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Seroprevalence of occult hepatitis B among Egyptian paediatric hepatitis C cancer patients

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SUMMARY. Occult hepatitis B infection is characterized by the presence of hepatitis B virus (HBV) DNA in the serum in the absence of hepatitis B surface antigen (HBsAg). Prevalence of hepatitis C virus (HCV) infections in Egypt is among the highest in the world. In this study, we aim at analysing the rates of occult HBV infections among HCV paediatric cancer patients in Egypt. The prevalence of occult HBV was assessed in two groups of paediatric cancer patients (HCV positive and HCV negative), in addition to a third group of paediatric noncancer patients, which was used as a general control. All groups were negative for HBsAg and positive for HCV antibody. HBV DNA was detected by nested PCR and real-time PCR. HCV was detected by real-time PCR. Sequencing was carried out in order to determine HBV genotypes to all HBV patients as well as to detect any mutation that might be responsible for the occult phenotype. Occult hepatitis B infection was observed in neither the non-HCV paediatric cancer patients nor the paediatric noncancer patients but was found in 31% of the HCV-positive paediatric cancer patients. All the detected HBV patients belonged to HBV genotype D, and mutations were found in the surface genome of HBV leading to occult HBV. Occult HBV infection seems to be relatively frequent in HCV-positive paediatric cancer patients, indicating that HBsAg negativity is not sufficient to completely exclude HBV infection. These findings emphasize the importance of considering occult HBV infection in HCV-positive paediatric cancer patients especially in endemic areas as Egypt.

Keywords: cancer, Egypt, HBV, HCV, occult HBV, paediatric.

INTRODUCTION

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are the major causes of chronic liver diseases worldwide, particularly cirrhosis and hepatocellular carcinoma (HCC) [1]. About two billion people globally have been infected with HBV, of whom more than 350 million are chronically infected with HCV [2,3]. Hepatitis B virus infection ranges from an asymptomatic state to an active stage of chronic hepatitis B (CHB), leading to cirrhosis and HCC [4]. HBV-related end-stage liver disease is responsible for over 0.5–1 million deaths annually and currently denotes to 5–10% of liver transplantation cases [5].

Nearly two to three million Egyptians are chronic carriers of HBV [6], but a decline in HBV incidence in Egyptian children is expected as 90% immunization coverage has been achieved [7]. On the other hand, eight to 10 million people among a population of 85 million Egyptians have been exposed to HCV, and five to seven million have active infections [8]. As HBV and HCV have similar transmission routes, dual infection may take place [9]. Hepatitis B virus and HCV coinfection may be associated with a more severe clinical presentation [10,11]. In most developing countries, the detection of hepatitis B virus surface antigen (HBsAg) in serum continues to be the foundation of diagnosis for the chronic HBV infection and screening for HBV [12]. Occult HBV (OBI) infection is a term used to describe HBV infection categorized by the existence of the HBV DNA in the absence of detectable HBsAg.

The gold-standard test for detection of OBI is the amplification of HBV DNA [13]. Currently, the optimal standard for diagnosis is the analysis of HBV DNA extracts from

Abbreviations: ALL, acute lymphoctic leukaemia; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHB, chronic hepatitis B; HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; MHR, major hydrophilic region; NASH, nonalcoholic steatohepatitis; OBI, occult hepatitis B infection.

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plasma performed by real-time and nested polymerase chain reaction (PCR) techniques [14].

In HBV, the core protein is coded for by gene C (HBcAg), the DNA polymerase is encoded by gene P and gene S is the gene that codes for the surface antigen (HBsAg) [15]. The HBV nonstructural X protein (HBx) is considered as the key regulatory protein of the virus that is at the intersection of HBV infection, replication, pathogenesis and possibly carcinogenesis [16].

The dominant epitopes of HBsAg, which are the targets of neutralizing B-cell responses, reside in the 'a' determinant which is a 2-loop structure that includes amino acids 124 to 147 within the major hydrophilic region (MHR) [17,18]. Amino acid substitutions in the MHR can cause reduced binding of anti-HBs antibodies, resulting in immune escape and consequently OBI [19].

Sequence analysis identified mutations in the surface gene, core gene and polymerase gene of occult HBV genomes from patients with chronic liver diseases [20]. Other studies identified mutations in the X gene which reduced viral replication in occult HBV OBI cases with chronic hepatitis [21]. OBI has also been reported in patients with nonalcoholic steatohepatitis (NASH) [22] and autoimmune hepatitis [23].

In this study, we monitor occult HBV infections in paediatric cancer patients who are HCV positive, with the aim of placing an emphasis upon the possible occurrence of occult HBV infection associated with HCV in endemic countries as Egypt.

MATERIALS AND METHODS

Statement of ethical approval

All experiments, involving any samples taken from personnel and done in this study, were carried out in accordance and approval of the Ethical Committee at Cairo University, Cairo, Egypt. In addition, all children who contributed any samples did this according to a written informed consent, from their parents or guardian.

Study design and population

This study was conducted during the period from September 2011 to January 2013 on two groups of 289 patients each (paediatric cancer and paediatric noncancer patients). The first group with cancer was from the Children's Cancer Hospital (CCHE) and the National Cancer Institute (NCI), both located in Cairo, Egypt. The second group (control group) of noncancer paediatric patients was collected and revised from (Imbaba Fevers Hospital) located in Giza (still part of the greater Cairo metropolitan area), Egypt. For both groups, inclusion criteria were age range of 7 month to 16 years, negativity for HBsAg and presence of HCV antibody (Ab).

Specimens

Blood samples were drawn from all enrolled patients; sera were separated and stored at −20 °C. For all the studied population, information about age, sex and cancer type was collected. In addition, laboratory diagnosis for hepatitis B and C was carried out: markers for HBV, hepatitis B surface antigen (HBsAg), antibodies to hepatitis B surface antigen (HbsAb), antibodies to hepatitis B core antigen [anti-HBc] and antibodies to HCV were all tested using AxSYM system (Abbot, Longford, Ireland) according to the manufacturer's instructions. Liver function tests such as serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase and bilirubin were also carried out using Synchron CX9 Pro (Beckman, Pasadena, CA, USA). White blood cell level was estimated using coulter LH750 Analyzer (Beckman).

Nucleic acid extraction

DNA/RNA was extracted from the serum using Ribo virus Extraction Kit (Sacace, Como, Italy) and RTP Virus Mini Kit (Stratec Molecular, Berlin, Germany) using 150 μL of the patient serum following the manufacturer's instructions.

Real-time reverse transcription PCR for HCV

Hepatitis C virus RNA was initially determined in all samples using real-time PCR (Hangzhou Bioer Technology, Zhejiang, China). The volumes per tube were 25 μL RT-PCR mix, 2.5 μL Mn^{2+} and 2.5 μL probe mix. Solutions were mixed and 30 μL was pipetted into the corresponding real-time PCR tubes. To each tube, 20 μL of the corresponding HCV RNA templates (specimen, controls) and 20 μL of the four standard controls (1–4) were added, and tubes were capped and centrifuged at 3000 rpm for 5 s.

Detection of occult HBV DNA by nested PCR

All positive samples, regardless of their viral load levels, were selected for nested PCR using three primers sets (Table 1) specific for surface, core and X viral genomic regions as previously described by Kazemi *et al.* [24].

The first round of the nested PCR was performed using Dream Taq Green PCR Master Mix (Fermentas, Thermo Scientific, St. Leon-Rot, Germany) and the outer primers. Components for each 50 μL reaction included: DreamTaq™ Green PCR Master Mix (2x): 25 μL, forward primer: 10 pmole, reverse primer: 10 pmole, template DNA: 1 ng and nuclease-free water: 50 μL. Samples were gently vortexed and spun down. PCR was performed using the recommended thermal cycling conditions as follows: initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and automated fluorescent extension at 72 °C for

Table 1 Sequences of primer pairs used for HBV PCR detection

Gene	Primer sequence	Nucleotide position
Core gene	C1s: 5′CTGGGAGGAGTTGGGGGA3′	(1730–1747) (outer)
	C2a: 5′GTAGAAGAATAAAGCCC3′	(2503–2487) (outer)
	C3s: 5′GGTCTTTGTACTCGGAGGCTG3′	(1763–1783) (inner)
	C4a: 5′ATACTAACATTGACATTCCC3′	(2455–2436) (inner)
Surface gene	S-1s:5′AGAACATCGCATCAGGACTC3′	(159–178) (outer)
	S-2a: 5′CATAGGTATCTTGCGAAAGC3′	(642–623) (outer)
	S-3s: 5′AGGACCCCTGCTCGTGTTAC3′	(181–200) (inner)
	S-4a: 5′AGATGATGGGATGGGAATAC3′	(619–600) (inner)
X gene	X1s: 5′CTAGCCGCTTGTTTTGCTCG3′	(1282–1301) (outer)
	X2a: 5′TTATGCCTACAGCCTCCTAG3′	(1666–1647) (outer)
	X3s:5′GGTCTTACATAAGAGGACTC3′	(1518–1537) (inner)
	X4a: 5′GTTCACGGTGGTCTCCAT3′	(1625–1600) (inner)

s: sense; a: antisense.

2 min/kbp followed by a final extension at 72 °C for 5–15 min. The second round of nested PCR was conducted by transferring 2 μL of the first PCR product to a second PCR tube containing the inner primers (Table 1), and the cycles were repeated.

Real-time PCR for HBV

All the reagents (Hangzhou Bioer Technology Co.) were centrifuged for a few seconds before preparing PCR reagents. PCR reagents were prepared per test as follows: 37.7 μL HBV PCR solution, 0.3 μL Taq polymerase and 0.1 μL UDG. After mixing, the PCR reagents were aliquoted into 0.2-mL PCR tubes as 38 μL per tube, the extracted samples, controls and references were added as per 2 μL each into PCR tubes above. Thermal cycler profile was set as follows:

Thirty-seven degree Celsius: 5 min, 94 °C: 2 min, 95 °C: 5 s, 60 °C: 40 s, 40 cycles and 30 °C: on hold in (CFX96; BioRad, Hercules, CA, USA). The channel of instrument for testing was selected and (FAM) dye F1 channels were chosen when collecting fluorescence signals and set fluorescence signals at 60 °C and before running, the FAM background was adjusted again to be between 10 and 20.

Sequence analysis of PCR products

DNA sequencing was carried out on PCR products using the same HBV DNA-specific primers. PCR products were purified using (QIAquick Purification Kit; Fermentas/Thermo Scientific, Pittsburgh, PA, USA). Cycle sequencing PCR was performed as described by Cuchacovich *et al.* [25] using Big Dye Terminator v3.1 cycle sequencing kit and Centri-Septm spin columns (Applied Biosystems, Foster City, CA, USA) for purification of cycle sequencing products. Sample electrophoresis was then carried out using automated sequencer ABI PRISM 310 Genetic Analyzer (Applied Biosystems), followed by sequencing analysis by specific analysis software. Comparative data analysis was also performed by Basic Local Alignment Search Tool (BLAST application) to generate alignments between the PCR nucleotide sequences and nucleotide sequences within the National Center for Biotechnology Information (NCBI) database to determine the viral genotype.

Statistical analysis

Data were analysed using IBM SPSS advanced statistics version 20 (SPSS Inc., Chicago, IL, USA). Numerical data were expressed as mean, standard deviation and range. Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was carried out using Student's *t*-test. A *P*-value <0.05 was considered significant.

RESULTS

Of the 289 paediatric cancer patients enrolled in the present study, 193 were men and 96 were women, and among the 289 noncancer patients, 157 were men and 132 were women. The mean age within the cancer group was 7.8 ± 4.7 years and within the noncancer group (control group) was 8.6 ± 4.5 years.

Detection of HCV infection and its levels among paediatric patients

Hepatitis C virus quantitative real-time PCR was carried out to all the studied populations. It was found that in the caner group, 50 of the 289 patients were HCV positive, and among the noncancer patients, only three patients

Table 2 HCV infection incidence among cancer and noncancer patients

	Cancer patients		Noncancer patients		
	No.	%	No.	%.	*P*-value
HCV					
+ve	50	17.3	3	1.0	*<0.001
−ve	239	82.7	286	99.0	
Total	289		289		

*$P < 0.001$ (Highly significant).

were HCV positive, which indicates that HCV infections are more prevalent among cancer patients (P-value < 0.01; Table 2). Quantitative real-time PCR was performed on all cancer patients to detect HCV levels, and it was found that of the 50 HCV-positive paediatric cancer patients, 13 patients had low viral load, six patients had a moderate viral load and 31 patients had a high viral load.

Detection of occult HBV in HCV and non-HCV cancer patients

Hepatitis B virus DNA PCR was performed by nested PCR using three primer sets specific for surface, core and X viral genomic regions (Fig. 1). It was found that among the 50 cancer patients who were HCV positive, 16 patients of the 50 (32%) were HBV positive (Fig. 2).

In order to test the relationship between occult HBV and HCV, 50 patients from the cancer group who were HCV negative and matched for sex and age with the HCV-positive group, were chosen to be tested for HBV. Among these 50 HCV-negative cancer patients, none had HBV DNA. This result shows a potential association between HCV and occult HBV among paediatric cancer patients.

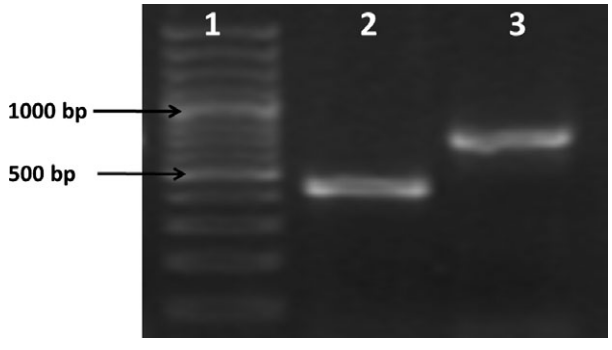

Fig. 1 DNA amplification by PCR using conserved nature of nucleotides sequences in core and surface regions. Lane 1 is the ladder marker, lane 2 represents a positive case for surface genome (400 bp) and lane 3 represents a positive case for core genome (800 bp) band.

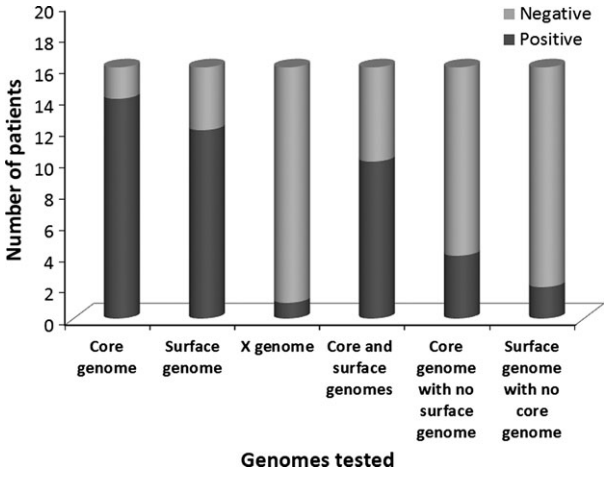

Fig. 2 Presence of core genome, surface genome and x genome among HCV-HBV paediatric cancer patients.

According to the cancer type, there was an observed prevalence of occult HBV DNA in HCV-positive paediatric patient with acute lymphocytic leukaemia as a total of eight of 16 (50%) of these children had HBV DNA in their serum. Tables 3 and 4 show the biochemical, serological and molecular parameters as well as the cancer types associated with the 16 patients who tested positive for HBV DNA.

DNA sequencing of positive HBV DNA amplified PCR products

DNA sequencing of the positive PCR products for the surface genome and the core genome was carried out for eight and five samples, respectively, using the same HBV DNA-specific primers (forward and reverse), and sequence alignments were carried out to detect any mutations at the core genome or the surface genome and to determine the genotype of HBV.

Out of the sequenced samples with occult HBV, two patients have retrievable hepatitis B DNA at the surface genome. There was a point mutation at the surface genome in patient number 23, at nucleotide 145 (C145T) of the surface region converting lycine (L) to phenyl alanine (F) at position 49 (L49F), also in patient number 26, a deletion at position (A401) was found leading to a frame shift in the amino acid sequences converting tyrosine (Y) to phenyl alanine (F) at position 134 (Y134F) ('a' determinant region), and consequently, all the following amino acids were different from the wild-type sequence. These changes may be responsible for occult HBV. On the other hand, there was a silent mutation at the surface gene in patient number 32 at position 475 (A475C) resulting in no change at the corresponding amino acids. Same patient (number 32) showed silent mutations in the core gene at the nucleotides 2050 (T2050C), 2269 (G2269A) and

Table 3 Biochemical parameters and cancer types associated with the occult HBV cases

Patient number	Sex	Age	ALT (SGPT) (7–40) IU/L	AST (SGOT) (7–37) IU/L	ALP (ALKP) (45–122) IU/L	Bilirubin total (0.3–1.2) mg/dL	Bilirubin Direct (0–0.2) mg/dL	WBC'S (4–11)*10^3/μL	Cancer type
3	M	13	47	34	108	0.8	0.2	10.8	Acute lymphocytic leukaemia
12	F	5	120	240	89	0.6	0.1	8.2	Neuroblastoma
23	M	11	265	190	321	0.3	0.16	7.24	Acute lymphocytic leukaemia
24	M	9	179	173	622	0.7	0.16	2.56	Acute lymphocytic leukaemia
26	M	8	130	170	155	0.6	0.2	5	Wilms' tumour
27	F	10	40	45	40	0.4	0.2	3.2	Neuroblastoma
28	F	12	87	60	323	0.4	0.2	6.62	Acute lymphocytic leukaemia
29	F	6	63	50	317	0.4	0.2	6.88	Acute lymphocytic leukaemia
32	F	4	210	127	110	1.3	0.02	4.46	Acute lymphocytic leukaemia
33	F	9	613	272	144	1.2	0.32	1.45	Acute lymphocytic leukaemia
36	M	16	87	54	69	1.2	0.17	8.53	Non-Hodgkin's lymphoma
37	M	9	21	25	231	0.5	0.17	3.73	Hodgkin's lymphoma
38	F	4	830	327	120	2.4	1.14	4.39	Wilms' tumour
40	M	12	77	58	294	0.2	0.13	3.65	Acute lymphocytic leukaemia
41	M	7	20	25	220	0.4	0.16	3.5	Hodgkin's lymphoma
42	M	13	370	173	104	0.7	0.16	6.7	Wilms' tumour

Table 4 HCV and HBV Viral levels, molecular and serological parameters associated with the occult HBV cases

Patient number	HCV copies*10^3/mL	HBV copies*10^3/mL	HBV nested PCR			HBc-Ab	HBs-Ab
			Core	Surface	X		
3	21.5	7.5	−	+	−	+	+
12	14 000	2.1	+	−	−	−	−
23	4.27	8.69	+	+	−	−	+
24	1270	27	+	−	−	−	−
26	8.7	396	−	+	−	+	+
27	291	8.77	+	+	−	+	−
28	600	1.49	+	+	−	+	−
29	445	0.562	+	+	−	−	−
32	35	0.769	+	+	−	−	+
33	895	12.6	+	−	−	−	−
36	492	8.15	+	+	+	−	−
37	0.580	1.22	+	+	−	−	−
38	4.482	13.4	+	+	−	+	−
40	0.380	14.3	+	+	−	−	−
41	2.7	1.22	+	+	−	−	−
42	360	1.1	+	−	−	−	+

2370 (A2370G) resulting in no change at the corresponding amino acids. There was also a silent mutation with patient 27 in the surface genome at nucleotide 71 (C71A) with no change at the corresponding amino acids.

All HBV patients in this study were infected with HBV D genotype.

DISCUSSION

Egypt has one of the world's highest prevalence rates of hepatitis C. Because it is common for hepatitis C patients to have dual infection with hepatitis B, we find it essential to monitor the existence of occult HBV infection in various

segments of the Egyptian population. In this study, we analyse the prevalence of occult HBV among paediatric cancer patients who are also HCV positive.

Hepatitis B virus infection in Egypt shows higher prevalence among males, with a male/female ratio (9:1). In addition, there is a high prevalence rate of negative variant of HBeAg (produced by proteolytic processing of the precore protein) and inactive HBsAg carrier states. HBeAg-negative variant state accounts for more than 80% among older age group (22–45 years). Undetectable HBsAg carriers represent nearly 33% of CHB infection [18]. In many cases, despite the serologic indication of circulating HBV DNA, HBsAg is undetectable, representing occult hepatitis B infection (OBI) [26,27].

As for the prevalence of occult HBV in the current study, it was found that among HBsAg-negative HCV cancer patients, there were 16 of 50 (31%) who had HBV DNA in their serum (OBI), and no cases were detected among a similar population of HCV-negative cancer patients as well as among the noncancer HCV patients. In a previous study, OBI prevalence in HCV patients was reported to be 28.1–50% in liver tissue [28–30] and 26.2–43.6% in serum [31–33]. The study by Fang *et al.* [34] revealed that 28.3% of cryptogenic chronic liver disease patients and 70.4% of HBsAg-negative HCC patients were positive for HBV DNA in serum. However, other reports documented prevalence range for OBI from 6.7 to 14.8% among chronic HCV patients [35,36], whereas in the study of Roman *et al.* [37], OBI was detected in 14.2% of the native population sample in Mexico. Moreover, De Matos *et al.* [38] detected serum occult HBV DNA in 12.7% of injecting drug users in Brazil.

In accordance with the study of Rossi *et al.* [39], which detected OBI in 10% among patients with chronic lymphocytic leukaemia, OBI was found in this study to be more frequent in patients with acute lymphoctic leukaemia (ALL). This finding may be because ALL is the most common childhood cancer and has a peak incidence from age 2 to 4 years as reported previously [40]. OBI may be prevalent among paediatric leukaemic patients, because blood transfusion for these patients is an obligatory therapeutic regime which may cause transmission of hepatotropic viruses as HCV and HBV.

As HBV and HCV have the same transmission routes, dual infection may occur [9]. The role of this dual infection in the pathogenesis of chronic liver disease is still controversial [3,9,24,41].

In this study, among all the sixteen HBV-HCV cancer patients, who were tested for HBc-Ab and HBs-Ab, only five patients had HBc-Ab, five patients had HBs-Ab, while only two patients were positive for both markers. This means that HBc-Ab and HBs-Ab could not be always taken as ideal markers for the detection of HBV DNA.

In our study, it was found that the majority of the HCV-HBV cancer patients (68.8%) had low level of HBV DNA (low viral load) due to suppression to both viruses; this matches with what have been reported by previous studies [25].

Our analyses revealed that there was a deletion at position (A401) leading to a frame shift in the amino acid sequences converting tyrosine (Y) to phenyl alanine (F) at position 134 (Y134F) ('a' determinant region), and consequently, the other amino acids beyond were changed in a different pattern compared with the wild-type sequence; this mutation might be leading to occult HBV infection because it can produce structural modifications in the S proteins, so that it is not recognized by current HBsAg immunoassays leading to a false or underestimated detection results. This result coincides with that found by Cheng *et al.* [42]. As reported by Raimondo *et al.*, a mutation in the 'a' determinant of the surface antigen was one of the earliest recognized mechanisms leading to occult HBV infection. Also, mutations in HBsAg lead to conformational changes rendering the protein undetectable by some of the commercially available HBsAg assays [19].

Carman *et al.* [17] reported the sG145R mutation in the 'a' determinant of HBsAg in a child who became infected with HBV despite active and passive immunoprophylaxis. In other studies, however, failure to detect HBsAg in OBI patients was not fully explained by S gene mutations [43,44]. Mutations outside the surface protein may also affect the HBV replication capacity; it is reported to be less 'replication fit' in comparison with the wild-type virus [45,46].

According to another study [47], the inability of some but not all commercial assays to detect HBsAg from samples could be attributed to mutations in the 'a' determinant. The emergence of 'a' determinant mutants is a serious health concern not only because they are not detectable by some commercial HBsAg assays but also because they can infect both unvaccinated and vaccinated individuals [47].

Another mutation was found at the surface region of one patient converting lycine (L) to phenyl alanine (F) at position 49 (L49F); we suggest that mutation at this position can be responsible for the occurrence of occult HBV. So, we recommend testing for occult HBV at patient with mutant surface antigen at this domain or region.

Our study showed that all of the occult HBV/HCV patients studied were infected by the D genotype of HBV. This result matches those obtained by Katsoulidou *et al.* [48] and Van *et al.* [49] who showed that the D genotype of HBV was prevalent in OBI patients from Greece, the Netherlands and Turkey (Europe and the Mediterranean region). Other HBV genotypes, including the C genotype, was found in OBI patients from China [50–52], as well as A genotype which has been identified in South Africa and G genotype has been found in France [52–54]. In the study of Hassan *et al.* [55], it was found that B or D genotype may influence the outcome of HBV infection, which may lead to the development of HCC among Egyptian OBI

patients. Previous studies have confirmed that HBV genotype D is more common in the Mediterranean area, the Middle East and India [53]. Consequently, it is not possible to conclude that the D genotype is the only genotype found in OBI. Clearly, other factors are to be considered and a more comprehensive study is needed in this field [56].

Hepatitis B virus occult infections have been reported in several clinical contexts, including (i) recovery from past infection indicated by the presence of hepatitis B surface antibody (anti-HBs); (ii) chronic hepatitis with surface gene escape mutants that are not recognized by current assays; (iii) chronic carriage without any marker of HBV infection other than HBV DNA (referred to as 'seronegative'); and (iv) most commonly in endemic areas, chronic carriage stage with HBsAg too low to be detected and recognized by the presence of anti-HBc as the only serological marker (referred to as 'anti-HBc alone' or 'isolated anti-HBc')[57]. Seropositive occult hepatitis B virus infection is characterized by the presence of anti-HBc and/or anti-HBs, while neither anti-HBc nor anti-HBs is detected in seronegative occult hepatitis B virus cases [19,58]. A good proportion of blood donors with occult hepatitis B virus infection have anti-HBc as the only serological marker of HBV infection [48,59]. Blood donors with isolated anti-HBc status are more infectious than those with low titres of anti-HBs [60].

CONCLUSION

We were able to detect occult HBV infection more frequently in HCV positive than in HCV-negative paediatric cancer patients or HCV paediatric noncancer patients. We detected the presence of OBI more frequently in leukaemic paediatric patients. HBsAg negativity is not sufficient to completely exclude HBV DNA presence. The presence of mutation in the 'a' determinant of the HBsAg genome confirmed previous finding that this determinant is highly likely to contribute to antibody binding in addition to the surface region where another mutation was found. Our findings emphasize the importance of considering occult HBV infection, through HBV DNA testing, in HCV-positive paediatric cancer patients in endemic areas as Egypt.

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AUTHORS' CONTRIBUTIONS

HER carried out the experimental procedures and participated in drafting the manuscript. ASY analysed the results and wrote the final version of manuscript. SAM suggested the concept, supervised the work and analysed the results. MSA and TM conceived and supervised the study. All authors read and approved the final manuscript.

CONFLICTS OF INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1 Rizzetto M. Hepatitis B vaccination: current status. *Minerva Gastroenterol Dietol* 1991; 45: 199–205.
2 World Health Organization (WHO): Annual meeting of the Safe Injection Global Network (SIGN). Injection Safety In Light Of Primary Health Care Reforms. WHO. Geneva, Switzerland, 2010, V 4.1, EB126/15.
3 Ayoola EA, Gadour MO. Hepatocellular carcinoma in Saudi Arabia: role of hepatitis B and C infection. *J Gastrenetrol Hepatol* 2004; 19: 665–669.
4 Hoofnagle JH. Reactivation of hepatitis B. *Hepatology* 2009; 49: S156–S165.
5 EASL–EORTC clinical practice guidelines: "management of hepatocellular carcinoma". *J Hepatol* 2012, 56: 908–943.
6 Shaaban FA, Hassanin AI, Samy SM, Salama SI, Said ZN. Long-term immunity to hepatitis B among a sample of fully vaccinated children in Cairo, Egypt. *East Mediterr Health J* 2007; 13: 750–757.
7 Zakaria S, Fouad R, Shaker O *et al.* Changing patterns of acute viral hepatitis at a major urban referral center in Egypt. *Clin Infect Dis* 2007; 44: e30–e36.
8 Frank C, Mohamed MK, Strickland GT *et al.* The role of parenteral antischistosomal therapy in the spread of HCV in Egypt. *Lancet* 2000; 355: 887–891.
9 Liu CJ, Chen DS, Chen PJ. Epidemiology of HBV infection in Asian blood donors: emphasis on occult HBV infection and the role of NAT. *J Clin Virol* 2006; 36: S33–S44.
10 Fattovich G, Tagger A, Brollo L *et al.* Hepatitis C virus in fection in chronic hepatitis B virus carriers. *J Infect Dis* 1991; 163: 400–402.
11 Crespo J, Lozano JL, de la Cruz F *et al.* Prevalence and significance of hepatitis C viremia in chronic active hepatitis B. *Am J Gastroeneterol* 1994; 89: 1147–1151.
12 Samal J, Kandpal M, Vivekanandan P. Molecular mechanisms underlying occult hepatitis B virus infection. *Clin Microbiol Rev* 2012; 25(1): 142.
13 Chisari FV, Klopchin K, Moriyama T *et al.* Molecular pathogenesis of hepatocellular carcinoma in hepatitis B virus transgenic mice. *Cell* 1989; 59: 1145–1156.
14 Besisik F, Karaca C, Akyüz F *et al.* Occult HBV infection and YMDD

variants in hemodialysis patients with chronic HCV infection. *J Hepatol* 2003; 38: 506–510.
15 Beck J, Nassal M. Hepatitis B virus replication. *World J Gastroenterol* 2007; 13(1): 48–64.
16 Bouchard MJ, Schneider RJ. The enigmatic X gene of hepatitis B virus. *J Virol* 2004; 78(23): 12725–12734.
17 Carman WF, Zanetti AR, Karayiannis P *et al.* Vaccine-induced escape mutant of hepatitis B virus. *Lancet* 1990; 336: 325–329.
18 El-Zayadi AR, Ibrahim EH, Badran HM *et al.* Anti-HBc screening in Egyptian blood donors reduces the risk of hepatitis B virus transmission. *Transfus Med* 2008; 18: 55–61.
19 Raimondo G, Allain JP, Brunetto MR *et al.* Statements from the Taormina expert meeting on occult hepatitis B virus infection. *J Hepatol* 2008; 49: 652–657.
20 Chaudhuri V, Tayal R, Nayak B, Acharya SK, Panda SK. Occult hepatitis B virus infection in chronic liver disease: full-length genome and analysis of mutant surface promoter. *Gastroenterology* 2004; 127: 1356–1371.
21 Saito T, Shinzawa H, Uchida T *et al.* Quantitative DNA analysis of low-level hepatitis B viremia in two patients with serologically negative chronic hepatitis B. *J Med Virol* 1999; 58: 325–331.
22 Berasain C, Betés M, Panizo A *et al.* Pathological and virological findings in patients with persistent hypertransaminasaemia of unknown aetiology. *Gut* 2000; 47: 429–435.
23 Honarkar Z, Alavian SM, Samiee S, Saeedfar K, Zali MR. Occult hepatitis B among chronic liver disease patients. *Saudi Med J* 2005; 26: 601–606.
24 Kazemi-Shirazi L, Petermann D, Muller C. Hepatitis B virus DNA in sera and liver tissue of HBsAg negative patients with chronic hepatitis C. *J Hepatol* 2000; 33(5): 785–790.
25 Cuchacovich R, Japa S, Huang WQ *et al.* Detection of bacterial DNA in Latin American patients with reactive arthritis by polymerase chain reaction and sequencing analysis. *J Rheumatol* 2002; 29: 1426–1429.
26 Allain JP. Occult hepatitis B virus infection. *Hepatitis B Ann* 2005; 2: 14–30.
27 De Mitri MS, Cassini R, Bernardi M. Hepatitis B virus-related hepatocarcinogenesis: molecular oncogenic potential of clear or occult infections. *Eur J Cancer* 2010; 46: 2178–2186.
28 Hou J, Wang Z, Cheng J *et al.* Prevalence of naturally occurring surface gene variants of hepatitis B virus in nonimmunized surface antigen-negative Chinese carriers. *Hepatology* 2001; 34(5): 1027–1034.
29 Jang JY, Jeong SW, Cheon SR *et al.* Clinical significance of occult hepatitis B virus infection in chronic hepatitis C patients. *Korean J Hepatol* 2011; 17: 206–212.
30 Lok A. Chronic hepatitis B. *N Engl J Med* 2002; 346(22): 1682–1683.
31 Georgiadou SP, Zachou K, Rigopoulou E *et al.* Occult hepatitis B virus infection in Greek patients with chronic hepatitis C and in patients with diverse nonviral hepatic diseases. *J Viral Hepatol* 2004; 11: 358–365.
32 Matsuoka S, Nirei K, Tamura A *et al.* Influence of occult hepatitis B virus coinfection on the incidence of fibrosis and hepatocellular carcinoma in chronic hepatitis C. *Intervirology* 2008; 51: 352–361.
33 Selim HS, Abou-Donia HA, Taha HA, El Azab GI, Bakry AF. Role of occult hepatitis B virus in chronic hepatitis C patients with flare of liver enzymes. *Eur J Intern Med* 2011; 22: 187–190.
34 Fang Y, Shang QL, Liu JY *et al.* Prevalence of occult hepatitis B virus infection among hepatopathy patients and healthy people in China. *J Infect* 2009; 58(5): 383–388.
35 Giannini E, Ceppa P, Botta F, Fasoli A, Romagnoli P, Ansaldi F. Previous hepatitis B virus infection is associated with worse disease stage and occult hepatitis B virus infection has low prevalence and pathogenicity in hepatitis C virus-positive patients. *Liver Int* 2003; 23: 12–18.
36 Kao JH, Chen PJ, Lai MY, Chen DS. Occult hepatitis B virus infection and clinical outcomes of patients with chronic hepatitis C. *J Clin Microbiol* 2002; 40: 4068–4071.
37 Roman S, Tanaka Y, Khan A *et al.* Occult hepatitis B in the genotype H-infected Nahuas and Huichol native Mexican population. *J Med Virol* 2010; 82(9): 1527–1536.
38 De Matos MA, Ferreira RC, Rodrigues FP *et al.* Occult hepatitis B virus infection among injecting drug users in the Central-West region of Brazil. *Mem Inst Oswaldo Cruz* 2013; 108(3): 386–389.
39 Rossi D, Sala L, Minisini R *et al.* Occult hepatitis B virus infection of peripheral blood mononuclear cells among treatment-naive patients with chronic lymphocytic leukemia. *Leuk Lymphoma* 2009; 50(4): 604–611.
40 MacDonald T. Pediatric cancer: a comprehensive review. Part I: biology, epidemiology, common tumours, principles of treatment and late effects. *Can Pharm J* 2010; 143(4): 176–183.
41 Shi J, Zhu L, Liu S, Xie WF. A meta-analysis of case control studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma in China. *Brit J Cancer* 2005; 92: 607–612.
42 Cheng AL, Hsiung CA, Su IJ *et al.* Steroid-free chemotherapy decreases risk of hepatitis B virus (HBV) reactivation in HBV-carriers with lymphoma. *Hepatology* 2003; 37: 1320–1328.
43 Banerjee A, Chandra PK, Datta S *et al.* Frequency and significance of hepatitis B virus surface gene variant circulating among 'antiHBc only' individuals in Eastern India. *J Clin Virol* 2007; 40: 312–317.
44 Brojer E, Grabarczyk P, Liszewski G, Mikulska M, Allain JP, Letowska M. Characterization of HBV DNA+/HbsAg- blood donors in Poland identified by triplex NAT. *Hepatology* 2006; 44: 1666–1674.
45 Pollicino T, Belloni L, Raffa G *et al.* Hepatitis B virus replication is regulated by the acetylation status of hepatitis B virus cccDNA-bound H3 and H4 histones. *Gastroenterology* 2006; 130: 823–837.
46 Sheldon J, Rodes B, Zoulim F, Bartholomeusz A, Soriano V. Mutations affecting the replication capacity of

the hepatitis B virus. *J Viral Hepat* 2006; 13: 427–434.
47 La'ulu SL, Roberts WL. The analytic sensitivity and mutant detection capability of six hepatitis B surface antigen assays. *Am J Clin Pathol* 2006; 125: 748–751.
48 Katsoulidou A, Paraskevis D, Magiorkinis E *et al.* Molecular characterization of occult hepatitis B cases in Greek blood donors. *J Med Virol* 2009; 81(5): 815–825.
49 Van Hemert FJ, Zaaijer HL, Berkhout B, Lukashov VV. Occult hepatitis B infection: an evolutionary scenario. *Virol J* 2008; 5(146): 146.
50 Fang Y, Teng X, Xu WZ *et al.* Molecular characterization and functional analysis of occult hepatitis B virus infection in Chinese patients infected with genotype C. *J Med Virol* 2009; 81(5): 826–835.
51 Fang ZL, Zhuang H, Wang XY, Ge XM, Harrison TJ. Hepatitis B virus genotypes, phylogeny and occult infection in a region with a high incidence of hepatocellular carcinoma in China. *World J Gastroenterol* 2004; 10(22): 3264–3268.
52 Datta S, Banerjee A, Chandra PK, Chowdhury A, Chakravarty R. Genotype, phylogenetic analysis, and transmission pattern of occult hepatitis B virus (HBV) infection in families of asymptomatic HBsAg carriers. *J Med Virol* 2006; 78(1): 53–59.
53 Kumar GT, Kazim SN, Kumar M *et al.* Hepatitis B virus genotypes and hepatitis B surface antigen mutations in family contacts of hepatitis B virus infected patients with occult hepatitis B virus infection. *J Gastroenterol Hepatol* 2009; 24(4): 588–598.
54 Candotti D, Grabarczyk P, Ghiazza P *et al.* Characterization of occult hepatitis B virus from blood donors carrying genotype A2 or genotype D strains. *J Hepatol* 2008; 49(4): 537–547.
55 Hassan ZK, Hafez MM, Mansor TM, Zekri AN. Occult HBV infection among Egyptian hepatocellular carcinoma patients. *Virol J* 2011; 8(1): 90.
56 Arababadi MK, Pourfathollah AA, Jafarzadeh A *et al.* Hepatitis B virus genotype, HBsAg mutations and co-infection with HCV in occult HBV infection. *Hepatol Gastroenterol* 2011; 35: 554–559.
57 Allain JP. Occult hepatitis B virus infection. *Transfus Clin Biol* 2004; 11: 18–25.
58 Torbenson M, Thomas DL. Occult hepatitis B. *Lancet Infect Dis* 2002; 2: 479–486.
59 Silva CM, Costi C, Costa C *et al.* Low rate of occult hepatitis B virus infection among anti-HBc positive blood donors living in a low prevalence region in *Brazil*. *J Infect* 2005; 51: 24–29.
60 Allain JP, Cox L. Challenges in hepatitis B detection among blood donors. *Curr Opin Hematol* 2011; 18: 46–466.