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Hepatitis B Core Antibody Testing among Blood Donors in Khartoum- Sudan

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Abstract:

Hepatitis B virus transmission among hepatitis B surface antigen negative blood donors continues to be major problem. Hepatitis B surface antigen is still the only hepatitis B virus screening test of blood donors in Sudan, but this marker cannot be detected during the window period of infection.

A total of 74 blood donors who attended blood bank were enrolled in this study to detect hepatitis B anti core, they were negative for hepatitis B surface antigen , all of them were males and their age ranged between 11-50 years. Serum specimens were tested by ELISA IgM for anti core Hepatitis B virus. The result showed 4 (5.4%) positive for anti core Hepatitis B virus. Data were analyzed by chi squared test in SPSS software.

Key words: Hepatitis B Core Antibody, Blood donors, ELISA, Khartoum -Sudan.

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INTRODUCTION:

Hepatitis B is an infectious disease caused by the hepatitis B virus (HBV) which affects the liver. It can cause both acute and chronic infections. Many people have no symptoms during the initial infection. Some develop a rapid onset of sickness with vomiting, yellow skin, feeling tired, dark urine and abdominal pain.[1] Often these symptoms last a few weeks and rarely does the initial infection result in death.[1][2] It may take 30 to 180 days for symptoms to begin.[1] In those who get infected around the time of birth 90% develop chronic hepatitis B while less than 10% of those infected after the age of five do.[3] Most of those with chronic disease have no symptoms; however, cirrhosis and liver cancer may eventually develop.[4] These complications results in the death of 15 to 25% of those with chronic disease.[1]

The virus is transmitted by exposure to infectious blood or body fluids. In areas where the disease is rare intravenous drug use and sexual intercourse are the most frequent routes of infection.[1] Other risk factors include: working in healthcare, blood transfusions, dialysis, living with an infected person, travel in countries where the infection rate is high, and living in an institution. [1] [3] The hepatitis B viruses cannot be spread by holding hands, sharing eating utensils, kissing, hugging, coughing, sneezing, or breastfeeding. [3] The infection can be diagnosed 30 to 60 days after exposure. Diagnosis is typically by testing the blood for parts of the virus and for antibodies against the virus. It is one of five known hepatitis viruses: A, B, C, D, and E. [1]

The hepatitis B surface antigen (HBsAg) is most frequently used to screen for the presence of this infection. It is the first detectable viral antigen to appear during infection. However, early in an infection, this antigen may not be present and it may be undetectable later in the infection as it is being cleared by the host. The infectious virion contains an inner

"core particle" enclosing viral genome. The icosahedral core particle is made of 180 or 240 copies of core protein, alternatively known as hepatitis B core antigen, or HBcAg. During this 'window' in which the host remains infected but is successfully clearing the virus, IgM antibodies specific to the hepatitis B core antigen (anti-HBc IgM) may be the only serological evidence of disease. Therefore most hepatitis B diagnostic panels contain HBsAg and total anti-HBc (both IgM and IgG). [5]

Shortly after the appearance of the HBsAg, another antigen called hepatitis B e antigen (HBeAg) will appear. Traditionally, the presence of HBeAg in a host's serum is associated with much higher rates of viral replication and enhanced infectivity; however, variants of the hepatitis B virus do not produce the 'e' antigen, so this rule does not always hold true. [6] During the natural course of an infection, the HBeAg may be cleared, and antibodies to the 'e' antigen (anti-HBe) will arise immediately afterwards. This conversion is usually associated with a dramatic decline in viral replication. If the host is able to clear the infection, eventually the HBsAg will become undetectable and will be followed by IgG antibodies to the hepatitis B surface antigen and core antigen (anti-HBs and anti HBc IgG). [7] The time between the removal of the HBsAg and the appearance of anti-HBs is called the window period. A person negative for HBsAg but positive for anti-HBs either has cleared an infection or has been vaccinated previously. [8]

Individuals who remain HBsAg positive for at least six months are considered to be hepatitis B carriers. [8] Carriers of the virus may have chronic hepatitis B, which would be reflected by elevated serum alanine aminotransferase (ALT) levels and inflammation of the liver, if they are in the immune clearance phase of chronic infection. Carriers who have seroconverted to HBeAg negative status, in particular those who acquired the infection as adults, have very little viral

multiplication and hence may be at little risk of long-term complications or of transmitting infection to others. [9]

In India Mohammad Asim*, et al* (2010) were evaluated the Significance of anti-HBc screening of blood donors & its association with occult hepatitis B virus infection, Implications for blood transfusion.[10]

MATERIALS AND METHODS:

This was descriptive- cross sectional study which had been conducted in Khartoum state during period from March to June 2015, 74 blood donors who attended blood bank) were enrolled, Data was collected by using direct interviewing questionnaire; ethical clearance was obtained from research ethical committee of Faculty of Graduate studies Al-Neelain University and verbal consent also was obtained from each patient.

EXPERIMENTAL WORK:

Specimens' collection:
Blood samples were collected from donors, under direct medical supervision by vein puncture using 5 ml syringe into plain tube to obtain serum by centrifugation at

5000 rpm for 10 min. serum was kept in -20°C till serological study was performed.

Specimens were processed by using Enzyme linked immune sorbent assay (ELISA) (WKEA MED SUPPLIES CORP_ China),(4th generation ELISA) for detection Hepatitis B anti core.

Enzyme linked immune sorbent assay for detection Hepatites B anti core.

All reagents and samples were allowed to reach room temperature for 15minutes before use washing buffer was prepared 1:20 from buffer concentrate with distilled water.

100µl of sample diluents was added into appropriate wells except the blank well and negative well. 100µl from each sample was added to the appropriate well and mixed by pipette repeatedly until liquids turn blue. 100µl from negative and positive control were dispensed and added to the negative and positive wells separately without dispensing liquid into the blank control well. Micro titer wells was flicked for 30 seconds and mixed well, then plate was covered and incubated for 30 minutes at 37° C. plate was taken out and wash buffer was added to each well (washing 1) and aspirated off after 20 seconds. This step was repeated for 5 times until each well become dry, and 100µl of 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 min at 37°C.
The plate cover was removed and discarded. The liquid was aspirated and each well was rinsed in wash buffer. This step was repeated for 5 times until each well become dry (washing 2).

Fivity µl of substrate A and 50µl substrate 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 min. 50 µl Stop solution was added into each well and mixed gently and read within 5 minutes.

Measuring the absorbance:

The plate reader was calibrated with blank well and the absorbance was read 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.

The absorbance was read with micro well reader at 450nm.

INTERPRETATION OF RESULTS:

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

Positive result: sample giving absorbance equal to or greater than Cut-off considered initially reactive.

Borderline: sample with absorbance to Cut-off value are considered borderline and retesting of these samples in duplicate is recommended.

Data analysis:
Data was analyzed by SPSS (Statistical Package of Social Science) software program version 16.

RESULT:

A total of 74 blood donors who attended Blood bank of Khartoum Teaching Hospital during the period from March-June 2015, consented to the study were included, they were hepatitis B surface antigen negative, all of them were males and their age ranged between 11-50 years and mean age of them was 30.5 years , most of donors (37 (50.0%)) were belonged to the age group (21-30)(fig1).The overall result showed that seropositive for Hepatitis B virus anticore IgM antibodies was detected in 4(5.4 %) and 70 (94.6%) were negative (Fig2).

Study population was divided into 5 groups according to their occupation (Table1).

Regarding risk factors, the highest seropositivity was observed among who had previous donation.

DISSCUSION:

Hepatitis B Virus (HBV) transmission via hepatitis B surface antigen (HbsAg) negative blood donors has been reported.

While many countries have implemented screening antibodies to hepatitis B core antigen (anti-HBc) to further enhance transfusion safety, HbsAg is still the only obligatory HBV screening test of blood donors. [11]

The present study result revealed that 4 blood donors (5.4%) were seropositive and 70 (94.6%) seronegative. When compared with other studies in Sudan, there was no similar published study. It was similar to the study in Nigeria (2011) obtained by Margaret *et al* who found Hepatitis B Core IgM antibody (anti-HBcIgM) among hepatitis B Surface antigen (HBsAg) negative blood donors 5 (5.4%) positive and 87 (94.6%) of blood donors negative [12]

However the present study result was lower result than the previous study in Delhi (2010) which obtained by Mohammad Asim *et al* who found significance of anti-HBc screening of blood donors & its association with occult hepatitis B virus infection :was detected in 413(19.8%) of 2175 blood donors [10]. also give lower result than the previous study in Syria(2013) obtained by Muselmani *et al* who found significance of screening antibodies to hepatitis B virus core antigen among Syrian blood donors was detected in 215 (11.2%) of 1913 donors [11] In Iran, Delavari *et al*. studied 1535 blood donors in Kerman in (2010) They reported that 8% of the blood samples negative for HBsAg, were positive for HBcAb [13]

On other hand this study gives higher result than the previous study in Iraq obtained by Abdulrazak SH. Hasan *et al.* who found 3.4% positive out of 178 blood donors [14]

CONCLUSION:

This study support the presence of anti core HBV positive in blood donors emphasizes on the need for establishing sensitive screening modalities for blood transfusion, The discrepancies of these results may be due to small sample size and differences

in the used techniques, for this large scale screening is recommended.

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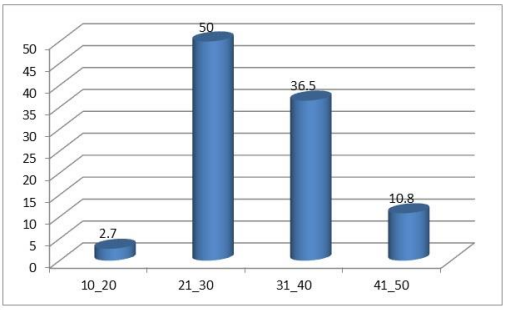

Fig(1) : Distribution of study population(n=74) according to their age.

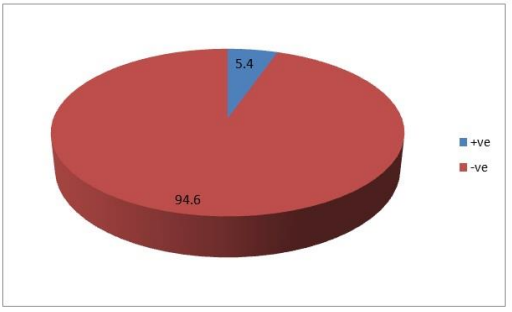

Fig 2: Seroprevalence of Hepatitis B virus anticore IgM antibodies among blood donors n=74

Table 1: **Distribution of study population (n=74) according to their occupation.**

			ELISA		Total
			+ve	-ve	
Occupation	employed	Count	1	15	16
		% within ELISA	25.0%	21.4%	21.6%
		% of Total	1.4%	20.3%	21.6%
	free jobs	Count	1	18	19
		% within ELISA	25.0%	25.7%	25.7%
		% of Total	1.4%	24.3%	25.7%
	student	Count	1	16	17
		% within ELISA	25.0%	22.9%	23.0%
		% of Total	1.4%	21.6%	23.0%
	merchant	Count	1	8	9
		% within ELISA	25.0%	11.4%	12.2%
		% of Total	1.4%	10.8%	12.2%
	worker	Count	0	13	13
		% within ELISA	.0%	18.6%	17.6%
		% of Total	.0%	17.6%	17.6%
Total		Count	4	70	74
		% within ELISA	100.0%	100.0%	100.0%
		% of Total	5.4%	94.6%	100.0%

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