International Journal of Scientific Research in Science, Engineering and Technology (www.ijsrset.com)
© 2020 IJSRSET | Volume 7 | Issue 1 | Print ISSN: 2395-1990 | Online ISSN : 2394-4099
DOI : https://doi.org/10.32628/IJSRSET207112

Prevalence of Occult Hepatitis B Virus Infections Among Kidney Transplant Patients in Khartoum State, Sudan

Mashaer M Mustafa[1], Abdel Rahim M El Hussein[2], Isam M Elkhidir[3] and Khalid A Enan[2*]

[1]Department of Medical microbiology, Faculty of Medical Laboratory Science, El Neelain University, Khartoum, Sudan

[2]Department of Virology, Central Laboratory- The Ministry of Higher Education and Scientific Research, P.O. Box 7099, Khartoum, Sudan

[3]Department of Microbiology and Parasitology, Faculty of Medicine, University of Khartoum, Khartoum, Sudan

***Corresponding Author**: Khalid A Enan, Department of Virology, Department of Virology Central Laboratory, The Ministry of Higher, Education and Scientific Research, P.O. Box 7099, Khartoum, Sudan

ABSTRACT

This study was carried out to detect occult hepatitis B virus (OHB) among renal transplant patients in Khartoum State, Sudan. Antigen capture enzyme linked immunosorbent assay (ELISA) competitive ELISA and polymerase chain reaction (PCR) were used to respectively detect hepatitis B surface antigen (HBsAg), ; Hepatitis B core antibody (HBcAb) and hepatitis B virus (HBV) DNA in 100 plasma samples collected from patients during the period from May to October 2018 . Out of the 100 patients sampled, 70 were males and 30 were females (age 15 to 65 years) and none of these patients showed signs of clinical hepatitis. The results showed that 3 out of the 100 samples were positive for HBsAg, and were subsequently excluded from the study. Out of the remaining HBsAg negative 97 samples, 39(40.2%) showed positive HBcAb and none (0%) tested positive to HBV DNA using competitive ELISA and PCR, respectively. These results indicated that more investigations including more patients from other transplant centres are needed to fully elucidate the situation of occult hepatitis B in renal transplant patients in Sudan.

Keywords : HBV, ELISA, PCR, Renal transplant, Sudan.

I. INTRODUCTION

Hepatitis B virus (HBV) is a species of the genus Orthohepadnavirus, which belongs to the family of Hepadnaviridae(1).

HBV is highly contagious, and is the most commonly transmitted blood borne virus in the health care setting. In such setting, transmission generally occurs from patient to patient or from patients to health care personnel via contaminated instruments or accidental needle-stick or sharps injuries (2).

Despite the availability of an efficacious vaccine;Hepatitis B virus (HBV) infection still represents a major global problem; with approximately 2 billion people infected worldwide with 600 000 deaths each year as a result of either acute or chronic infections with the virus.

The diagnosis for HBV infection is made following serologic tests for the virus such as ELISA that are used to detect HBsAg, HBsAb and HBcAb or by molecular biological techniques such as polymerase

chain reaction (PCR) used to detect HBV DNA. (Ke-Qin, 2002) [3].

Occult hepatitis B virus infection (OBI) is defined as the presence of hepatitis B virus (HBV) DNA in the blood and/or in the liver samples of individuals testing negative to hepatitis B surface antigen (HBsAg) with or without antibodies to hepatitis B core antigen (anti-HBc) or antibodies to HBsAg (anti-HBs) in both index and follow up samples[4-5.] OBI differs from the window period (WP) of the acute primary infection although both are recognized by the absence of HBsAg in index blood samples; however subsequent follow- up samples after WP will usually test positive for HBsAg but negative for OBI.[5-6]. End-stage renal disease (ESRD) patients may be at risk for OHB infection due to their use of hemodialysis and history of blood transfusions[7]. Diagnosis of occult HBV infection always requires the use of sensitive HBV-DNA PCR assay.

The aim of this study was to determine the prevalence of occult HBV infection among renal transplant patients in Bahari Hospital, Khartoum State , Sudan.

II. METHODS AND MATERIAL

Study area and period:

This study was conducted on renal transplant patients in Khartoum State, during the period from May to October 2018.

Study design and study subjects:

A cross-sectional study that was carried out to investigate the prevalence of OBI in 100 renal transplant patients of between 15 to 65 years old, at Bahari Hospital, Khartoum State, Sudan .All participating patients were given written informed consent.

The demographic data from the patients including age, gender, date of renal transplant, and patient residence in addition to date of sample collection were recorded during sample collection. Plasma was separated from patients blood samples by centrifugation and stored frozen at -20°C until further analyses.

Serology: Commercial ELISA kits (VIDAS , Biomerieux ,SA & prechek Bio, Inc, USA respectively) were used to detect HBsAg and HBc antibodies respectively according to the procedures described by the manufacturers.

DNA extraction

DNA was extracted from patient's materials using commercial Kit (innuPREP , Germany) according to manufacturer's instructions. The extracted DNA was stored at -20°C till used.

Polymerase chain reaction (PCR):

The PCR was performed by processing the extracted DNA with primers specific for the HBsAg gene of HBV. The primers used consisted of forward primer 5'-TCGGAAATACACCTCCTTTCCATGG-3' (HBVgenome1353-1377) and reverse primer, 3'GCCTCAAGGTCGGTCGTTGACA-5' (HBV genome1 702-1681). The reaction was performed in 20 µl total volume using Solis Bio dyne master mix. The volume included: 4 µl master mix, 1 µl forward primer, 1 µl reverse primer, 5 µl extracted DNA and 9 µl distilled water. The DNA was amplified in thermo cycling conditions using PCR machine (Techno Japan) as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 62°C for 1 min and extension at 72°C for 1 min, with a final extension 72°C for 7 min.

10 µl of the amplified product was subjected to direct analysis by gel electrophoresis in 2% Agarose with 0.15% Ethidium bromide . The gel was prepared by adding 0.5 g of agarose to 25 ml 5X Tris Borate EDTA buffer. The product was visualized using UV gel documentation system (INGeNius). The

expected size of surface antigen gene amplicon was 350 bp.

III. RESULTS AND DISCUSSION

RESULTS

Detection of HBsAg

Out of the 100 samples tested for HBsAg using Ag detection ELISA, ninety seven(97%) samples (68 males and 29 females) were negative for HBsAg while three(3%) samples (2 males and 1 female) were positive (Table 1). None of the negative HBsAg 97 patients was proved positive for either HBcAb or HBV DNA using Elisa or PCR respectively.

Table 1. Frequency of HBsAg in renal transplant patients at Khartoum Bahri hospital, Sudan

Gender	Frequency of HbsAg		Total
	Positive	Negative	
male	2 (2%)	68(68%)	70
female	1(1%)	29(29%)	30
total	3(3%)	97(97%)	100

DISCUSSION

The present study was aimed to determine the presence of occult hepatitis B among renal transplant patients in Khartoum, Sudan.

No HBV DNA was detected in any of the patients investigated (0%) in this study. Around the world, the incidence of occult HBV was reported to range between 0 to 58% ,it was reported as 1% in Brazil[8] and 2.3% in Korea[9], ,but higher prevalence of occult HBV is described in patients with chronic HCV infection, and a higher prevalence is observed in certain geographic regions, such as in Asia[10-11]

The incidence of occult HBV in Sudan was reported to range between 0 to 14.5% in haemodialysis patients [12-13-14-15-16] but no published data about OBI in Sudanese renal transplant patients are available. None of the patients in our study group proved to be positive for OBI.

In general, the differences in the reported incidences of occult HBV infection between several studies, including the present study, could be attributed to several factors including differences in the sensitivity of the various molecular techniques used in detection of HBV DNA, differences in the prevalence of HBV in geographical areas, and differences in the storage and age of samples used in the studies [17-18]However, it should be noted that in some studies which displayed high prevalence of occult HB , the study of viral DNA was performed in liver tissue where the PCR is more efficient, possibly because of the normally higher viral load in the liver. [19].

It is worth mentioning that the risk of post-transplantation reactivation of HBV disease is negligible [20]. however, a significant worsening of patient survival is found beginning by the 5th year post transplantation[21].

In conclusion, we found that the prevalence of OHB infection is very low(less than 1%) among our sample of kidney transplant recipients. However, the low level of occult HBV infection reported in this study clearly showed that serological markers of HBV infection should always be backed up with molecular tests to investigate possible occult HBV infection. Currently, molecular tests such as PCR, is not in use as a routine laboratory investigation tool for HBV in most of the health centres in Sudan, But a PCR method such as the one described in this study should be used as routine test for HBV infections in the hospitals in the country.

Finally, although the results of the present study do not support the existence of appreciable prevalence of OHB in renal transplant patients, it is clear that more studies are needed to fully elucidate the incidence of OHB in these patients in Khartoum as well as in other parts of Sudan.

IV.Acknowledgements

We would like to thank Bahari Hospital staff for allowing us to collect samples from patients.

We would also like to acknowledge the Central Laboratory, Ministry of Higher Education and Scientific Research, Khartoum, Sudan, and the Faculty of Medical Laboratory Sciences, Alneelain University, Khartoum, Sudan for their help and support..

V. REFERENCES

[1]. Dr Richard Hunt (2007). Hepatitis viruses. University of Southern California, Department of Pathology and Micro Biology, 2213.Availableat:http://pathmicro.med.sc.edu/virol/hepatitis-virus.htm.

[2]. Mast EE, Alter MJ .Prevalence of hepatitis B virus among health Care personnel. In Ellis RW, ed. Hepatitis B vaccine in clinical practice. New York, Marcel Dekker.199;. pp. 295-307.

[3]. Ke-Qin Hu, Occult hepatitis B virus infection and its clinical implications. J. Viral Hepatitis.2002, 9:243-257.

[4]. Allain JP, Cox L. Challenges in hepatitis B detection among blood donors. Curr Opin Hematol. 2011;18:461-6

[5]. Gonzalez R, Torres P, Castro E, Barbolla L,. et al. Efficacy of hepatitis B virus (HBV) DNA screening and characterization of acute and occult HBV infections among blood donors from Madrid, Spain Transfusion.2010;50: 221-30.

[6]. Gerlich W, Wagner F, Chudy M, Harritshoj L, et al HBsAg non-reactive HBV infection in blooddonors: transmission and pathogenicity. J Med Virol. 2006;79: 32-6.

[7]. Di Stefano M, Volpe A, Stallone G, Tartaglia L, et al, . Occult HBV infection in hemodialysis setting is marked by presence of isolated antibodies to HBcAg and HCV. J Nephrol.2009; 22: 381-386.

[8]. Cibele Franz, Renata de Mello Perez, Mariano Gustavo Zalis, et al, of occult hepatitis B virus infection in kidney transplant recipients. Mem. Inst. Oswaldo Cruz [online]. 2013, 108:657-660. http://dx.doi.org/10.1590/0074-0276108052013019

[9]. EunsinBae, Chang-Hun Park, Chang-Seok Ki, Sung-Joo Kim, et al(2012) Prevalence and clinical significance of occult hepatitis B virus infection among renal transplant recipients in Korea, Scandinavian Journal of Infectious Diseases.2012; 44: 788-792, DOI: 10.3109/00365548.2012.680488.

[10]. Kazemi-Shirazi L, Petermann D, Müller CA . Hepatitis B virus DNA in sera and liver tissue of HBsAg negative patients with chronic hepatitis C. J Hepatol. 2000; 33: 785-790.

[11]. Grob P, Jilg WH.. BornhakG. Gerken BG, Gerlich W et al Serological pattern anti HBc alone: report on workshop. J Med Virol 2000; 62: 437-455.

[12]. Hassanein EH. Molecular Detection of Occult Hepatitis B Virus DNA in Haemodialysis Patients in El Gazeera State, Sudan. MSc Thesis. Sudan University of Science Technology, Khartoum,2013.

[13]. Nafisa AI, Wafa IE . Occult Hepatitis B Virus infection in Sudanese haemodialysis patients. Lab. Med. J.2003; 1:43-49.

[14]. Mohammed AA, Enan KA, Khair OA . Hussien MO, El Hussein AM2 and Elkhidir IM. Prevalence of occult hepatitis B virus (HBV) infections in haemodialysis patients in

Khartoum State, Sudan from 2012 to 2015. 2015 6: 22-26,

[15]. Ali R N, Rahmat Allah T, Osman T E, Almugadam B S. Serological detection of hepatitis B virus infection in patients under hemodialysis MOJ Biol Med. 2018;3:186–188.

[16]. Sahr Hagmohamed SA*, Isam ME, M El Hussein AR and Khalid AE. Prevelance of occult Hepatitis B Virus (HBV) Infection among Hemodialysis Patients in Northern State, Sudan. Virol I mmunol J,2019,3(2): 000212.

[17]. Ismail H,Soliman M, Ismail N . Occult hepatitis B virus infection in Egyptian hemodialysis patients with or without hepatitis C virus infection. Pathol. Lab. Med. Int.2010; 2:113-120.

[18]. Ana Cecilia C, Maria RC, Marcílio FL, Regina CM et al. “Occult hepatitis B virus infection in hemodialysis patients in Recife, State of Pernambuco, Brazil”. Revista da Socieda de Brasileira de MedicinaTropical,2012; 45): 558-562.

[19]. Cacciola I, Pollicino T, Squadrito G, Cerenzia G,., et al,. Occult hepatitis B virus infection in patientswith chronic hepatitis C liver disease N Engl J Med. 1999; 341: 22-26.

[20]. Duhart Jr. BT, Honaker MR, Shokouh-Amiri MH, Riely CA, et al Retrospective evaluation of the risk of hepatitis B virus reactivation after transplantation.Transpl Infect Dis. 2003;5:1-6.

[21]. Sengar DP, Couture RA, Lazarovits AI, Jindal SL Long-term patient and renal allograft survival in HBsAg infection: a recent update.Transplant Proc. 1989; 21: 3358-3359.

Cite this article as :

Mashaer M Mustafa, Abdel Rahim M El Hussein, Isam M Elkhidir, Khalid A Enan, "Prevalence of Occult Hepatitis B Virus Infections Among Kidney Transplant Patients in Khartoum State, Sudan", International Journal of Scientific Research in Science, Engineering and Technology (IJSRSET), Online ISSN : 2394-4099, Print ISSN : 2395-1990, Volume 7 Issue 7, pp. 80-84, January-February 2020. Available at doi : https://doi.org/10.32628/IJSRSET207112

Crossref Cited-by

Journal URL : http://ijsrset.com/IJSRSET207112