

CHAPTER ONE

1. INTRODUCTION

1.1. Background

Hepatitis B virus (HBV) infection is a global health problem. The frequency of hepatitis B viral infection in a subset of population is a function of several factors, which increases the risk of viral entry. These include environmental and life style related factors contributing to the acquisition of HBV infection. Health care workers (HCWs) constitute a high risk group for acquiring infection with blood borne pathogens due to occupational contact with infected body fluid (Varsha *et al*; 2010) The World Health Organization (WHO) estimates that at least 2 billion persons have been infected with hepatitis B virus (HBV) worldwide, with more than 378 million persons being chronic carriers 6% of the world population (George and Ioannou, 2014).

Approximately 350 million people are infected with Hepatitis B virus (HBV) around the world. It is responsible for a high proportion of the world cases of liver cirrhosis, and is the cause of up to 80% of all cases of hepatocellular carcinoma (Ghendon, 1990). Sudan is one of countries with high HBV, seroprevalence in the Africa (Itoshima *et al* ,1989, Yousif *et al* ;1998).

Hepatitis B virus (HBV) the commonest risk factor for developing chronic hepatitis, liver cirrhosis (Yousif *et al*; 1998) and hepatocellular carcinoma (Omer *et al*; 2001).

Vaccination has been proved to be effective in the control of this disease (Fahal *et al*; 1995). The discovery of etiologic agent of hepatitis B and development of safe and effective vaccines against HBV constitute one of the remarkable scientific achievements of the 20th century (Egemen *et al*; 1998 and Kurugol *et al*; 1997).

Since HBV vaccination was introduced, many advances (*e.g.*, use recombinant technology) have been made in order to increase its safety and immunogenicity (Williams, 2006). HBV vaccine is able to stimulate a long-term immune response in healthy individuals, since the antibody response occurs in over 90% of the immune-competent individuals after three doses of 20 µg HBV vaccine administered at 0, 1, and 6 month intervals (Mast *et al*; 2005, Chang, 2007). Considering this percentage of vaccine

efficacy in healthy subjects, it has been postulated that between 537000 and 660000 HBV-related deaths could be prevented each year with a mass vaccination (Pungpapong *et al*; 2007).

The incidence of new HBV infections might be expected to have decreased in recent years as a result of more widespread vaccination in children, as well as improvements in needle-using practices and effective exclusion of HBV infected persons from blood donation. In addition many antiviral agents that effectively suppress viral replication have become available, although they lead to elimination of HBsAg only rarely. In contrast, persons of Asian and Pacific Islander descent, racial groups with a particularly high prevalence of HBV infection, constitute an increasing proportion of the U.S. population. Therefore, it is unclear whether the prevalence of HBV infection in the United States has decreased in recent years (George and Ioannou, 2014).

1.2. Rationale

Vaccination is the principal strategy to reduce the morbidity and mortality caused by hepatitis B virus. Vaccinated subjects with a hepatitis B surface antibody titer must be more than 20mIU/mL after three doses of vaccine .Because there is a few literature about vaccination in Sudan, this study was conducted to evaluate the efficiency of HBV vaccine in medical staff in some hospitals in Khartoum State.

1.3. Objectives

1.3.1. General objective

To evaluate the efficiency of HBV vaccine in Health Care Workers in some hospitals in Khartoum State.

1.3.2 Specific objective

- a) To collect serum from vaccinated individual, who had three doses.
- b) To screen HBV in vaccinated medical staff in some hospitals in Khartoum State using Immuno Chromatography Test (ICT) to detect HBV antigen.
- c) To measure titer of Anti-Hepatitis B antibody using ELISA.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Hepatitis B infection

Hepatitis B virus causes epidemics in parts of Asia and Africa, and it is endemic in China (Williams, 2006). Approximately 45% of the world population live in hyper-endemic areas where the prevalence of hepatitis B surface antigen (HBsAg) is greater than 8%; 43% live in mid-endemic areas where HBsAg prevalence is 2% to 7%; and 12% live in hypo endemic areas where HBsAg prevalence is less than 2% (Mast *et al*; 2005). The acute illness causes liver inflammation, vomiting, jaundice and rarely, death. Chronic hepatitis B may eventually cause liver cirrhosis and liver cancer, it is a fatal disease with very poor response to current chemotherapy (Chang, 2007). However, the infection is prevented by vaccination (Pungpapong *et al*; 2007). The WHO strategy for effective control of HBV infection and its sequel is mass vaccination of neonates and children within the framework of Expanded Program on Immunization (EPI) (Kao and Chen, 2002). It has been recommended that all countries should integrate hepatitis B vaccine into national immunization by the year 1997 (Kao and Chen, 2002). Hepatitis B virus (HBV) infections represent the leading cause of illness and death in China. Every year, an estimated 300,000 persons in China die from HBV-related liver cancer or cirrhosis, accounting for 37–50% of HBV-related deaths worldwide (Liaw and Chu, 2009, Liu and Fan, 2007). This disease results in tremendous economic and health care burdens. The Chinese National Hepatitis Sero epidemiological Survey found that the prevalence of hepatitis B surface antigen (HBsAg) in individuals aged 1–59 years was 7.2%, and that the hepatitis B epidemic in China shifted from high to intermediate endemic following implementation of effective nationwide vaccination programs (Bennet *et al*; 1996). However, the incidence of HBV in Western China remains high with a prevalence of HBs Ag of 8.2% in 2006 (Liang *et al*; 2009), which was significantly higher than rates observed in Eastern China and most western countries (Liang *et al*; 2009, Ioannou 2011). In order to prevent and control HBV transmission, a comprehensive prevention

strategy was initiated in 1992 that included universal vaccination of infants; screening of all pregnant women for HBV, administration of post exposure prophylaxis to infants born to HBsAg positive women, catch-up vaccination of children and adolescents, and vaccination of adults who were at increased risk of infection(Sun *et al*;2002). However, many newborns, children, and adolescents were not vaccinated due to high vaccination costs, especially in economically underdeveloped regions in Western China (Cui *et al*;2007,Liang *et al*;2009). However, analysis of surveillance data has not always resulted in similar conclusions. For example, one study indicated that the incident rate of HBV infections was lower than there ported incident rate described by the NDSIMS and that theinfection rates did not increase between 2005–2007 in China(Cui *et al*; 2008,Wang, 2007) Other studies that focused on high-risk groups showed different HBV prevalence(Ding *et al* ;2003, Zhang *et al*; 2008). Therefore, it is unclear whether the prevalence of HBV infections in western China has decreased in recent years because population-based studies have been rather sparse. This report describes a population-based study designed to determine the HBV infection baseline rates in Wuwei City and to estimate HBV infection and exposure rates in addition to characterizing the immune status in the population.

2.2. Hepatitis B vaccine

It is developed for the prevention of hepatitis B virus infection. The vaccine contains one of the viral envelope proteins, hepatitis B surface antigen (HBsAg). A course of two to three (2–3) vaccine injections is given, the second injection at least one month after the first dose and the third injection being administered six months after the first dose. The first and second dose offer complete protection. 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 cells then provide immunity to hepatitis B infection (CDC,2011).The first vaccine became available in 1981.A range of vaccines are available in the market. Presently recombinant DNA vaccines are available, which means they are produced by inserting the gene for HBV into common baker's yeast where it is grown, harvested, and purified. HBV infection cannot occur from receiving hepatitis B vaccine. The common brands available are Recombivax HB (Merck), Engerix-B (GSK),

Elovac B , Shanvac B, etc. These vaccines are given intra muscularly.It is on the World Health Organization's List of Essential Medicines, a list of the most important medication needed in a basic health system (CDC, 2011).

2.2.1. Medical uses

Babies born to mothers who've had the hepatitis virus are vaccinated with hepatitis B vaccine and injected with hepatitis B immunoglobulin (HBIG) (Mast *et al.*, 2005).In many areas, vaccination against hepatitis B is also required for all health-care and laboratory staff (CDC, 2011). The Centers for Disease Control (CDC) and Prevention have issued recommendations for vaccination against hepatitis B among patients with diabetes mellitus .

2.2.2. Effectiveness

Those who still do not respond to a second course of vaccination may respond to intradermal administration or to a high dose vaccine or to a double dose of a combined Hepatitis A and B vaccine(Cardell *et al.*; 2008).At least one study suggests that hepatitis B vaccination is less effective in patients with HIV (Pasricha *et al.*, 2006).

2.2.3. Duration of protection

It is now believed that the hepatitis B vaccine provides in definite protection. However, it was previously believed and suggested that the vaccination would only provide effective cover of between five and seven years (Petersen *et al.*,2004,Gabbuti *et al.*, 2007) 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 (Damme and Herck ,2007).

2.2.4. Safety

Several studies looked for a significant association between recombinant hepatitis B vaccine (HBV) and multiple sclerosis (MS) in adults. Most published scientific studies do

not support a causal relationship between hepatitis B vaccination and demyelinating diseases such as MS (Zuckerman, 2006) reported a significant increase in risk within 3 years of vaccination. Some of these studies were criticized for methodological problems. This controversy created public misgivings about HB vaccination, and hepatitis B vaccination in children remained low in several countries. A 2006 study concluded that evidence did not support an association between HB vaccination and sudden infant death syndrome, chronic fatigue syndrome, or multiple sclerosis (Mikaeloff *et al*; 2007).

A 2009 study of the hepatitis B vaccine and associated risk of CNS inflammatory demyelination was conducted. The hepatitis B vaccine was found to be generally safe, however the Engerix B vaccine appeared to triple the risk of CNS inflammatory demyelination in infant boys (Mikaeloff *et al*, 2008).

2.2.5. Route of administration

The skin is the more immunogenic site for vaccination due to presence in the dermis of dendritic cells, capable of presenting antigens and stimulating innate and adaptive immune responses. In fact skin protects the body from microbial infection using both its physical barrier and its immunological function performed by dendritic cells (Kupper and Fuhlbrigge, 2004). The main target of intradermal vaccination is represented by Langerhans cells and macrophages in the dermis that are specialized in antigen presentation due to their ability to express high levels of class II Major Histocompatibility Complex (MHC) and CD1 molecules (Kupper and Fuhlbrigge, 2004). These dendritic cells process the antigens released in the dermis, re-expressing part of them as peptide-MHC complexes on the surface and after they have acquired immune stimulatory capacity, migrate to the Para-cortical area of the regional lymph nodes, where they present the antigens to CD8⁺ and CD4⁺ T-lymphocytes (Flacher *et al*; 2006, Nicolas and Guy, 2008). Different signaling pathways are involved in promoting this mechanism, such as increased expression of MHC antigens, interleukin (IL)-1 β , IL-6, IL-12 and tumor necrosis factor- α (Lambert and Laurent, 2008). Furthermore, the release of antigens directly in the dermis affects the migration of dendritic cell precursors from the blood stream to the dermis (Flacher, 2006, Borgn *et al*; 2006, Allan *et al*; 2006). CD8⁺ T

cells clonally expand and become effect or and memory T cells, while CD4+ T cells promote the differentiation of B cells into antibody producing plasma cells (Nicolas and Guy, 2006). This route is more immunogenic, due to the direct release of antigens to the skin immune system, compared with intramuscular vaccination that stimulates T-cell response, due to the lack of dendritic cells in muscles. Studies conducted on smallpox, rabies, *Bacillus Calmette-Guerin* and hepatitis B vaccines supported this hypothesis (Plotkin, 2009). After the introduction of the Mantoux method (Icardi *et al*; 2012).

2.3. Vaccination protocol

The subjects who fulfilled the following selection criteria were enrolled into the vaccination protocol: Age between 18-45 years; seronegativity for HBsAg, anti- HBs, IgG

anti-HBc, anti-HBe and anti-HCV. Exclusion criteria included history of prior exposure to HBV or HCV, incomplete or complete course of vaccination, blood transfusion or presence of any serious systemic disease such as chronic renal failure, congestive heart failure, bleeding diathesis, pregnancy, lactation, overt malignancy, lack of consent. 20 µg of vaccine (recombinant HBsAg) was administered intramuscularly in the deltoid region at 0, 1, and 6 months. The first vaccination was given within seven days after the availability of the screening results. The subjects were asked to report any adverse effects and monitored on the first 3 days. One month after each vaccination, the serum anti-HBs titer was quantitated, at months 1, 2 and 7, with an automated immunoassay analyzer (IMX from Abbott Lab, North Chicago, IL., USA), based on micro particle enzyme immunoassay (MEIA). The analyte (anti-HBs) was captured on coated micro particles. The immune complex was detected with alkaline phosphatase labeled conjugate and fluorogenic substrate. The protocol was approved by the Institutional Ethics Committee of the hospital and the use of the vaccine was approved by the Drug Controller General of India.

The results of vaccination were defined: Sero conversion presence of detectable anti-HBs >1 MIU/ml antibody; sero protection: presence of >10 MIU/ml of anti-HBs titres; unsatisfactory response: absence or presence of <1 MIU/ml anti-HBs titres (non-response) or anti-HBs titres <10 MIU/ml (hypo-response) one month after completion of

the full vaccination schedule; and reactogenicity: nature and incidence of reaction/adverse event after each vaccination. Local and general symptoms including fever, pain, edema, rash, redness, fatigue, headache, body ache, allergy, nausea and vomiting after each vaccination (Thakur *et al*; 2010).

2.4. Previous studies

In Sudan study by (Mohammed *et al*; 2012), showed that HBV rDNA vaccine is immunogenic in Sudanese people when received as three complementary doses. Ninety percent of subjects who received three doses mounted protecting HBsAg specific antibodies while 45% and 55% of those who received one and two doses mounted sufficient antibody immune responses to hepatitis B surface antigen (HBsAg), respectively.

Study in Limburg by Angelique *et al*; (2013) was found from the age of 29 on in men and 43 on in women, more than 5% of subjects did not respond compared with women, men had a higher risk of non-response and exhibited a steeper decline in antibody titer produced with increasing age.

Study in United States by (George and Ioannou, 2014), Results: among persons aged 6 years or older, 0.27% (95% CI, 0.20% to 0.34%) had chronic HBV infection (corresponding to approximately 704 000 persons nationwide), and 4.6% (CI, 4.1% to 5.0%) had been exposed to HBV (approximately 11 993 000 persons). These estimates are lower ($P=0.001$) than estimates of HBV infection (0.42%) and exposure (5.1%) in the United States reported from 1988 to 1994. Infection and past exposure were very uncommon among persons aged 6 to 19 years. Children aged years have high rates of immunity (68.6% [CI, 64.1% to 73.2%]). Adults, including those at high risk for infection, have much lower rates of immunity. Study by Martina *et al*; (2014) about Hepatitis B vaccine by intradermal route in non responder patients. To treat this group of patients at high risk of hepatitis B infection. Recent studies seem to indicate that the administration of HBV recombinant vaccine by the intradermal route is very effective and could represent a more useful strategy than intramuscular route. This review focuses on the use

of anti hepatitis B vaccine by intradermal route as alternative to conventional intramuscular vaccine in all non responder patients. A comprehensive review of the literature using PubMed database, with appropriate terms, was undertaken for articles in English published since 1983.

Study in New Delhi, India, (Varsha *et al*; 2010). After vaccination, 32 males (67%) and 76 females (72%) showed seroconversion; finally 12 (25%) of the males and 47 (45%) of the females were seroprotected. Seroprotection at 2 and 7 months was more dominant in the females than in the males (96% vs. 56%, $P=0.001$, 100% vs. 85%, $P=0.0001$), respectively. Geometric mean titres of anti-HBs after vaccination were also higher in the females than in the males (257 ± 19.7 vs. 29 ± 1.88 MIU/ml, $P=0.01$, 1802 ± 35.2 vs. 306 ± 13.6 MIU/ml, $P\leq0.05$, 6465 ± 72 vs. 2142 ± 73.6 MIU/ml, $P<0.05$). Seven male HCWs showed unsatisfactory response, non-response ($n=3$, 6%) and hypo-response (≤ 10 MIU/ml, $n=4$, 8%) at the end of vaccination. Smoking and alcoholism were significantly correlated with unsatisfactory response. No significant adverse effects of vaccination were observed in any HCW.

Study in China (Zhaohua *et al*; 2014), showed among individuals 1 year of age, 7.2% (95%CI: 6.3–8.1%) had chronic HBV infections, 43.9% (CI: 40.4–47.4%) had been exposed to HBV, and 23.49% (CI: 21.6–25.3%) had vaccine-induced immunity. Multi-factor weighted logistic regression analysis showed that having household contact with HBV carriers (OR = 2.6, 95%CI: 2.3–3.0) and beauty treatments in public places (OR = 1.2, 95%CI: 1.1–1.3) were the risk factors of HBV infection in whole population. Having household contact with HBV carriers (OR = 3.8, 95% CI: 2.2–6.5) and lack of hepatitis vaccination (OR = 2.0, 95% CI: 1.4–3.3) were the risk factors for HBV infection in children aged 1–14 years.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

The present work is a cross-sectional study which was conducted on Health Care Workers vaccinated against hepatitis B virus in some hospitals in Khartoum State.

In this study a total 89 samples (n= 89) were collected from Health Care Workers previously vaccinated with three doses of hepatitis B vaccine in some hospitals in Khartoum State. Khartoum Teaching hospital, Khartoum Bahree hospital, Ear, Nose and Throat Hospital, Alrebat National hospital, from Al raid hospital, Alban jaded Teaching Hospital, from Best care Hospital, Alengaz centre (table1).

Table 1.Number of medical staff from different hospitals

Hospitals	Number
Khartoum Teaching Hospital	7
Khartoum Bahree Hospital	8
E.N .T Hospital	5
Alrebat National hospital	21
Alraed hospital	12
Alban jaded Teaching Hospital	21
Best Care Hospital	7
Alengaz Centre	9
Total	89

3.1.2. Study period

The study was carried during the period from Septamber to Desember 2014

3.1.3. Study population

Sudanese medical staff vaccinated by three doses against hepatitis B virus.

3.1.4. Study area

Khartoum State, The study was carried out in Reach laboratory in Sudan University for Science and Technology Khartoum.

3.1.5. Criteria for selection of cases

3.1.5.1. Inclusion criteria

Participant vaccinated by three doses of hepatitis B vaccine.

3.1.5.2. Exclusion criteria

Health Care Workers with positive surface antigen were excluded from this study.

Health Care Workers vaccinated by one or two doses were excluded from this study.

Health Care Workers infected in the past were excluded from this study.

3.1.6. Data collection

The required data was obtained by a questionnaire (appendix 1), which was designed to obtain data and information which might help in either including or excluding certain individuals.

3.1.7. Ethical consideration

Oral consent was taken from all participants. The study was approved by the Research Board of the Faculty of Medical Laboratory, Sudan University of science and Technology, and full permission was obtained from the different hospitals.

3.2. METHODS

3.2.1. Screening of HBsAg by ICT

It is a qualitative detection of Hepatitis B surface Antigen (HBsAg) in serum (appendix (3)).

3.2.2. PRINCIPLE OF ICT

It is a qualitative, lateral flow immunoassay for the detection of HBsAg in serum or plasma. The membrane is pre-coated with anti-HBsAg antibodies on the test line region of the test. During testing, the serum specimen reacts with the particle coated with anti-HBsAg antibody. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-HBsAg antibodies on the membrane and generate a colored line. The presence of this colored line in the test region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

3.2.3. Test procedures

Test device, serum specimen, and/ or controls were allowed to equilibrate to room temperature (15-30°C) prior to testing.

1. The pouch was brought at room temperature before opening it. The test device was removed from the sealed pouch and it was used before one hour.
2. The test device was placed on a clean and level surface. The dropper was held vertically and 3 full drops of serum (approx. 100ul) was transferred to the specimen well (S) of the test device, and then the timer started. The result was taken within 15 minutes.

Note: A low HBsAg concentration might result in a weak line appearing in the test region (T) after an extended period of time; therefore, do not interpret the result after 30 minutes.

3.2.4. Quantitative methods

3.2.5. Principle of the assay by ELISA

The anti-HBs ELISA kit uses polystyrene microwell strips pre-coated with recombinant HBs Ag. Patient's serum sample added to the microwell together with a second recombinant HBsAg conjugated with horseradish peroxidase (HRP). In case of presence of anti-HBs in the sample the two antigens will be bound to the two variable domains of the antibody and during reaction, the specific immunocomplex formed, is captured on the solid phase.

After washing to remove sample and unbound conjugates, chromogen solutions containing tetramethylbenzidine (TMB) and urea peroxide added to the wells. in presence of the antigen-antibody-antigen sandwich complex ,the colorless chromogens are hydrolyzed by the bound HRP conjugate to a blue colored product. The blue color turn yellow after stopping the reaction with sulfuric acid. The amount of color measured is proportional to the amount of antibody in the sample. Wells containing samples negative for anti-HBs remained colorless.

$\text{Ag(p)} + \text{Ab(s)} + (\text{Ag})\text{ENZ} \rightarrow [\text{Ag(p)} - \text{Ab(s)} + (\text{Ag})\text{ENZ}] \rightarrow \text{blue} \rightarrow \text{yellow color}$

$\text{Ag(p)} + (\text{Ag})\text{ENZ} \rightarrow [\text{Ag(p)}] \rightarrow \text{No color. Incubation(60 min) immobilization complex coloring.}$

3.2.6. Assay / procedure

3.2.2.1. Sampling collection

After informed consent, and use of antiseptic for the skin (70% alcohol), a sample of venous blood (5ml) was collected from each individuals included in this study. The sample was transferred to plain container and left for 30 minute, serum was separated after clotting by centrifugation at 3000 r.p.m, for 5minutes and serum was stored at -20° C until used.

3.2.2.2 Reagents preparation

The reagents were allowed to reach room temperature (18—25 °C).The wash buffer concentration was checked for the presence of salt crystals. If crystals were formed in the solution, resolubilized by warming at 37 °C until crystals dissolved. The stock wash buffer was diluted 20 times with distilled water in clean vessels.

3.2.2.3. Numbering wells

The strips were numbered with sufficient number of wells including six calibration curve standers wells (B1-G1;B2-G2)and one Blank (A1,neither samples nor HRP-Conjugate were added into the blank well).Only one number of strips required for blank was used

and only one number of strips required for it was also used. The standers was used in duplicates.

3.2.2.4. Sampling

Fifty microliter of calibration curve standards and 50 μ l specimen were added into their respective wells. separate disposal pipette tip was used for each specimen to avoid cross-contaminated.

3.2.2.5. HRP-conjugate

Fifty μ l of HRP Reagent was added each well except the blank, and then mixed gently.

3.2.2.6 Incubation

The plate was covered with the plate sealer and incubated for 60 min at 37°C. It dry incubator was used, do not open the door frequently.

3.2.2.7. Washing

After the end of the incubation, the plate sealer was removed and discard. Each well was washed 5 times, allowed the microwells to soak for 30—60sec (appendix(4)).After the final washing cycle, the strips plate was turned in blotting paper to remove any remainders.

3.2.2.8. Coloring

Fifty μ l of chromogen A/B solutions was dispensed and the HRP-Conjugate, blue color in calibration curve standards wells and in anti-HBs positive sample wells (appendix (2)).

3.2.2.9. Stopping Reaction

Used a multichannel pipette 50 μ l of stop solution was added3 into each well and mixed gently. The blue color will turn yellow after stopping the reaction (appendix (1)).

3.2.2.10. Measuring the absorbance

The plate reader was calibrated with the blank and the absorbance was read at 450nm. The cut-off value was calculated automatically by machine.

3.2.3. Quality controls

1-The Optical Density (OD) value of the Blank well, which contains only chromogens and stop solution, is less than 0.080 at 450nm.

2- The Optical Density (OD) value of 0 mIU/ml standard must be less than 0.100 at 450nm after blanking.

3-The Optical Density (OD) value of 160 mIU/ml standard must be higher 1.500 at 450nm after blanking.

3.2.4. Test performance data and expected results

Analytical Endpoint Sensitivity (lower detection limit):in the follow-up of vaccinated individuals the value of 20WHOmIU/ml is the minimum concentration at which the recipient is consider protected. This anti-HBs ELISA kit shows sensitivity of 5mIU/ml.

3.2.5. Data analysis

The data collected in this study were analyzed using Statistical Package For Social Science (SPSS version 11.5) computer software program.(significant levels were set at $p < 0.05$)

CHAPTER FOUR

4. RESULTS

The efficiency of vaccine was determined by measure the titer of antibodies using quantitative Enzyme Linked Immuno Sorbent Assay (ELISA).

The vaccinated subject at different group of ages of the participants show 20-30years 54 participants (60.6%), 31-40years 26(29.2%), 41-50years 4(4.5%),51-60 years 3(3.4%), 61-70 years2(2.3%).(table2).

Table 2: Relation between the efficiency of the vaccine and age by years

Age by years	>20WHO mIU/ml	<20WHO mIU/ml	Total
20-30	41	13	54
31-40	18	8	26
41-50	4	0	4
51-60	2	1	3
61-70	1	1	2
Total	66	23	89

The vaccinated subjects were 28 (31.5%) males, and female 61(68.5%). Relation between the gender of the participant and the efficacy of vaccine, females was positive anti-HBsAg(45),females negative anti-HBsAg(11).and males was positive anti-HBsAg21,males negative anti-HBsAg(7). show there are no effect on efficacy of vaccine in both gender Table 8.

Table 3: Relation between the efficiency of the vaccine and gender

		WHO mIU/ml		Total
		>20	<20	
Gender	Female	45	11	61
	Male	21	7	28
Total		66	18	89

The smoker and protective (8), smokers and non protective (3).

Non smokers and protective (58), no smokers and non protective (20).

Table 4: Relation between the efficiency of the vaccine and smoking

		WHO mIU/ml		Total
		>20	<20	
Smoking	Yes	8	3	11
	NO	58	20	78
Total		66	23	89

P value 0.204 not significant

Period after last dose of HBV vaccine of participants varied from six month to ten years.

The efficacy of the vaccine at different period from last dose of the vaccine show result: short period was more positive anti-HBs Ag 6-20month effective 26, not effective 4, 21-35month effective 12, not effective 7, 36-50 month effective 9, not effective 6, 51-65month effective 7, not effective 1, 66-80month effective 3, not effective 0, 81-95month effective 2, not effective 1, 96-110month effective 4, not effective 2, 111-125month effective 4, not effective 2.

Table 5: Relation between the efficiency of the vaccine and period from the last dose of vaccine.

period after last dose per month	WHO mIU/ml		Total
	Effective	not effective	
6-20	26	3	29
21-35	12	7	19
36-50	9	6	15
51-65	7	1	8
66-80	3	0	3
81-95	1	2	3
96-110	4	2	6
111-125	4	2	6
Total	66	23	89

The results showed that 66(74.2%) of the vaccinated subject found to be protective read by optical density more than (more than 20 WHO mIU/ml) ,24(26.7%) of the vaccinated subject found to be positive (more than 20 WHO mIU/ml

The efficacy of the vaccine was found 100% in participants of age rang 41-50 years while it was found 33% in participants 51-60 years.

The vaccine was found effective in 87.5% in participant of period last dose 51-65 month while it was found effective in 33% in participant of period 81-95 month after the last dose of vaccination(table 8)

positive anti-HBsAg= seroprotective >20WHOmIU/ml.

Negative anti-HBsAg= not seroprotective<20WHOmIU/ml.

Table6: the efficiency of the vaccine

efficacy	Frequency	Percentage%
Effective	66	74.2
Not effective	23	25.8
Total	89	100.0

P value 0.204 not significant

CHAPTER FIVE

DISCUSSION

Hepatitis B virus (HBV) infection is a global health problem. The frequency of hepatitis B viral infection in a subset of population is a function of several factors, which increase the risk of viral entry. These include environmental and life style related factors contributing to the acquisition of HBV infection. Health Care Workers (HCWs) constitute a high risk group for acquiring infection with blood borne pathogens due to occupational contact with infected body fluid.

This study was conducted to assess efficiency of hepatitis B vaccine in Health Care Workers (HCWs). The results of study show efficient antibody immune responses to recombinant rDNA vaccine following the third dose 74.2% of Sudanese health care worker built efficient antibody these in agreement with (Bolad *et al*; 2012) who demonstrated 90% of Sudanese individuals have built efficient antibody immune responses to recombinant DNA vaccine following the third dose. found few variation in the result referred to small sample size in my research and large sample size Bolad.

In this study 89 HCWs screened, all have not shown HBsAg markers these results disagree with that obtain by Thakur *et al*;(2010) who reported that out of 597 HCWs screened, 216 (36.2%) had at least one of the markers of HBV or HCV infection.

The gender of the participant and the efficacy of vaccine, females was positive anti-HBsAg(45),females negative anti-HBsAg(16) males was positive anti HBsAg (21),males negative anti-HBs(8) show there are no effect on efficacy of vaccine in both gender these result disagree with that obtain by (Thakur *et al*;2010) The response to this vaccine was better in female HCWs than in male ones. The percentage of subjects who were seroprotected (anti-HBs titres >10 MIU/ml) at the first month was slightly higher in females than in males (44% vs. 21%), though the difference was not significant. However, the proportion of seroprotected female HCWs was significantly higher after the 2nd and 3rd vaccinations at month 2 and 7 than that the male HCWs (95% vs. 57% and 100% vs. 85%).

show there are no effect on efficacy of vaccine associated with age and obesity,

smoking, and gender these result disagree with that obtain by (Ferraz *et al*;1995) showed that the decreased immunogenicity of HBV

vaccination was associated with increasing age, obesity, smoking, and male gender.

In this study no effect on efficacy of vaccine in different age groups this disagrees with (Hussain *et al*;2005)who found that 85.7% seroconversion was in those > 40 years old and disagree from the study by Angelique *et al.*, 2013, who found that the age of 29 in men and 43 in women, and more than 5% of subjects did not respond compared with women, men had a higher risk of non-response and exhibited a steeper decline in antibody titer produced with increasing age .

CONCLUSION AND RECOMMENDATION

5.1. Conclusion

It is concluded from this study that 74.2% HCWs build immunity against Hepatitis B virus when vaccinated with recombinant DNA hepatitis B virus the result varied among age, gender, period after last dose of vaccine and smokers

5.2. Recommendation

1. Incorporation of rDNA HBV vaccine in the routine immunization for Sudanese people.
2. Preferably individuals should receive at least three doses of the rDNA vaccine in order to achieve long term protection.
3. More knowledge about prevention is needed among HCWs and appropriate educational programs regarding HBV and its modes of transmission, infection and the way of prevention.
4. Implementing extensive educational programs about HBV risks and vaccinations are mandatory.
5. Constant policy for most of our universities to vaccinate their students who are going to work in the medical field.

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بسم الله الرحمن الرحيم

Sudan University of Science and technology

College Of medical Laboratory science

Questionnaire about the Efficiency of Recombinant DNA Vaccine Against Hepatitis B Virus in Health Care Workers in Some Hospital in Khartoum State

Date:.....telephone

Sample number. :.....

Name:.....

Age:.....

Sex: male

☐

female

☐

Occupation

Residence

No of doses: three

☐

or more

☐

Did you have history of jaundice?

Before

☐

during

☐

after

☐

Screening before vaccination

Yes

☐

No

☐

Smoking

Yes ☐

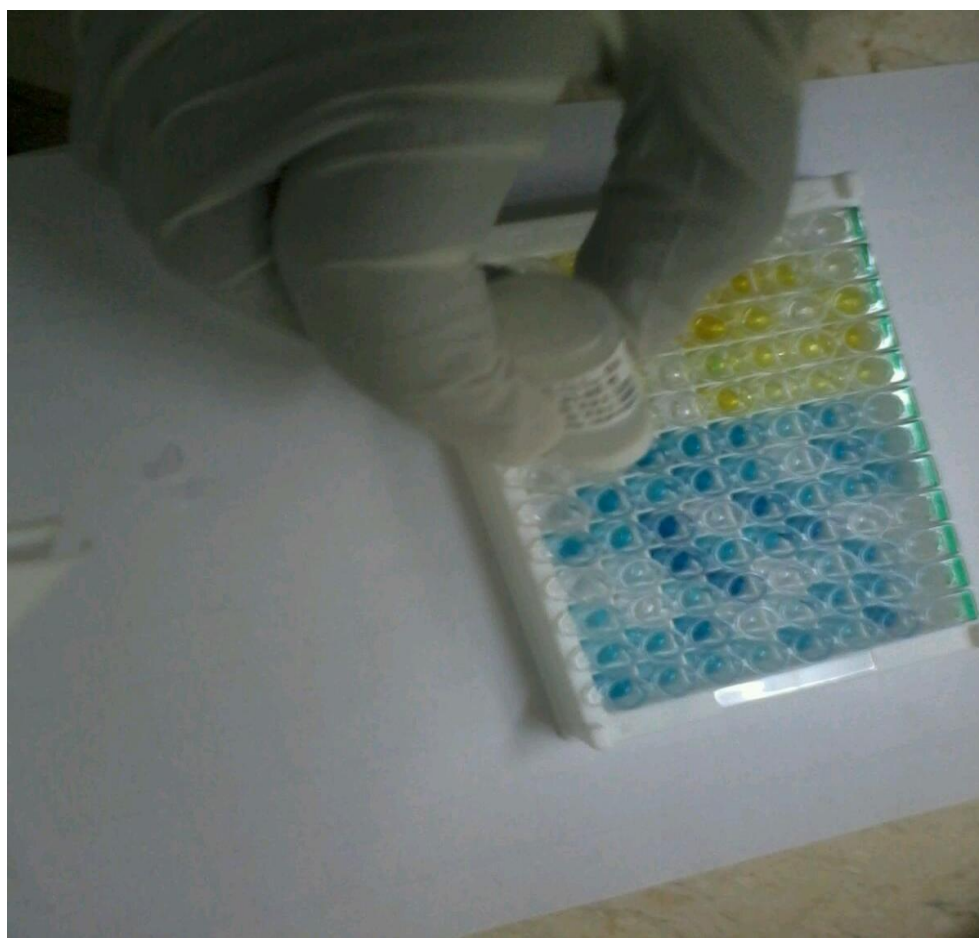
No ☐

Alcoholic

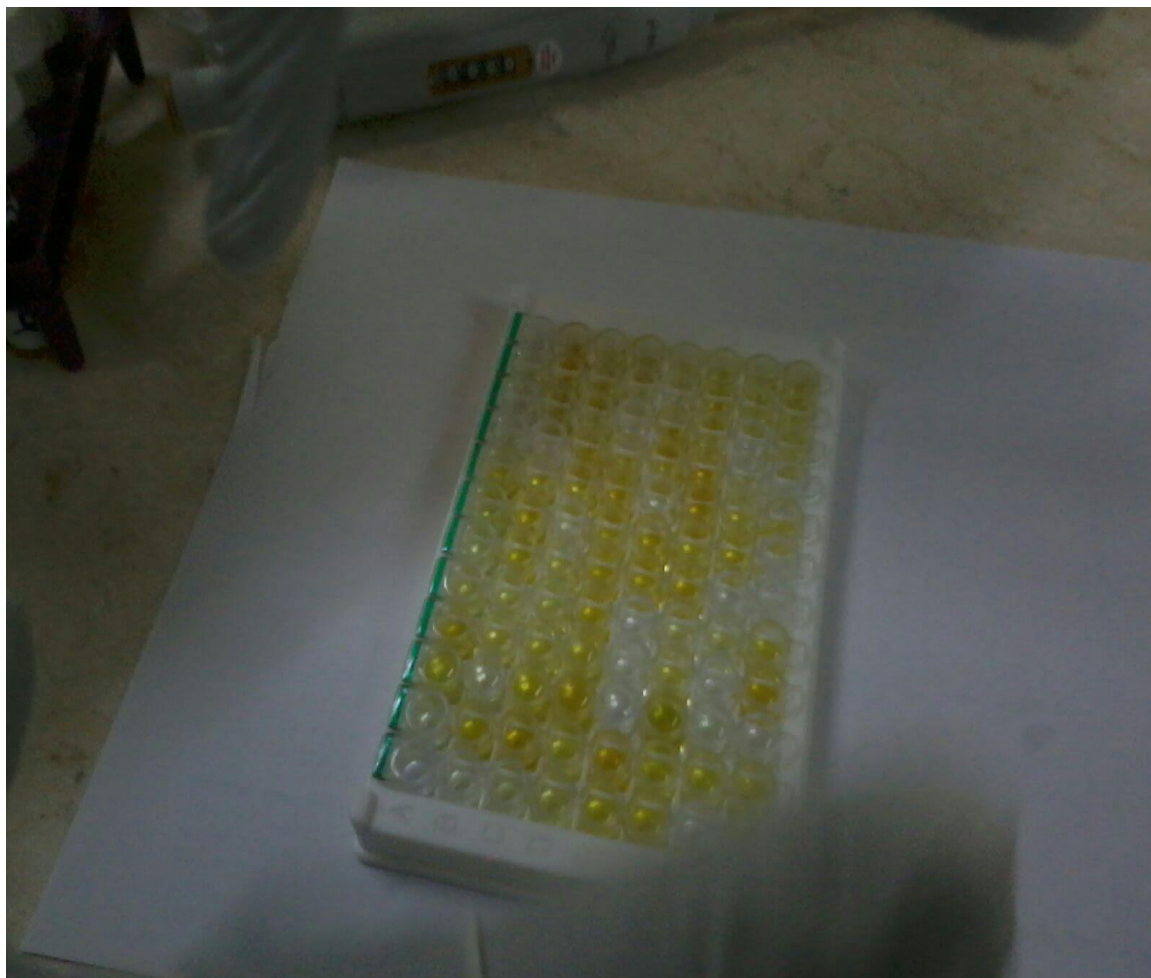
Yes ☐

No ☐

Appendix (1): Stop solution



Appendix (2): Coloring (Chromogen Solution)



Appendix(3):Rapid Immuno Chromatography Test for screening HBsAg



Appendix(4):washer machine

