a large study in a Greek National Reference Center for Congenital Bleeding Disorders in order to identify OBI cases among our study population, using sensitive molecular and serology methods. To the best of our knowledge this is the first study in Greece that evaluates the prevalence and significance of occult HBV infection in patients with inherited bleeding disorders.

2. Materials and methods

2.1. Subjects and sample collection

The study was conducted in the National Reference Center for Congenital Bleeding Disorders in Laiko General Hospital of Athens between April 2013 and June 2014. This study was designed and performed in accordance with the Declaration of Helsinki and was approved by the institution's Hospital's Review Board. One hundred and fourteen patients with inherited bleeding disorders were enrolled in the study and their data were fully anonymised. All subjects included in the study had at least once received non-inactivated factor concentrates before 1986. Serum and K_2-EDTA plasma samples were drawn from each subject. All study participants signed an informed consent.

2.2. Laboratory methods

Serum samples from all patients were analyzed in order to investigate HBV serology markers, namely HBsAg, total anti-HBc, anti-HBs, HBeAg and anti-HBe by enhanced chemiluminescence (Architect i2000sr, Abbott Laboratories, Chicago, USA). All tests were conducted in accordance with the manufacturer's instructions. K_2-EDTA plasma samples were also individually tested for HBV DNA using the Cobas TaqScreen MPX Test v2.0 (Roche Molecular Diagnostics, USA), a qualitative multiplex in vitro test for the direct detection of HCV RNA, HIV-1 group M, HIV-1 group O, HIV-2 RNA and HBV DNA, with an average 95% Limit of Detection (LOD) for HBV plasma DNA of 2.3 IU mL^{-1}. Presence of HBV DNA was further confirmed by quantification of viral load that was performed with an ultrasensitive in house Real Time PCR (LC II HBV DNA sensitive v1.3). The 95% LOD of this method is 22 IU mL^{-1}(Paraskevis et al., 2010).

2.3. Statistical analysis

All statistical calculations were performed using SPSS software version 19.0. Results are expressed as mean ± SD and as number and percentage as appropriate. Student's *t*-test was used to examine differences between continuous variables. The chi-square test (or Fisher's exact where applicable) was used to analyze differences between categorical variables. A two-sided *P* value <0.05 was considered as statistically significant.

Table 1
Demographic and clinical characteristics of the study sample.

Age (years)			Bleeding disorder	N	(%)
Median	46		Haemophilia A	88	77.2
Mean (SD)	48.5 (±13.3)		Haemophilia B	21	18.4
Range	23–77		Von Willebrand disease	4	3.5
			Factor VII deficiency	1	0.9
Gender	**N**	**(%)**	**Severity**[a]	**N**	**(%)**
Male	114	100	Severe	60	54.5
Female	0	0	Moderate	17	15.5
			Mild	33	30.0
			Viral infection[b]	**N**	**(%)**
			HCV	57	50.0
			HIV	1	0.9
			HCV/HIV	26	22.8
			HBV/HCV	1	0.9
			HCV/HIV/HBV	1	0.9
			None	28	24.5

[a] Severity of the bleeding disorders refers to patients with haemophilia A and B and with factor VII deficiency.
[b] Patients' viral status as characterized by the presence of HBsAg and corresponding antibodies. HIV: Human Immunodeficiency Virus, HCV: Hepatitis C Virus, HBV: Hepatitis B Virus.

3. Results

The sociodemographic and clinical characteristics of the participating patients are shown in Table 1. The patients' age ranged between 23 and 77 years old, with a median age of 46 and mean value of 48.5 ± 13.3 years. Haemophilia A and B were present in 88 (77.2%) and 21 (18.4%) of the screened patients, respectively. Four patients (3.5%) had von Willebrand disease and one patient (0.9%) was factor VII deficient. Regarding their viral status, 57 (50%) and 1 (0.9%) patients were HCV and HIV mono-infected, respectively. 28 patients (24.5%) had different co-infections. More specifically, one patient (0.9%) was HBV/HCV co-infected and 26 (22.8%) had HCV/HIV co-infection. One patient had a triple infection with HBV, HIV and HCV (0.9%). 28 patients (24.5%) of our study sample had no detectable viral markers (Table 1).

The relation between the presence of anti-HBs and anti-HBc status is shown in Table 2. Among the 114 patients tested, anti-HBc positive patients comprised 46.5% ($n = 53$) of the study population, while 53.5% ($n = 61$) were found negative for anti-HBc. Among the anti-HBc negative patients, 36 have been vaccinated and 30 (49,1%) were anti – HBs positive. Out of the 53 anti-HBc positive patients, 35 (66%) were also anti-HBs positive, whereas 18 (34%) were anti-HBs negative. As shown in Table 2, anti-HBe presence was observed in almost half (49.1%) of anti-HBc-positive subjects; more specifically, 26 patients were anti-HBe positive, while 24 (45.3%) were anti-HBe negative. For four patients screening for anti-HBe was not performed. Presence of anti-HBc and/or anti-HBe is concordant with HBV past infection. A trend for lower percentage of anti-HBs was found in HIV positive patients (p:0.066). Among 28 HIV infected patients, 15 (53.6%) were found positive for anti-HBs compared to 64 out of 86 HIV negative

Table 2
Anti-HBs and anti-HBe profile in patients with and without anti-HBc.

	Anti-HBc (+) *N = 53*	Anti-HBc (−) *N = 61*	Total *N = 114*
Anti-HBs			
Negative	18 (34.0%)	17 (27.9%)	35
Vaccinated	0	6 (9.8%)	
Positive	35 (66.0%)	44 (72.1%)	79
Vaccinated	0	30 (49,1%)	
Anti-HBe			
Positive	26 (49.1%)	0 (0%)	26
Negative	24 (45.3%)	60 (98.4%)	84
Not done	3 (5.7%)	1 (1.6%)	4