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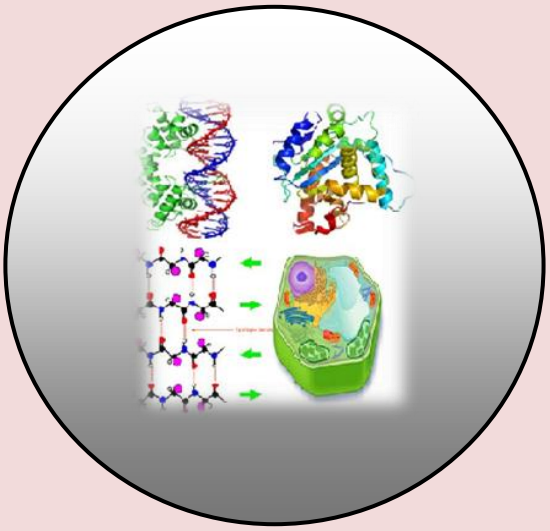

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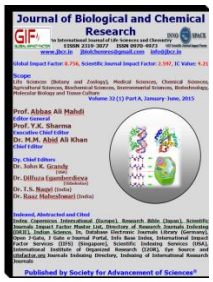

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RESEARCH PAPER

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Estimation of *Hepatitis B virus* Antibody Titer due to Vaccination among Medical field professionals in Khartoum State

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ABSTRACT

Hepatitis B vaccine is a vaccine that prevents hepatitis B. The first dose is recommended within 24 hours of birth with either two or three more doses given after that. This includes those with poor immune function such as from HIV/AIDS and those born premature. The vaccine contains one of the viral envelope proteins, hepatitis B surface antigen (HBsAg). It is now produced by yeast cells, into which the genetic code for HBsAg has been inserted. This is descriptive study conducted in Sudan cardiac center, Khartoum state-Sudan, in period from May to august 2018. The objective was to estimate the titer of hepatitis B antibody among medical field professionals who were vaccinated by hepatitis B vaccine. A total of 89 whole blood samples were collected from the study participants, serum was harvested by centrifugation at (2000 rpm) for 5 minutes. The levels of HBV antibody titer were determined by using sandwich ELISA Assay. The study revealed that 65.2% of participants have strong immune response (antibody titer was > 100 IU/ml), 30.3% develop poor immune response (antibody titer was 10- 100 IU/ml) and 4(4.5%) develop no immune response (antibody titer was < 10 IU/ml) against HBV vaccine. The study concluded that the strong immune reactivity against HBV vaccine could not be attributed to gender or a particular age group, and all Vaccinated people should be tested for estimation of Anti-HBs antibodies titer to insure Immunity.
Key words: HBV, Antibody Titer, Vaccination and Khartoum State.

INTRODUCTION

Hepatitis B vaccine is a vaccine that prevents hepatitis B. The first dose is recommended within 24 hours of birth with either two or three more doses given after that. This includes those with poor immune function such as from HIV/AIDS and those born premature (Burton *et al*, 2009). It is also recommended for health-care workers to be vaccinated (Chen, 2005). In healthy people routine immunization results in more than 95% of people being protected (Burton *et al*, 2009). The vaccine contains one of the viral envelope proteins, hepatitis B surface antigen (HBsAg). It is now produced by yeast cells, into which the genetic code for HBsAg has been inserted (Liao and Liang, 2015). Afterward an immune system antibody to HBsAg is established in the bloodstream.

The antibody is known as *anti-HBs*. This antibody and immune system memory then provide immunity to HBV infection (Thun *et al*, 2009). Hepatitis B vaccination, hepatitis B immunoglobulin, and the combination of hepatitis B vaccine plus hepatitis B immunoglobulin, all are considered as preventive for babies born to mothers infected with HBV. The combination is superior for protecting these infants (Cowan and Qureshi, 2007). Many countries now routinely vaccinate infants against hepatitis B. In countries with high rates of hepatitis B infection, vaccination of newborns has not only reduced the risk of infection, but has also led to marked reduction in liver cancer. This was reported in Taiwan where the implementation of a nationwide hepatitis B vaccination program in 1984 was associated with a decline in the incidence of childhood hepatocellular carcinoma (Chang *et al*, 1997). It is now believed that the hepatitis B vaccine provides indefinite protection. However, it was previously believed and suggested that the vaccination would only provide effective cover of between five and seven years (Krugman and Davidson, 1987: Petersen *et al*, 2004), but subsequently it has been appreciated that long-term immunity derives from immunological memory which outlasts the loss of antibody levels and hence subsequent testing and administration of booster doses is not required in successfully vaccinated immunocompetent individuals (Gabbuti *et al*, 2007; Kane *et al*, 2000). Hence with the passage of time and longer experience, protection has been shown for at least 25 years in those who showed an adequate initial response to the primary course of vaccinations (Van Damme and Van Herck, 2007) and UK guidelines now suggest that for initial responders who require ongoing protection, such as for healthcare workers, only a single booster is advocated at 5 years (Ashor, 2011). Serious side effects from the hepatitis B vaccine are very uncommon. Pain may occur at the site of injection. It is safe for use during pregnancy or while breastfeeding. The current vaccines are produced with recombinant DNA techniques. They are available both by themselves and in combination with other vaccines (Burton *et al*, 2009). Blood testing to verify that the vaccine has worked is recommended in those at high risk. Additional doses may be needed in people with poor immune function but are not necessary for most people. In those who have been exposed to the hepatitis B virus but not immunized, hepatitis B immune globulin should be given in addition to the vaccine. The vaccine is given by injection into a muscle (Burton *et al*, 2009).

MATERIAL AND METHODS

Specimen collection

A total of 89 blood specimens were collected from medical care professionals who were vaccinated by hepatitis B vaccine at Sudan cardiac center. Samples were collected in plain container from each study participant; then serum was harvested by centrifugation at (2000 rpm) for 5 minutes.

Procedure of ELISA Technique

- Reagents preparation: the reagents were allowed to reach room temperature (18-25 °C), and the wash buffer concentration was Checked for the presence of salt crystals.
- The stock wash buffer was diluted 20 times with distilled.
- Numbering wells: The strips needed were set in strip-holder and numbered sufficient number of wells including six calibrations curve standards wells (B1-G1; B2-G2) and one Blank (A1, Neither samples nor HRP-Conjugate should be added into the Blank well).
- Adding Sample: 50 µl calibration curve standards and 50 µl of specimen were added into their respective wells.
- Adding HRP-Conjugate: 50 µl of HRP-Conjugate Reagent was added into each well except the blank, and mixed gently.
- Incubation : The plate was covered with the plate sealer and incubated for 60 min. at 37 ^{O}C
- Washing: After the end of the incubation, the plate sealer was removed and discarded. And the wells were washed, each well 5 times with diluted Wash buffer. After the final washing cycle, the strips plate was turned onto blotting paper or clean tower, and taped to remove any remainders.
- Coloring: 50µl of Chromogen A and 50µl Chromogen B solution were Dispensed into each well included the Blank, covered the plate with plate sealer and mixed gently by tapping the plate. The plate was incubated at 37 ^{O}C for 15 minutes.

o Stopping Reaction: The plate sealer was removed and discarded. And by using a multichannel pipette 50 µl Stop Solution was added into each well and mixed gently.

o Measurement of the Absorbance: The plate reader was calibrated with the Blank well and read the absorbance at 450 nm.

Reading and Interpretation of the Results

The optical density (OD) of microplate was obtained at the 450 nm. The presence or absence of antibody to hepatitis B surface antigen was determined by comparing the absorbance measured for each sample to the calculated cut- off value.

According to the measured anti-HBs Ab level, the participants were classified into three groups:

Non responders: anti-HBsAb level ≤ 10 IU/mL.

Acceptable responders: anti-HBsAb level of 10-100 IU/mL.

Good responders: anti - HBsAb level of ≥ 100 IU/mL.

The protection level was considered when anti-HBsAb titers were above 10 IU per mL (Wood *et al.*, 1993).

RESULTS

In this study a total of (89) participants were included the majority of them (59.7%) were females and (40.3%) were males. The age of study participants ranged from (21) to (60) years, more than one half were within the age group (25 – 30 years). Out of the 89 participants of the healthcare workers, 58(65.2%) show strong immune response (antibody titer was > 100 IU/ml), 27 (30.3%) show poor immune response (antibody titer was 10- 100 IU/ml) and 4(4.5%) show no immune response (antibody titer was < 10 IU/ml) (see Table 1).

Table 1. Shows the degree of immune response against HBV vaccine among study participants.

Vaccine response	Frequency	Percentage	P value
Strong response (> 100 IU/ml)	58	65.2%	.000
Poor response (10-100 IU/ml)	27	30.3%	
No response (< 10 IU/ml)	4	4.5%	
Total	89	100 %	

Table 2. Shows the degree of immune response against HBV vaccine according to gender.

Gender	No response < 10 IU/ml		Poor response 10-100 IU/ml		strong response > 100 IU/ml		P value
	NO	%	NO	%	NO	%	
Male (n=36)	1	2.7%	13	36.2%	22	61.1%	0.545
Female (n=53)	3	5.6%	14	26.5%	36	67.9%	
Total (n=89)	4	4.5%	27	30.3%	58	65.2	

Table 3. Shows the degree of immune response against HBV vaccine according to Age group.

Age group	No response < 10 IU/ml		Poor response 10-100 IU/ml		Full response > 100 IU/ml		P value
	NO	%	NO	%	NO	%	
< 25 Year (n=19)	1	5.3%	5	26.3%	13	68.4%	0.169
25 -30 Year (n=54)	2	3.8%	21	38.8%	31	57.4%	
>30 Year (n=16)	1	6.2%	1	6.2%	14	87.6%	

In this study only 1 (2.7%) of male participants, and 3 (5.6%) of female participants failed to develop immunity against HBV vaccine (antibody titer was < 10 IU/ml) (see Table 2). The active age group (more than 30 years) revealed remarkable elevation in HBV antibodies titer level which was 14 (87.6%) and only 1(6.2%) showed a reduction in HBV antibodies titer level (see Table 3).

Table 4. Shows the degree of immune response against HBV vaccine according to Vaccine duration.

Vaccine duration	No response < 10 IU/ml		Poor response 10-100 IU/ml		Full response > 100 IU/ml		P value
	NO	%	NO	%	NO	%	
> Year (n=23)	0	0%	5	21.7%	18	78.3%	.277
1 -2 Year (n=40)	2	5%	11	27.5%	27	67.5%	
<2 Year (n=26)	2	7.7%	11	42.3%	13	50%	

18 (78.3%) of the participants who got the vaccine less than one year before the study showed strong immunity against HBV vaccine in comparison to only 13 (50%) of the participants who got the vaccine more than two years before the study (see Table 4).

DISCUSSION

In this study 58 (65.2%) of all participants showed strong response to HBV vaccine (antibody titer was more than 100 IU/ml), 27 (30.3%) showed weak response to HBV vaccine (antibody titer was 10- 100 IU/ml), and only 4(4.5%) failed to show response to HBV vaccine (antibody titer was less than 10 IU/ml). these findings were in accordance with Abe *et al.* (2006), Xiao-wen *et al.* (2005) and Kane *et al.* (1999) reported that about 5-10% of those vaccinated people fail to develop antibody to the vaccine.

In this study, the female showed higher response to HBV vaccine (67.9 %) than male (61.1%), however statically there was insignificant relation (P=0.545). This finding is in difference with results reported by Zeeshan *et al.* (2007) at Pakistan and this could be related to genetic factors.

In our study the strong reactivity could not be attributed to a particular age range (P value 0.169), as 68.4% of the age group (less than 25 years), 57.4% of the age group (25-30 years), and 87.6% of age group (more than 30 years) showed remarkable response to HBV vaccine. This result not in accordance with the reports of Tohme *et al.* (2011) results, who evaluated the seroprotection conferred by hepatitis B vaccine among older adults. Their reports stated that age was a significant determinant of seroprotection conferred by Hepatitis B vaccination.

The participants who received the vaccine more than two years before this study showed failure to response (7.7%) higher than those who received the vaccine less than one year before this study. This finding indicating that HBsAb titer may decrease over time after vaccination, which is in agreement with the reports of Platkov *et al.* (2003) and Hosseini *et al.* (2009).

The study concluded that 4.5% of study participants who were vaccinated against HBV show no response to HBV vaccine, this findings highlights that it is essential to check the post vaccination status of all Medical field professionals as it does not only ensure safety of employee but also reduces rate of transmission.

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