

## Seroprevalence of hepatitis B virus among blood donors attending Omdurman teaching hospital

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### Abstract:

*Hepatitis B virus infection is the most common cause of liver disease worldwide, Sudan is classified among the countries with high hepatitis B virus seroprevalence, this study was carried out to determine seroprevalence of hepatitis B virus among blood donors in Khartoum (Sudan) , a total of ninety blood donors' males who attending Omdurman Teaching Hospital blood bank during the period from November to December 2014, were enrolled in this study, their age range from 18 to 60 years with mean {27}, the aim of this study was to detect seroprevalence of hepatitis B surface antigen, serum specimens were collected from blood donors, and analyzed by Enzyme linked Immune Sorbent Assay (ELISA) technique, the result showed that( 9/90, 10%) were positive for hepatitis B surface antigen, high frequency of hepatitis B surface antigen was observed among 40 – 60 years age groups and among whom had history of previous dental operation. Large-scale studies in different settings and studies are required.*

**Key words:** seroprevalence, HBsAg, blood donors, ELISA technique, Khartoum, Sudan.

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## **Introduction:**

Hepatitis B virus (HBV) is a small, partially double-stranded DNA virus of the hepadnavirus Family, genus Orthohepadnavirus which replicates through a reverse transcription phase, unlike HCV, HBV integrates into the host hepatocyte genome early in infection, usually at sites of DNA damage <sup>(1)</sup>, the virus carries no known Oncogenes and sites of integration are not consistent from cell to cell, but the Presence of multiple integrated viral genes is thought to create genomic instability in the host and may lead to loss of heterozygosity for tumor suppressor genes, moreover, hepatitis B x antigen acts as a transactivator that may lead to increased transcriptional activity of cellular oncogenes as well as interference with the function of tumor suppressor genes, such as p53 <sup>(2)</sup>.

In populations where HBV infection occurs in early childhood, the lifetime risk of hepato-cellular carcinoma (HCC) in HBV-infected person has been estimated at 27% for males and 4% for females<sup>(3)</sup>, among endemic populations, however, there may be substantial disparities in HCC risk due to differences in the natural history of chronic HBV infection between populations <sup>(4)</sup>.

HBV is classified into eight genotypes (A-H) that are related to their geographical origins, the genotypes are based on the greater than 8% sequence divergence that exists throughout the entire genome of the virus <sup>(5)</sup>.

Most genotypes have been divided into sub genotypes with distinct virological and epidemiological properties, recently, two new genotypes, designated I <sup>(6)</sup> and J <sup>(7)</sup> have also been proposed, in Africa, genotypes A, D and E are the most predominant, It is well established that genotype A is prevalent in Northwest Europe, Africa and the Americas, genotype A, which circulates in Africa, was initially divided into two subgroups: A1 and A2, subgroup A1 has been previously identified in three countries in sub-Saharan Africa, including

South Africa, Zimbabwe and Malawi which suggests that subgroup A1 evolved within the indigenous populations of some African countries <sup>(8)</sup>.

Hepatitis B virus is a major public health problem, particularly in developing countries <sup>(9)</sup>, the Prevalence of HBV in a population can be predicted by risk factors associated with the transmission of infection such as injections, blood and blood products transfusion, surgical procedures, body tattooing, occupational injury, sexual and vertical transmission <sup>(10)</sup>, many infected individuals deny history of any of these risks so the probability of inter-familial transmission is possible in these subjects <sup>(11)</sup>, however, the prevalence varies from area to area and population to population due to variability in ethnicity and socioeconomic conditions <sup>(12)</sup>.

HBV is a serious public health problem worldwide and major cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma, it was estimated that approximately 2 billion people have serological evidence of past or present HBV infection, more than 350 million are chronic carriers of HBV <sup>(13)</sup>, approximately 15–40% of infected patients will develop cirrhosis, liver failure, or hepatocellular carcinoma <sup>(14)</sup>.

HBV infection accounts for 500 000 to 1.2 million deaths each year <sup>(15)</sup>, and is the 10th leading cause of death worldwide, hepatocellular carcinoma incidence has increased worldwide, and the disease is now the 5th most frequent cancer, killing 300 000 – 500 000 people each year <sup>(16)</sup>.

Screening of blood and blood products for HBV was only introduced to blood banks throughout the country in 2002, before which, screening was only performed in a very few centers in the capital Khartoum, Vaccination for HBV was included as part of the extended program of immunization in 2005, Sudan was classified among the countries with high HBV endemicity of more than 8% <sup>(17)</sup>, seroprevalence ranging from as low as 6.8% in central Sudan <sup>(18)</sup> to as high as 26% in southern Sudan <sup>(19)</sup>.

Identified risk factors for HBV infection in Sudan include living in southern Sudan, parenteral antischistosomal therapy, sexual promiscuity and scarification which are a common ritual in southern Sudan <sup>(20)</sup>.

These rates are comparable to some African countries where seroprevalence of HBsAg was reported at rates of 15.6%, 10% in Burundi and Uganda respectively <sup>(21)</sup>, 14% in Central African Republic <sup>(22)</sup>.

Lower rates were however found in other countries such as Tanzania (4.4%) <sup>(23)</sup>, Nigeria (4.98%) <sup>(24)</sup> and Ethiopia (7%) <sup>(25)</sup>.

The aim of this study was to detect seroprevalence of hepatitis B surface antigen among blood donors at Omdurman Teaching Hospital blood bank.

## **Materials and methods:**

This was a descriptive cross-sectional study which had been conducted in Khartoum state during period from November to December 2014, ninety blood donors males apparent healthy were enrolled in this study, data was collected by using direct interviewing questionnaire, ethical clearance was obtained from research ethical committee of faculty of graduate studies Al-Née lain University and ministry of health Khartoum state, written consent also was obtained from blood donors.

## **Experimental work and Samples collection:**

Blood samples were collected from ninety blood donors males, under direct medical supervision by medical vein puncture using 5 ml syringe into plain tube to obtain serum by centrifugation at 5000 rpm for 10 minutes, serums was kept in -20°C till serological study was performed, Specimens were processed by ELISA (4th generation ELISA) (fortress-diagnostics limited, United Kingdom) for detection hepatitis B surface antigen.

All reagents and samples were allowed to reach room temperature for 15 minutes before use, washing buffer was prepared 1:20 from buffer concentrate with distilled water, 20µl of sample diluents was added into appropriate wells except the blank well and negative well, 100µl from each sample was added to the appropriate wells and mixed by tapping the plate gently, 100µl from negative and positive control was dispense and added to the negative and positive wells separately without dispensing liquid into the blank control well, then plate was covered and incubated for 60 minutes at 37°C, plate cover was removed and discarded, each well washed 5 times with diluted wash buffer each time (washing 1), 50µl of Horseradish Peroxidase (HRP)-Conjugate Reagent was added in to each well except the blank, the plate was mixed well and covered with the plate cover and incubated for 30 minutes at 37°C.

The plate cover was removed and discarded, the liquid was aspirated and each well was rinsed in wash buffer (washing 2), this step was repeated for 5 times until each well became dry, 50µl of chromogen A and 50µl of chromogen B solution were added in to each well including the blank and mixed by tapping the plate gently, the plate was incubated at 37°C for 15 minutes, 50 µl of Stop solution was added into each well and mixed gently, blue color developed in a positive control and hepatitis B surface antigen positive samples wells.

### **Measuring the absorbance:**

The plate reader was calibrated with blank well and the absorbance was read with micro well reader at 450 nm, the results were calculated by relating each sample optical density (OD) value to the Cut off value of plate.

Calculation of Cut off (C.O) value:  $C.O = *Nc *2.1$

\*Nc = the mean absorbance value for the three negative controls.

### **Interpretation of results:**

**Negative results:** samples giving absorbance less than Cut-off value are negative for this assay.

**Positive results:** samples giving absorbance equal to or greater than Cut-off value considered initially reactive.

**Borderline:** samples with absorbance O.D.  $\leq$  Cut-off \* 2 are considered borderline and retesting of those samples in duplicates is recommended.

### **Data analysis:**

Data was analyzed by SPSS (Statistical Package of Social Science) software program version 13.

### **Result:**

A total of ninety blood donors' males who attending Omdurman Teaching Hospital blood bank during the period from November to December 2014, were enrolled in this study, their age ranges from 18-60 years with mean (27), all of them had no history of jaundice.

The result revealed that seropositivity of HBsAg was (9/90, 10%) as showed in fig (1), high frequency was observed among previous dental operation (2/18, 11.1%), previous surgical operation (1/10, 10%), and blood donors without risk factor (6/61, 9.8%), statistical analysis showed significant differences among blood donors (Table-1).

Most of the donors aged from 20 to 29 years old (52/90 , 57.8%), and most of positive results was also observed among this age group, (Table-2).

### **Discussion:**

Blood transfusion is a branch of medicine in the healthcare sector, an incorporated strategy for blood safety is required for

elimination of transfusion transmitted infections and for provision of safe and adequate blood, the infectious agents such as HBV, HIV, HCV and syphilis are important blood born and transfusion transmitted infections throughout the world, the previous research statistical data has been established that prevalence rate of HBsAg among blood donors on the general population varied from country to another <sup>(26)</sup>.

All subjects examined in this study were males, because usually females do not donate blood in Sudan, this is because socially and culturally women are not favored to donate blood either to men or women, usually men of young or middle age are those who can willingly donate their blood.

The present study result revealed that the seropositivity of hepatitis B surface antigen among blood donors attending Omdurman teaching hospital was (10%), which is typical to result of *Elfaki et al, (2008)* in Sudan-Elobeid who found it as 10% <sup>(27)</sup>, and closely similar to result of *Shaza Salih and Wafa Ibrahim (2013)* in Sudan -Khartoum whose found it 10.2% <sup>(28)</sup>, however the present study was higher than 8.2% reported in eastern Sudan <sup>(29)</sup>, 5.1% in the north of the country <sup>(30)</sup> and 5.6% in the central regions <sup>(31)</sup>, Whereas it was low if compared to southern Sudan (26%) <sup>(32)</sup>, and in other country 18.6% reported in Nigeria by *Buseri et al., 2009* <sup>(33)</sup>.

## **Conclusion:**

The study concluded that the seroprevalence of hepatitis B surface antigen among blood donors in Omdurman teaching hospital was (10%) in a high rate, moreover high seropositivity of HBsAg observed among the age group of 40-60 years and among whom had previous dental operation.

Preventive measures should be delivered to the community through the different media and HBV immunization should be strongly supported and sustained by the Ministry of Health.

### **Recommendations:**

Further recent confirmatory techniques like Western blot, Southern blot and Polymerase Chain Reaction (PCR) should be considered.

Quality assured screening of all donated blood for transfusion transmissible infections.

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### **REFERENCES:**

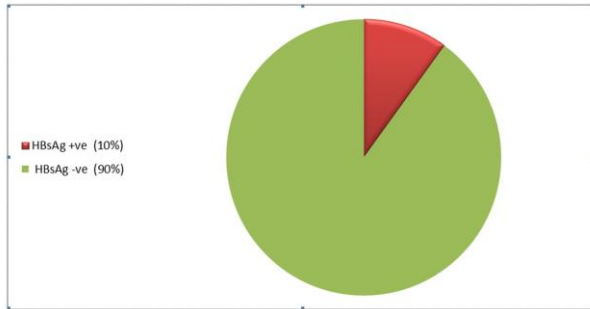
- 1- Bill CA, Summers J. Genomic DNA double-strand breaks are targets for hepadnaviral DNA integration. Proceedings of the National Academy of Sciences of the United States of America 2004; 101:1
- 2-Feitelson MA,Sun B, Satiroglu Tufan NLS, et al. Genetic mechanisms of hepatocarcinogenesis. Oncogene. 2002; 212593-2604.
- 3- Dickinson JA, Wun WT, Wong SL. Modeling death rates for carriers of hepatitis B. Epidemiology and Infection. 2002;128:83-92.
- 4- Evans AA, O'Connell AP, Pugh JC, et al. Geographic variation in viral load among hepatitis B carriers with differing risks of hepato-cellular carcinoma.Cancer Epidemiol Biomarkers.1998; 7:559-565.
- 5- Kramvis A, Restorp K, Norder H, Botha JF, Magnus LO, Kew MC 2005. Full genome analysis of hepatitis B virus genotype E strains from South-Western Africa and Madagascar reveals low genetic variability. *J Med Virol* 77: 47-52.



- 6-Olinger CM, Jutavijittum P, Hübschen JM, Yousukh A, Samounry B, Thammavong T, Toriyama K, Muller CP 2008. Possible new hepatitis B virus genotype, southeast Asia. *Emerg Infect Dis* 14: 1777-1780.
- 7- Tatematsu K, Tanaka Y, Kurbanov F, Sugauchi F, Mano S, Maeshiro T, Nakayoshi T, Wakuta M, Miyakawa Y, Mizokami M 2009. A genetic variant of hepatitis B virus divergent from known human and ape genotypes isolated from a Japanese patient and provisionally assigned to new genotype J. *J Virol* 83: 10538-10547.
- 8- Sugauchi F, Orito E, Kato H, Suzuki S, Kawakita S, Sakamoto Y, Fukushima K, Akiba T, Yoshihara N, Ueda R, Mizokami M 2003. Genotype, serotype, and phylogenetic characterization of the complete genome sequence of hepatitis B virus isolates from Malawian chronic carriers of the virus. *J Med Virol* 69: 33-40.
- 9- Haider Z, Khan AA, Rehman K, Janjua MI, Iqbal J, Chishti MA, *et al.*, 1994 Sero-diagnosis of Viral hepatitis in 93 patients admitted with acute hepatitis in three different teaching hospitals in Lahore. *J Pak Med Assoc*; 44: 182-4.
- 10- Abildgaard N, Peterslund NA (1991).Hepatitis C virus transmitted by tattooing needle. *Lancet*; 338: 460.
- 11- Roy KM, Goldberg D, Taylor A, Mills P (2003).Investigating the source of hepatitis C virus Infection among individuals whose route of infection is undefined: a study of ten cases. *Scand J Infect Dis*; 35: 326-328.
- 12- Munoz.PS, Robert. S, Sheffieldd. J et al 6.3% Prevalence of hepatitis B and C in pregnant women who are infected with HIV, American Journal of Obstetrics and Gynecology Vol 2005; 193, (3) Supplement 1, September: 1270-3.
- 13- World Health Organization. Hepatitis B. World Health Organization Fact Sheet 204 (Revised October 2000).*WHO Web site*. 2000.
- 14- Lok AS. Chronic hepatitis B.N Engle J Med 2002; 346(22):1682–1683.

- 15- Lee WM. Hepatitis B virus infection's Engle J Med 1997; 337(24): 1733–1745.
- 16- Expanded program on immunization, hepatitis B vaccine, making global progress. EPI update, WHO: October 1996.
- 17- Expanded program on immunization, hepatitis B vaccine, making global progress: EPI update. Geneva, Switzerland: World Health Organization; October, 1996.
- 18- Mudawi HM, Smith HM, Rahoud SA, Fletcher IA, Saeed OK, Fedail SS. Prevalence of hepatitis B virus infection in the Gezira state of central Sudan.Saudi J Gastroenterol.2007; 13:81–3.
- 19- McCarthy MC, el-Tigani A, Khalid IO, Hyams KC. Hepatitis B and C in Juba, southern Sudan: results of a serosurvey.Trans R Soc Trop Med Hyg.1994; 88:534–6.3.
- 20- McCarthy MC, Hyams KC, el-Tigani el-Hag A, et al. HIV-1 and hepatitis B transmission in Sudan.AIDS.1989; 3:725–9.
- 21- de Lalla F, Rizzardini G, Rinaldi E, Santoro D, Zeli PL, Verga G. HIV, HBV, delta-agent and Treponema pallidum infections in two rural African areas.Trans R Soc Trop Med Hyg.1990; 84:144–7.
- 22-.Pawlotsky JM, Bélec L, Grésenguet G, et al. High prevalence of hepatitis B, C, and E markers in young sexually active adults from the Central African Republic.J Med Virol. 1995;46:269–72.8.
- 23- Pellizzer G, Blè C, Zamperetti N, et al. Serological survey of hepatitis B infection in Tanzania.Public Health.1994;108:427–31.9.
- 24- Ejele OA, Ojule AC.The prevalence of hepatitis B surface antigen (HBsAg) among prospective blood donors and patients in Port Harcourt, Nigeria. Niger J Med. 2004;13:336–8.10.
- 25- Abebe A, Nokes DJ, Dejene A, Enquselassie F, Messele T, Cutts FT. Seroepidemiology of hepatitis B virus in Addis Ababa, Ethiopia: transmission patterns and vaccine control. Epidemiol Infect.2003; 131:757–70.

- 26-Luksamijarulkul P, Thammata N, Tiloklurs M. Seroprevalence of hepatitis B, hepatitis C and human immunodeficiency virus among blood donors, Phitsanulok Regional Blood Centre, Thailand. *Southeast Asian J Trop Med Public Health*. 2002; 33:272-9.
- 27- Abdelsalam Elfaki, Ahmed Agab Eldour, Nasir Elsheikh Sero-prevalence of immunodeficiency virus, hepatitis B and C and syphilis among blood donors at ElObeid Teaching Hospital, West Sudan December 2008.
- 28- Shaza Salih Gafir and Wafa Ibrahim Elhag. Frequency of hepatitis B surface antigen and syphilis among blood donors attending Omdurman teaching hospital 2013 (thesis).
- 29- Abdallah, T. M., M. H. Mohamed and A. A. Abdel Aziem (2011). Seroprevalence and epidemiological factors of hepatitis B virus (HBV) infection in Eastern Sudan.” *International Journal of Medicine and Medical Sciences* 3(7): 239-241.
- 30- Nagi, A. M., H. A. Altyeb and A. M. Ahmed (2007). Seroprevalence of Hepatitis B and C Viral Infections among blood donors in Shendi, River Nile State, Sudan.” *Research Journal of Medicine and Medical Sciences* 2: 122-1
- 31- Nakwagala, F. N. and M. M. Kagimu (2002). Hepatitis B virus and HIV infections among patients in Mulago hospital. *East Afr Med J* 79(2): 68-72
- 32- McCarthy MC, El-Tigani and Khalid IO, Hyams KC: Hepatitis B and C in Juba, southern Sudan: results of a serosurvey, *transactions of Royal Society of Tropical Medicine and Hygiene* 1994, 88:534-536.
- 33- Buseri FI, Muhibi MA, Jeremiah ZA (2009). Sero-epidemiology of transfusion transmissible infectious diseases among blood donors in Osogbo, south- west Nigeria. *Blood Transfuse.* (7):293-9.



**Figure (1): Seroprevalence of hepatitis B surface antigen among blood donors (n=90) attending Omdurman teaching hospital**

**Table (1): Seroprevalence of hepatitis B surface antigen among blood donors according to their risk factors:**

Risk factor	Frequency	percent	HBV (+ve)	HBV (-ve)	p. value
Previous surgical operation	<b>10</b>	(11.1%)	1	9	0.011
Previous dental operation	<b>18</b>	(20%)	2	16	0.001
Previous blood transfusion	<b>0</b>	(0%)	<b>0</b>	0	0.000
Cupping	<b>1</b>	(1.1%)	<b>0</b>	1	0.000
Blood donors with no of the above	<b>61</b>	(67.8%)	6	55	0.000
<b>Total</b>	<b>90</b>	(100%)	9 (10%)	81(90%)	

**Table (2): Seroprevalence of Hepatitis B surface antigen among blood donors according to their age:**

Age	Frequency	percent	HBV (+ve)	HBV (-ve)
Less than 20	6	(6.7%)	<b>0</b>	6
20_____29	52	(57.8%)	<b>5</b>	47
30_____39	<b>25</b>	(27.8%)	<b>2</b>	23
40_____60	<b>7</b>	(7.8%)	<b>2</b>	5
<b>Total</b>	<b>90</b>	(100%)	<b>9 (10%)</b>	81 (90%)