Palestinian Medical and Pharmaceutical Journal (PMPJ). 2020; 5(1): 55-64

Prevalence of Occult Hepatitis B among Multi-Transfused Patients in the Northern Districts of the Occupied Palestinian Territories

Adham Abu Taha[1, 2], Mays Inirat[3], Nehaya Al-Kharraz[3], Momen Nassar[3], Qusay Abdoh[3, 4]*

[1]Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, Palestine. [2]Department of Anatomic and Clinical Pathology, An-Najah National University Hospital, Nablus, Palestine. [3]Department of Medicine, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, Palestine. [4]Unit of Gastroenterology, Department of Internal Medicine, An-Najah National University Hospital, Nablus, Palestine

*Corresponding author: aabutaha@najah.edu

Received: (18/6/2018), Accepted: (7/9/2019)

ABSTRACT

Transfusion-transmitted infections including hepatitis B virus (HBV) have been a major public health concern worldwide. Occult HBV infection (OBI) is defined as the presence of HBV DNA in the liver (with detectable or undetectable HBV DNA in the serum) of individuals who tested negative for HBsAg. Clinically, OBI is considered a risk for viral reactivation and possible transmission of infection among individuals. It also contributes to liver disease progression and development of hepatocellular carcinoma. Better knowledge and strategies to prevent, diagnose and manage must be implemented in this field. The aim of this study was to estimate the prevalence of OBI among multi-transfused patients in the northern districts of the occupied Palestinian territories. A prospective cross-sectional study was conducted in the period from August 2016 to May 2017 on 169 who were negative for HBsAg and received more than 10 units of packed RBCs throughout their lives were included in the study. Blood samples were tested for HBsAg, anti-HBs and anti-HBc by ELISA. Those positive to anti-HBc were then retested for HBV DNA, using an automated real-time PCR method. Among the 169 HBsAg- negative sera, anti-HBc was detected in 35 patients (20.7%). All anti-HBc-positive patients were HBV DNA-negative, and 27 (77.1%) of them were anti-HBs-positive. Based on our results, it seems that there were no cases of OBI among poly-transfused patients with thalassemia and sickle cell anemia. However, to improve decisions concerning OBI screening, especially in transfusion centers, more original studies with more precise laboratory techniques and larger sample sizes are needed.

Keywords: Occult HBV; Anti-Hbc; HBV-DNA; Multi-Transfused Thalassemia.

INTRODUCTION

Over the past few decades, regular blood transfusions and iron chelating therapies in multiply-transfused patients such as patients of thalassemia major, hemophilia or sickle cell disease, have substantially improved their overall survival [1]. However, these recurrent transfusions may result in iron overload and carry a definite risk of infection with blood-borne viruses such as human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) [2]. Among these infections, HBV infection is the most common disease commuted through blood transfusion [3] and it remains the primary cause of liver cirrhosis and hepatocellular carcinoma (HCC), which are major contributors to global mortality.

While most infectious blood units are removed by screening for hepatitis B surface antigen (HBsAg), these assays remain unable to detect infection in the pre-seroconversion window period and in samples with very low viral load after decades of chronicity or clinical recovery which is known as occult HBV infection (OBI) [4]. OBI defined as the presence of hepatitis B virus DNA (HBV DNA) without HBsAg, with or without the presence of HBV antibodies [5]. Most OBIs are asymptomatic and would only be detected by systematic screening of large populations [4]. It has been a matter of debate for years and may impact in several clinical aspects, in-

cluding the liver disease progression, risk of HCC, transmission of the infection by blood transfusion or organ transplantation and its acute reactivation when an immunosuppressive status occurs, all these clinical impact make it very important to detect it especially in high risk patients such as HCV patients and multiply-transfused patient.

As the prevalence of OBI tends to be higher where the prevalence of overt HBV infection is high [6], there may be a relatively high prevalence of OBI in Palestine since it is categorized as an HB virus moderate endemic area, with the HB carrier rate ranging from 2-6 % [7]. All of these translating to an increasing number of patients who may be at risk of chronic HBV infection and its consequences. Against this background, we sought to investigate the prevalence of occult hepatitis B infection among multiply-transfused patients in the North of Palestine, to enable us make evidence-based recommendations for effective HBV screening helping prevent and control this prevalent health problem.

METHODS

A prospective cross-sectional study was conducted in the period from August 2016 to May 2017 on 169 patients of different hematological disorders. Patients who received blood transfusions at three blood transfusion centers located in the governmental hospitals of Tulkarm (Thabet thabet governmental Hospital), Nablus (Al-Watani governmental Hospital) and Jenin (Shaheed Dr. Khalil Suleiman Governmental Hospital) cities were included in the study.

Ethical consideration

Approvals were obtained from the Institutional Review Board Committee (IRB) at Najah National University and the Palestine Ministry of Health (MOH).

Participation in the study was voluntary and informed written consent was obtained from the patients before the study.

Selection of study population

Recipients of at least 10 units of Packed Red blood cells (RBCs) throughout their lives and are negative for HBsAg were included in the study. With the aid of structured data sheet, relevant demographic data were obtained from participants, including age, gender, age at the first transfused unit, history of splenectomy, and use of chelating agents. Lab tests results were also obtained from the medical records and included AST/ALT, and ferritin levels.

Selected participants were tested for the presence of anti-Hepatitis B core antibodies, anti- Hepatitis B surface antibodies, and Hepatitis B Virus DNA. (Figure 1).

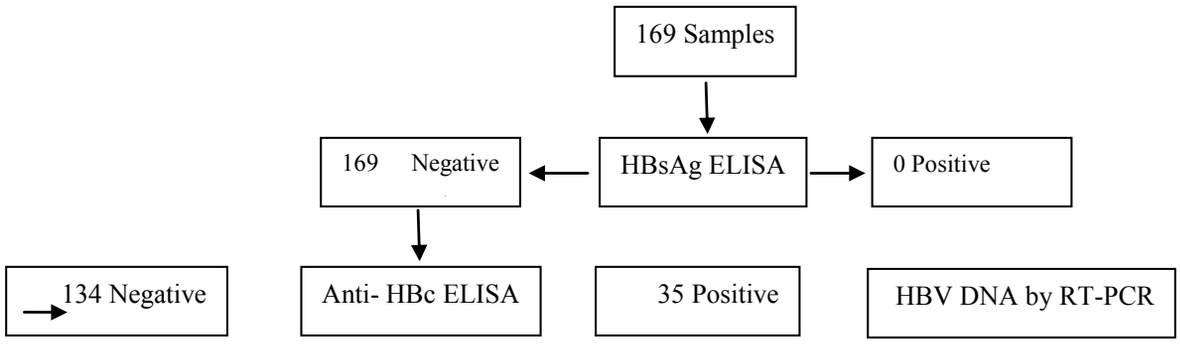

Figure (1): Flowchart of the Study.

Serologic analysis

The serological studies were done at the Laboratory of An-Najah National University Hospital in Nablus. All samples were screened again for HBsAg using Dialab® HBsAg ELISA kit (Dialab, GmbH, Austria). Samples were screened for the presence of total anti-HBc antibodies using One-step ELISA kit (MBS, Milan Italy). Titers of anti-HBs antibodies were measured using Dialab® HBsAb ELISA kit (Dialab, GmbH, Austria). All serological tests were done according to manufacturers' instructions.

Hbv dna analysis

Plasma of participants who tested positive for anti-HBc antibodies were subjected to Nucleic Acid Amplification Testing (NAT). Viral DNA was extracted from plasma samples by Spin Column method using an QIAamp Ultrasense Virus Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Plasma HBV DNA was quantified by real-time Polymerase chain reaction (PCR) using automated system. Real-time PCR was performed with an ABI 7500 Detection System (Applied Biosystems, Foster City, CA) using universal thermal profile. Real Time PCR results were also confirmed with another available Real Time PCR assay: Abbott Real-Time HBV assay with a lower detection limit of 10 IU/m (Abbott Laboratories, USA).

Data analysis

Data were statistically described in terms of range, mean ± standard deviation (SD), frequencies, and percentages when appropriate. Comparison of quantitative variables between the study groups was done using the Independent-Sample T Test. For comparing categorical data, a Chi-square ($\chi 2$) test was performed, and a probability value (P value) less than 0.05 was considered statistically significant. Binary logistic regression was used, to asses significance among variables.

All statistical calculations were done using SPSS (SPSS Inc., Chicago, IL, USA) version 21 for Microsoft Windows.

RESULTS

This study included 169 patients with different hematological disorders who receive multiple blood units, of whom 83 (49.1%) were males and 86 (50.9%) were females. Their ages ranged from 2- 71years (mean of 20.2 ± 10.1) and half of them were 19 years of age and younger (Table 1)

Three centers were enrolled in our study; 86 patients (50.9%) from Nablus, 43 (25.4%) from Jenin, 40 (23.7%) from Tulkarm. Regarding the diagnosis, there were 117 patients (69.2%) with thalassemia major, 15 (8.9%) thalassemia intermedia, 11 (6.5%) sickle cell anemia, and 26 (15.4 %) sickle-thalassemia. The mean number of transfused-units was 235.5 ± 204.280. 82 patients (48.5%) had undergone splenectomy. 140 patients (82.8%) were on chelating therapy. (Table 1)

Table (1): Characteristics of participants.

Characters		N	%
Center	Jenin	43	25.4
	Nablus	86	51
	Tulkarm	40	23.7
Disorder	Thalassemia major	117	69.2
	Thalassemia intermedia	15	8.9
	Sickle cell	11	6.5
	Sickle-Thalassemia	26	15.4
Gender	Male	83	49.1
	Female	86	50.9
Age	24 or less	120	71
	More than 24	49	29
The mean number of transfused units		235.5 ± 204.3	
Splenectomy	Yes	82	48.5
	No	87	51.5
Use of chelating agents	Yes	140	82.8
	No	29	17.2

Liver function tests were at the upper limit of normal while ferritin levels were extremely high despite the fact the majority (82.8%) of participants were on chelating agents. (Table 2 &1)

Table (2): Results of biochemistry lab tests of participants.

Lab test	Mean ± SD
ALT	42.7 ± 36.9
AST	48.9 ± 33.3
Ferritin	4187.7 ± 5292.1

Serological lab tests showed that seventy-five percent of patients were positive for anti-HBsHBcAb was detected in 35 patients (20.7%). (Table 3)

Table (3): Results of Serological lab tests of participants.

		N	%
Anti-HBc	Positive	35	21
	Negative	134	79
Anti-HBs	Positive	127	75.1
	Negative	42	24.9

All anti-HBc-positive patients were HBV DNA-negative; therefore, the prevalence of occult HBV is zero. Among the anti-HBc-positive patients, 27 (77.1%) of were positive for anti-HBs-positive. (Table 4)

In this study, no significant difference was found between negative and positive anti-HBc patients regarding their distribution according to center, hematologic disorder, gender, ferritin levels, ALT, or AST. (Table 4 &5)

However, after binary logistic regression was used, no significance was found with any variable.

Table (4): Characteristics of positive and negative HBcAb patients.

Variables				***P* value**
		Anti-HBc		
		Positive: n (%)	**Negative: n (%)**	
Center	Jenin	10 (28.6)	33 (24.6)	0.341
	Nablus	20 (57.1)	66 (49.3)	
	Tulkarm	5 (14.3)	35 (26.1)	
Disorder	Thalassemia major	29 (82.9)	88 (65.7)	0.11
	Thalassemia intermedia	0	15 (11.2)	
	Sickle cell	1 (2.9)	10 (7.5)	
	Sickle-Thalassemia	5 (14.3)	21 (15.7)	
Gender	Male	21 (60)	62 (46.3)	0.148
	Female	14 (40)	72 (53.7)	
Age	24 or less	20 (57.1)	100 (74.6)	0.042*
	More than 24	15 (42.9)	34 (25.4)	
Number of transfused units		322.97± 325.4	212.63± 151.98	0.004*
Splenectomy	Yes	23 (65.7)	59 (44)	0.022*
	No	12 (34.3)	75 (56)	
Use of chelating agents	Yes	33 (94.3)	107 (79.9)	0.044*
	No	2 (5.7)	27 (20.1)	
Anti-HBs	Positive	27 (77.1)	100 (74.6)	0.759
	Negative	8 (22.9)	34 (24.4)	
ALT		41.84± 42.886	42.86± 35.293	0.885
AST		52.84± 43.741	47.81± 30.173	0.428
Ferritin		3307.64± 3202.010	4417.60± 5700.776	0.27
		Anti-HBs		
		Positive	Negative	
Age	24 or less	91 (71.7)	29 (69)	0.747
	More than 24	36 (28.3)	13 (31)	

DISCUSSION

Hepatitis B virus (HBV) infection is the most common disease commuted through blood transfusion and it is considered a global public health issue. Worldwide, approximately two billion people showed serological evidence of past or current HBV infection. Investigations have reported that a lot of individuals suffer from the long term forms of HBV infection including chronic, asymptomatic and occult hepatitis B infection (OBI) [3]. According to the World Health Organization (WHO), more than 780 thousand people die every year as a consequence of acute or chronic HBV infection [8]. HBV has a higher residual risk of transmission by transfusion than HCV or HIV. Also HBV is second only to tobacco as a known human carcinogen [9].

While most infectious blood units are discarded by screening for hepatitis B surface antigen (HBsAg)- the initial test used in screening for HBV infection in Palestine and many regions in the world- anti-HBc is not being used as a screening test to determine previous exposure to the HBV. However, there is clear evidence that transmission by HBsAg negative components occurs during the serologically negative window period and the late stages of infection [10].

The clinical impact of OBI especially in high risk groups as described previously, lead to the conduction of many studies in this field aiming to gain better understanding and improving methods of detection. Although The gold standard for OBI diagnosis is the detection of HBV DNA in the DNA extraction from the liver, as covalently closed circular HBV DNA (ccc) DNA (cccDNA) persists in the hepatocytes and HBV DNA is sometimes detected in the liver in the absence of HBV DNA in the serum, obtaining liver tissue is an invasive procedure and difficult to obtain in clinical practice. Hence, serum HBV DNA assays are widely used to diagnose OBI [11].

Many studies indicate that the potential risk of acquiring occult hepatitis B virus infection is higher in patients receiving multiple and frequent blood transfusions. The prevalence rates are widely variable reported to range from 1% to 95% worldwide among different target groups and are influenced by several factors (table 4) as follows: 1) geographic differences (endemicity) (2) different patient characteristics, including the presence of comorbid diseases such as chronic hepatitis C; and (3) and the different diagnostic techniques used, which have different sensitivity. The type of sample used (liver or serum) or number of samplings can also have some effect on the diagnosis of OBI. Indeed, as serum HBV DNA levels seem to fluctuate in OBI, serial sample is more useful to identify OBI [4].

To the best of our knowledge, this is the first study that investigates the OBI prevalence among multi-transfused patients in Palestine. We included 169 multiply - transfused patients diagnosed with thalassemia major, thalassemia intermedia, sickle cell disease, and sickle-thalassemia; who were HBsAg negative. 35 patients (20.7%) found to be positive anti-HBc. All anti-HBc-positive patients were HBV DNA-negative, and 27 (77.1%) of them were anti-HBs-positive. This suggests a cleared previous HBV infection. Similar to our results, an Iranian cross-sectional study showed that there was no detectable HBV DNA among thalassemic patients who have chronic hepatitis C infection [12]. In 2015, a cohort Australian study found that only 0.69% of patients were HBsAg negative, anti-HBc positive and HBV DNA positive [13].

On the other hand, Singh et al. found that OBI had a prevalence of 31.4% among thalassemic patients [14]. Another more recent Indian study reported that HBV DNA was detected in 50 % of thalassemic pediatric patients who had anti-HBc as the only marker, and it was detected in 16.12

% of cases who were sero-positive for both anti-HBs and anti-HBc [15].

Among the Arab world, most studies were done among different groups of Egyptian population with different diagnostic methods. A study conducted in 2010 revealed that the prevalence of OBI among thalassemic patients was 32.5% [9].

Despite no OBI cases were detected in this study, attention must be directed toward those with anti-HBC positive, 35 cases (20.7%), since it was observed that anti-HBc positive/HBV-DNA negative patients showed a similar prevalence of severe liver disease to anti-HBc positive/HBV-DNA positive patients and a significantly higher prevalence than anti-HBc negative cases. This notion raises the clinical significance of isolated positive anti-HBc antibody in relation to liver disease [16].

Isolated anti-HBc is defined as the presence of anti-HBc in the serum without HBsAg or HBsAb. In our study, 8 patients of the positive anti-HBc group (22.9%) were anti-HBs negative. This has importance in clinical practice especially in candidate patients for chemotherapy and immunosuppressive therapy that requires further investigation because of the risk of HBV reactivation. Isolated anti-HBc may be due to resolved HBV infection in which HBsAb had declined to an undetectable level, testing during the window period or chronic infection, in which HBsAg cannot be detected due to protein mutation, makes it undetectable by certain diagnostic assays [17] so all these causes of isolated anti-HBc only distinguishable if the additional assays are done and measures of liver damage are taken into account. Measurement of serum anti-HBs responses after the administration of HBV vaccination can be useful to distinguish this serological profile [18]. Newly published Egyptian study revealed that 15.19% of HBsAg negative patients were positive for anti-HBc and anti-HBs negative. Those anti-HBc positive patients were tested for HBV DNA, and it was detected in 41.67% of them. Concluding that OBI prevalence was 6.33% [17].

Patients who were born before 1992 had more positive anti-HBc results than the younger (30.6% vs. 16.7%, P = 0.042). This most likely supports the effectiveness of the HBV vaccination program that was adopted in Palestine in 1992. However, the anti-HBs titers were not significantly related with the age of patients neither with anti-HBc results.

Moreover, anti-HBc positive results were significantly linked to the number of transfused units (mean= 322.97 ± 325.421, P =0.004). This result is in agreement with that of Sabat et al. study, on 174 thalassemia patients in India, in which anti-HBc was 12, 26.8, and 71.4% in patients who received less than 40, 40-80, and greater than 80 units of transfusions, respectively. Their prevalence of anti-HBc was reported to be 21.8% and anti-HBc was found to be the only marker in six patients (3.45%) [19].

Splenectomy has been a common management strategy for reducing regular transfusion requirements, iron overload, and extramedullary hematopoiesis in patients with hemoglobinopathies (e.g., thalassemia major, sickle thalassemia). 82 of our patients (48.5%) had undergone splenectomy, and 28% of them were found to be positive anti-HBc (vs. 13.8% among patients who have spleen, P = 0.022). This raises a question about the clinical impact of splenectomy regarding increased risk of viral infections. A cohort Australian research studied different infection outcomes in 63 transfusion-dependent patients with different hemoglobinopathies and recorded 30 cases of blood-borne viral infections. The infection rate for hepatitis C, hepatitis B, and HIV was 1.7 per 100 person-years in splenectomized patients and 1.5 per 100 person-years in the non-splenectomized patients; however, the p-value was not significant. 5 patients had been diagnosed with HBV infection (acute and chronic) prior to implementing of blood units screening with HBsAg or HBcAb. 4 of them were splenectomized [20]. Our results showed that splenectomized patients had

significantly higher numbers of transfused-units than non-splenectomized patients (P = 0.0001). This can probably provide an explanation for the significance that was found between the anti-HBc and splenectomy.

Interestingly, anti-HBc was significantly (P = 0.044) associated with chelating agents use, which are being used among 82.8% of our patients. However, the mean of ferritin level among those on chelation therapy was slightly higher than non-users (4279.98 ± 5250.260 vs. 3742.37 ± 5563.354 ng/ml, P = 0.620) and as overall both means were inappropriately very high. Moreover, we noticed that the ferritin level and liver enzymes, ALT and AST, were significantly positively correlated (Table 5), which could be explained by hepatocytes damage due to transfusion-induced iron overload. These findings are concurrent with the results of Suman et al. [21], Asif M. et al. [22], and others. Based on our notes, we believe these results may reflect an inadequate chelation or poor compliance from the patients. Unfortunately, this makes them susceptible to iron-overload complications, ranging from multi-organ damage to vulnerability to bacterial and non-bacterial infections. There is an evidence confirming the persistence and resistance to treatment of viral hepatitis in the presence of excess iron. Blumberg et al. were the pioneers in describing a role for iron in the modulation of hepatitis B virus. They found that patients with higher levels of serum iron or ferritin were less likely to achieve spontaneous recovery after acute HBV infection [23].

Table (5): Correlations between different variables.

		ALT	AST	Ferritin	Patient's Age	HBsAb Titer
ALT	Pearson Correlation	1	.737**	.444**	-.043	.102
	Sig. (2-tailed)		.000	.000	.580	.187
	N	169	169	169	169	169
AST	Pearson Correlation	.737**	1	.324**	.019	.151
	Sig. (2-tailed)	.000		.000	.807	.050
	N	169	169	169	169	169
Ferritin	Pearson Correlation	.444**	.324**	1	.016	-.056
	Sig. (2-tailed)	.000	.000		.834	.471
	N	169	169	169	169	169
Patient's Age	Pearson Correlation	-.043	.019	.016	1	.000
	Sig. (2-tailed)	.580	.807	.834		.997
	N	169	169	169	169	169
HBsAb Titer	Pearson Correlation	.102	.151	-.056	.000	1
	Sig. (2-tailed)	.187	.050	.471	.997	
	N	169	169	169	169	169
**** Correlation is significant at the 0.01 level (2-tailed).**						

AST: Aspartate Aminotransferase, **ALT:** Alanine Aminotransferase, **HBsAb:** Hepatitis B Surface Antibody.

In this study, no significant difference was found between negative and positive anti-HBc patients regarding their distribution according to center, hematologic disorder, gender, ferritin levels, ALT, or AST.

However, after binary logistic regression was used, no significance was found with any variable. This indicates that there is no single significant predictor of the anti-HBc presence due to the interactions between different variables.

Limitations of the study

There was a discrepancy between the reported and actual number of patients attending these transfusion centres. The population of this study and its sample size is relatively small. In addition, sickle cell patients and those patients who had splenectomy were not on a regular schedule to get their transfusion. Moreover, patients' medical records lacked essential relevant information including vaccination status, presence of co-morbidities, and some laboratory tests. Lastly, although the detection of HBV DNA in liver biopsy is the gold standard for diagnosis of OBI, it was not practical or possible.

CONCLUSIONS

Our results showed no cases of OBI among multi-transfused thalassemia and sickle cell disease patients in the northern of districts of Palestine. However, to improve decisions concerning OBI screening and better understanding of the epidemiology of OBI and the significance of isolated anti-HBc, further studies with larger sample size are needed. We also suggest considering anti-HBc screening in high risk groups and a better follow up for such patients regarding proper vaccinations, regular physical examination and investigations, and prevention of transfusion related complications.

ACKNOWLEDGEMENT

We thank Mr. Issa Shtayeh head of molecular labs at the Palestine Ministry of Health for the DNA analysis, Prof. Waleed Sweileh for his help in statistical analysis, Mr. Ahmad Hussein from the lab department of An-Najah National University Hospital and all workers in blood banks and transfusion centers in Jenin, Nablus and Tulkarm for their valuable help in data collection.

CONFLICT OF INTEREST

Authors declare no conflict of interest in this study.

FUNDING

This work was funded by the Faculty of Medicine and Health Sciences at An-Najah National University.

REFERENCES

1) Asif M, Manzoor Z, Farooq MS, Munawar SH, Aziz A, Khan IA. Status of oxidant, antioxidantand serum enzymes in thalassaemic children receiving multiple blood transfusions. J Pak Med Assoc. 2015; 65(8): 838-43.

2) Chakrabarty P, Rudra S, Hossain MA. Prevalence of HBV and HCV among the multi-transfused beta thalassemic major patients in a day care centre of blood transfusion department of Mymensingh Medical College Hospital. Mymensingh Med J. 2014; 23(2): 235-41.

3) Arababadi MK, Nasiri Ahmadabadi B, Yousefi Daredor H, Kennedy D. Epidemiology of occult hepatitis B infection among thalassemic, hemophilia, and hemodialysis patients. Hepat Mon. 2012; 12(5): 315-9.

4) Kwak MS, Kim YJ. Occult hepatitis B virus infection. World J Hepatol. 2014; 6(12): 860-9.

5) Allain JP. Occult hepatitis B virus infection. Transfus Clin Biol. 2004; 11(1): 18-25.

6) Olotu AA, Oyelese AO, Salawu L, Audu RA, Okwuraiwe AP, Aboderin AO. Occult Hepatitis B virus infection in previously screened, blood donors in Ile-Ife, Nigeria: implications for blood transfusion and stem cell transplantation. Virol J. 2016; 13: 76.

7) Nazzal Z, Sobuh I. Risk factors of hepatitis B transmission in northern Palestine: a case - control study. BMC Res Notes. 2014; 7: 190.

8) Moraes TC, Fiaccadori FS, Souza M, Almeida TN, Cunha MD, de Araujo Castro I, et al. Hepatitis B virus infection among institutionalized mentally ill patients in Brazil. Braz J Infect Dis. 2015.

9) Shaker O, Ahmed A, Abdel Satar I, El Ahl H, Shousha W, Doss W. Occult hepatitis B in Egyptian thalassemic children. J Infect Dev Ctries. 2012; 6(4): 340-6.

10) Allain JP. Occult hepatitis B virus infection: implications in transfusion. Vox Sang. 2004; 86(2): 83-91.

11) Ocana S, Casas ML, Buhigas I, Lledo JL. Diagnostic strategy for occult hepatitis B virus infection. World J Gastroenterol. 2011; 17(12): 1553-7.

12) Arababadi MK, Hassanshahi G, Yousefi H, Zarandi ER, Moradi M, Mahmoodi M. No detected hepatitis B virus-DNA in thalassemic patients infected by hepatitis C virus in Kerman province of Iran. Pak J Biol Sci. 2008; 11(13): 1738-41.

13) Martinez MC, Kok CC, Baleriola C, Robertson P, Rawlinson WD. Investigation of occult hepatitis B virus infection in anti-hbc positive patients from a liver clinic. PLoS One. 2015; 10(3): e0117275.

14) Singh H, Pradhan M, Singh RL, Phadke S, Naik SR, Aggarwal R, et al. High frequency of hepatitis B virus infection in patients with beta-thalassemia receiving multiple transfusions. Vox Sang. 2003; 84(4): 292-9.

15) Sabat J, Dwibedi B, Dash L, Kar SK. Occult HBV infection in multi transfused thalassemia patients. Indian J Pediatr. 2015; 82(3): 240-4.

16) Emara MH. Occult hepatitis B: the Egyptian situation. Trop Gastroenterol. 2012; 33(4): 242-50.

17) Elbedewy T, Noreldin N, Hamam S. Prevalence and significance of hepatitis B core antibodies among hepatitis B surface antigen-negative Egyptian polytransfused adult patients. Egypt J Haematol 2015; 40(3): 143.

18) Wang Q, Klenerman P, Semmo N. Significance of anti-HBc alone serological status in clinical practice. Lancet Gastroenterol Hepatol. 2017; 2(2): 123-34.

19) Elbedewy TA, Elashtokhy HE, Rabee ES, Kheder GE. Prevalence and chemotherapy-induced reactivation of occult hepatitis B virus among hepatitis B surface antigen negative patients with diffuse large B-cell lymphoma: significance of hepatitis B core antibodies screening. J Egypt Natl Canc Inst. 2015; 27(1): 11-8.

20) Yapp AR, Lindeman R, Gilroy N, Gao Z, Macintyre CR. Infection outcomes in splenectomized patients with hemoglobinopathies in Australia. Int J Infect Dis. 2009; 13(6): 696-700.

21) Suman R, Sanadhya A, Meena P, Goyal S. Correlation of liver enzymes with serum ferritin levels in beta-thalassemia major. International Journal of Research in Medical Sciences. 2016: 3271-4.

22) Asif M, Manzoor Z, Farooq M, Kanwal A, Shaheen U, Munawar S, et al. Correlation between serum ferritin level and liver function tests in thalassemic patients receiving multiple blood transfusions. Int J Res Med Sci. 2014; 2(3): 988-94.

23) Khan FA, Fisher MA, Khakoo RA. Association of hemochromatosis with infectious diseases: expanding spectrum. Int J Infect Dis. 2007; 11(6): 482-7.