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Estimation of Hepatitis B virus Antibodies Titer due to Vaccination among Medical field professionals in sudan cardiac center

A dissertation submitted in Partial Fulfillment for the Requirements of M.Sc. Degree in Medical Laboratory Sciences (Microbiology)

By

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قال تعالى :(وَوَصَّيْنَا الْإِنْسَانَ بِوَالِدَيْهِ حَمَلَتْهُ أُمُّهُ وَهْنًا عَلَى وَهْنٍ وَفِصَالُهُ فِي عَامَيْنِ أَنِ اشْكُرْ لِي وَلِوَالِدَيْكَ إِلَيَّ الْمَصِيرُ (14)وَإِنْ جَاهَدَاكَ عَلَى أَنْ تُشْرِكَ بِي مَا لَيْسَ لَكَ بِهِ عِلْمٌ فَلَا تُطِعْهُمَا وَصَاحِبْهُمَا فِي الدُّنْيَا مَعْرُوفًا وَاتَّبِعْ سَبِيلَ مَنْ أَنَابَ إِلَيَّ ثُمَّ إِلَيَّ مَرْجِعُكُمْ فَأُنَبِّئُكُمْ بِمَا كُنْتُمْ تَعْمَلُونَ (15)يَا بُنَيَّ إِنَّهَا إِنْ تَكُ مِثْقَالَ حَبَّةٍ مِنْ خَرْدَلٍ فَتَكُنْ فِي صَخْرَةٍ أَوْ فِي السَّمَاوَاتِ أَوْ فِي الْأَرْضِ يَأْتِ بِهَا اللَّهُ إِنَّ اللَّهَ لَطِيفٌ خَبِيرٌ (16)يَا بُنَيَّ أَقِمِ الصَّلَاةَ وَأْمُرْ بِالْمَعْرُوفِ وَانْهَ عَنِ الْمُنْكَرِ وَاصْبِرْ عَلَى مَا أَصَابَكَ إِنَّ ذَلِكَ مِنْ عَزْمِ الْأُمُورِ (17)وَلَا تُصَعِّرْ خَدَّكَ لِلنَّاسِ وَلَا تَمْشِ فِي الْأَرْضِ مَرَحًا إِنَّ اللَّهَ لَا يُحِبُّ كُلَّ مُخْتَالٍ فَخُورٍ (18)وَاقْصِدْ فِي مَشْيِكَ وَاغْضُضْ مِنْ صَوْتِكَ إِنَّ أَنْكَرَ الْأَصْوَاتِ لَصَوْتُ الْحَمِيرِ (19))

صدق الله العظيم

سورة لقمان من الاية 14 الي الاية 19

Dedication

I dedicate this research to…………..
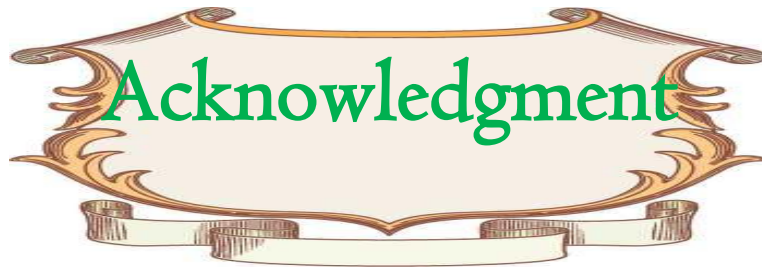
My parents………

Brothers………

Sisters ……….

My families and fiancée ……..

Friends……….

Colleagues………

All peoples who encourage, facilitate, forearm, and help us

OMER

Acknowledgment

All the gratitude and thank firstly for Allah.

who was sun enlighten I thanks full **Dr. Waseem Sameer Kwami**,

knowledge and didn't niggard by valuable notion, exclusive and expensive

time, I thank him for his condole and mummify for this continuous

supervision, advisor, valuable comments and suggestion.

I grateful to staff of Microbiology department at Shendi University,

teachers, technician and workers.

ABSTRACT

This is descriptive cross sectional study conducted in sudan cardiac center in period from April to July 2018 .The objective was to state   Khartoum estimate the titer of hepatitis B antibody among medical field professionals who were   vaccinated by hepatitis B vaccine and had no history of HBV infection or received hepatitis B immunoglobulin prophylaxis.

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.

المستخلص

أجريت هذه الدراسة الوصفية في مركز السودان للقلب ولاية الخرطوم في الفترة من شهر ابريل 2018 الى يوليو 2018 .

الغرض من هذه الدراسة هو تقدير العيار الكمي للأجسام المضادة لفيروس إلتهاب الكبد البائي (ب) في الاشخاص الذين يعملون في المجال الطبي نتيجة لاخذهم التطعيم ضد فيروس إلتهاب الكبد البائي (ب) ، وليس لهم تاريخ بالأصابة بهذا العدوى وايضا لم يتناولوا جرعات تطعيمية وقائية من الاجسام المضادة .

تم جمع عدد 89 عينة دم من المشاركين في الدراسة وتم فصل المصل من هذه العينات عن طريق إستخدام جهاز الطرد المركزي بسرعة 2000 دورة في الدقيقة . وتم تحديد مستويات العيار الكمي لهذه الاجسام المضادة عن طريق استخدام جهاز الاليزا (تقنية الساندوتش).

كشفت هذه الدراسة ان 65.2 % من المشاركين لديهم استجابة قوية للتحصين ضد فيروس إلتهاب الكبد البائي (ب) (المعدل اكثر من 100 وحدة عالمية امل) و30.3 لديهم استجابة ضعيفة (المعدل 10−100 وحدة عالمية امل) ,و 4.5% ليست لديهم استجابة مناعية للتحصين (المعدل اقل من 10 وحدات عالميةامل).

خلصت الدراسة الى أن الاستجابة المناعية القوية للتحصين ضد فيروس إلتهاب الكبد البائي لا تعزى الى النوع ولا الفئة العمرية . و على جميع الذين اخذوا تحصين ضد فيروس إلتهاب الكبد البائي ان تقاس كمية الاجسام المضادة الموجودة في أجسامهم للتاكد للتمنعهم من ضد الفيرس.

Table of contents

List of tables

List of abbreviations

Abbreviation	Full name
AIDS	Acquired immunedeficiency syndrome
ALT	Alaanine aminotransferase
Anti-HBs	Anti hepatitis B antibody
CTLs	Cytotoxic t lymphocytes
DNA	Deoxy ribonucleic acid
ELISA	Enzyme linked immune serpent assay
HBcAg	Hepatitis B virus core antigen
HBeAg	Hepatitis B envelope antigen
HBIG	Hepatitis B immunoglobulin
HBs Ag	Hepatits B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCWs	Health care worker
HIV	Human immuodeficiency virus
MGN	Membranous glomerulonephritis
MTCT	Mother to child transmission
PDV	Plasma -derived vaccine
RV	Recombinant vaccine
STD	Sexual transmitted diseased
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION and OBJECTIVES

1.1. Introduction

Hepatitis B vaccine is a vaccine that prevents hepatitis B infection . 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 *etal* ., 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).

1.2. Rationale:

Hepatitis B is a potentially life-threatening liver infection caused by the hepatitis B virus (HBV). It is a major global health problem. It can cause chronic infection and puts people at high risk of death from cirrhosis and liver cancer. Vaccine of hepatitis B prevents serious disease and decrease number of infection. (WHO ., 2015)

The current study is conducted to determine efficiency of vaccine in reducing the risk of hepatitis B in medical field professionals through estimation of antibodies level as indicator of immune response to hepatitis B vaccine.

1.3. Objectives:

1.3.1. General objective:

To estimate the titer of hepatitis B antibody among vaccinated medical field professionals.

1.3.2. Specific objectives

1. To determine the level of hepatitis B virus antibody titer in serum of vaccinated medical field professionals by using quantitative indirect ELISA assay.

CHAPTER TWO

LITERATURE REVIEW

2.1. Background:

Hepatitis B is an infectious disease caused by the hepatitis B virus (HBV) that 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, yellowish skin, tiredness, dark urine and abdominal pain , Often these symptoms last a few weeks and rarely does the initial infection result in death. It may take 30 to 180 days for symptoms to begin (Busch and Thimme ., 2015).

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 (Vincent ., 2016) . Most of those with chronic disease have no symptoms; however, cirrhosisand liver cancer may eventually develop (Chang,., 2007).

These complications result in the death of 15 to 25% of those with chronic disease (Busch and Thimme ., 2015).

The virus is transmitted by exposure to infectious blood or body fluids. Infection around the time of birth or from contact with other people's blood during childhood is the most frequent method by which hepatitis B is acquired in areas where the disease is common. In areas where the disease is rare, intravenous drug use and sexual intercourse are the most frequent routes of infection (Busch and Thimme ., 2015).

Other risk factors include in health care warker , blood transfusions, dialysis, living with an infected person, travel in countries where the infection rate is high, and living in an institution (Vincent ., 2016).

Tattooing and acupuncture led to a significant number of cases in the 1980s; however, this has become less common with improved sterility (Forton *et al* ., 2003). The hepatitis B viruses cannot be spread by holding hands, sharing eating utensils, kissing, hugging, coughing, sneezing, or breastfeeding (Vincent ., 2016).

The infection can be diagnosed 30 to 60 days after exposure. The diagnosis is usually confirmed by testing the blood for parts of the virus and for antibodies against the virus (Busch and Thimme ., 2015). It is one of five main hepatitis viruses: A, B, C, D, and E (WHO ., 2017).

The infection has been preventable by vaccination since 1982 (Pungpapong *etal* .,2007). Vaccination is recommended by the World Health Organization in the first day of life if possible. Two or three more doses are required at a later time for full effect. This vaccine works about 95% of the time (Busch and Thimme ., 2015). About 180 countries gave the vaccine as part of national programs as of 2006 (Williams ., 2006).

It is also recommended that all blood be tested for hepatitis B before transfusion and condoms be used to prevent infection. During an initial infection, care is based on the symptoms that a person has. In those who develop chronic disease , antivral medication such as tenofovir or interferon may be useful ; however , these drugs are expensive. Liver transplantation is sometimes used for cirrhosis (Busch and Thimme ., 2015).

About a third of the world population has been infected at one point in their lives, including 343 million who have chronic infections (Schilsky, 2013). Another 129 million new infections occurred in 2013 (Vos *et al* ., 2015). Over 750,000 people die of hepatitis B each year. About 300,000 of these are due to liver cancer (Abubakar*et al*, 2015). The disease is now only common in East Asia and sub-Saharan Africa where between 5 and 10% of adults are chronically infected. Rates in Europe and North America are less than 1%. It was originally known as "serum hepatitis" (Barker *et al*, 1996). Research is looking to create foods that contain HBV vaccine (Thomas *et al* ., 2002). The disease may affect other great apes as well (Kuschner*et al*, 2013).

2.2. General characteristics of hepatitis B virus:

Hepatitis B virus (HBV) is a member of the hepadnavirus family (Zuckerman, 1996). The virus particle (virion) consists of an outer lipid envelope and an icosahedral nucleocapsid core composed of protein. These virions are 30–42 nm in diameter. The nucleocapsid encloses the viral DNA and a DNA polymerase that has reverse transcriptase activity (Locarnini ., 2004).

The outer envelope contains embedded proteins that are involved in viral binding of, and entry into, susceptible cells. The virus is one of the smallest enveloped animal viruses. The 42 nm virions, which are capable of infecting liver cells known as hepatocytes, are referred to as "Dane particles" (Harrison, 2009). In addition to the Dane particles, filamentous and spherical bodies lacking a core can be found in the serum of infected individuals. These particles are not infectious and are composed of the lipid and protein

that forms part of the surface of the virion, which is called the surface antigens (HBsAg), and is produced in excess during the life cycle of the virus (Howard ., 1986).

2.3. Genome of hepatitis B virus:

The genome of HBV is made of circular DNA, but it is unusual because the DNA is not fully double-stranded. One end of the full length strand is linked to the viral DNA polymerase. The genome is 3020–3320 nucleotides long (for the full-length strand) and 1700–2800 nucleotides long (for the short length-strand) (Kay and Zoulim ., 2007).

The negative-sense (non-coding) is complementary to the viral mRNA. The viral DNA is found in the nucleus soon after infection of the cell. The partially double-stranded DNA is rendered fully double-stranded by completion of the (+) sense strand and removal of a protein molecule from the (−) sense strand and a short sequence of RNA from the (+) sense strand. Non-coding bases are removed from the ends of the (−) sense strand and the ends are rejoined. There are four known genes encoded by the genome, called C, X, P, and S. The core protein is coded for by gene C (HBcAg), and its start codon is preceded by an upstream in-frame AUG start codon from which the pre-core protein is produced. HBeAg is produced by proteolytic processing of the pre-core protein. In some rare strains of the virus known as Hepatitis B virus precore mutants, no HBeAg is present (Buti *et al* ., 2005).

The DNA polymerase is encoded by gene P. Gene S is the gene that codes for the surface antigen (HBsAg). The HBsAg gene is one long open reading frame but contains three in frame "start" (ATG) codons that divide the gene

into three sections, pre-S1, pre-S2, and S. Because of the multiple start codons, polypeptides of three different sizes called large (the order from surface to the inside: pre-S1, pre-S2, and S), middle (pre-S2, S), and small (S) (Glebe and Urban ., 2007) are produced (Beck and Nassal ., 2007).

The function of the protein coded for by gene X is not fully understood but it is associated with the development of liver cancer. It stimulates genes that promote cell growth and inactivates growth regulating molecules (Li, *et al* ., 2010).

2.4. Serotypes and genotypes:

The virus is divided into four major serotypes (adr, adw, ayr, ayw) based on antigenic epitopes presented on its envelope proteins, and into eight major genotypes (A–H). The genotypes have a distinct geographical distribution and are used in tracing the evolution and transmission of the virus. Differences between genotypes affect the disease severity, course and likelihood of complications, and response to treatment and possibly vaccination (Kramvis*et al*, 2005: Magnius and Norder ., 1995).

There are two other genotypes I and J but they are not universally accepted as of 2015 (Araujo ., 2015).

Genotypes differ by at least 8% of their sequence and were first reported in 1988 when six were initially described (A–F) (Norder *et al* ., 1994).

Two further types have since been described (G and H) (Shibayama*et al*, 2005). Most genotypes are now divided into subgenotypes with distinct properties (Schaefer., 2007).

2.5. Epidemiology of hepatitis B virus:

In 2004, an estimated 350 million individuals were infected worldwide. National and regional prevalence range from over 10% in Asia to under 0.5% in the United States and Northern Europe. Routes of infection include vertical transmission (such as through childbirth), early life horizontal transmission (bites, lesions, and sanitary habits), and adult horizontal transmission (sexual contact, intravenous drug use) (Custer *et al* ., 2004).

The primary method of transmission reflects the prevalence of chronic HBV infection in a given area. In low prevalence areas such as the continental United States and Western Europe, injection drug abuse and unprotected sex are the primary methods, although other factors may also be important (Redd*et al* ., 2007).

 In moderate prevalence areas, which include Eastern Europe, Russia, and Japan, where 2–7% of the population is chronically infected, the disease is predominantly spread among children. In high-prevalence areas such as China and South East Asia, transmission during childbirth is most common, although in other areas of high endemicity such as Africa, transmission during childhood is a significant factor (Alter ., 2003).

The prevalence of chronic HBV infection in areas of high endemicity is at least 8% with 10–15% prevalence in Africa/Far East (Komas *et al*, 2013). As of 2010, China has 120 million infected people, followed by India and Indonesia with 40 million and 12 million, respectively. According to World Health Organization (WHO), an estimated 600,000 people die every year related to the infection (Ge *et al* ., 2012).

2.6. Transmission:

Transmission of hepatitis B virus results from exposure to infectious blood or body fluids containing blood. It is 50 to 100 times more infectious than human immunodeficiency virus (HIV) (Sood *et al* ., 2013).

Possible forms of transmission include sexual contact (Israr *etal* ., 2017), blood transfusions and transfusion with other human blood products (Buddeberg *etal* ., 2008), re-use of contaminated needles and syringes (Hughes ., 2000), and vertical transmission from mother to child (MTCT) during childbirth. Without intervention, a mother who is positive for HBsAg has a 20% risk of passing the infection to her offspring at the time of birth. This risk is as high as 90% if the mother is also positive for HBeAg. HBV can be transmitted between family members within households, possibly by contact of nonintact skin or mucous membrane with secretions or saliva containing HBV (Sood *et al* ., 2018).

However, at least 30% of reported hepatitis B among adults cannot be associated with an identifiable risk factor (Shapiro, 1993). Breastfeeding after proper immunoprophylaxis does not appear to contribute to mother-to-child-transmission (MTCT) of HBV (Shi *etal* ., 2011).

The virus may be detected within 30 to 60 days after infection and can persist and develop into chronic hepatitis B. The incubation period of the hepatitis B virus is 75 days on average but can vary from 30 to 180 days (Hope *etal* ., 2014).

2.7. Signs and symptoms of hepatitis B virus:

Acute infection with hepatitis B virus is associated with acute viral hepatitis, an illness that begins with general ill-health, loss of appetite, nausea, vomiting, body aches, mild fever, and dark urine, and then progresses to development of jaundice. It has been noted that itchy skin has been an indication as a possible symptom of all hepatitis virus types. The illness lasts for a few weeks and then gradually improves in most affected people. A few people may have a more severe form of liver disease known as (fulminant hepatic failure) and may die as a result. The infection may be entirely asymptomatic and may go unrecognized (Terrault *etal* ., 2005).

Chronic infection with hepatitis B virus either may be asymptomatic or may be associated with a chronic inflammation of the liver (chronic hepatitis), leading to cirrhosis over a period of several years. This type of infection dramatically increases the incidence of hepatocellular carcinoma (HCC; liver cancer). Across Europe, hepatitis B and C cause approximately 50% of hepatocellular carcinomas (El–Serag and Rudolph, 2007; Davila *et al* ., 2011). Chronic carriers are encouraged to avoid consuming alcohol as it increases their risk for cirrhosis and liver cancer. Hepatitis B virus has been linked to the development of membranous glomerulonephritis (MGN) (Gan *et al* ., 2005).Symptoms outside of the liver are present in 1–10% of HBV-infected people and include serum-sickness–like syndrome, acute necrotizing vasculitis (polyarteritisnodosa), membranous glomerulonephritis, and papularacrodermatitis of childhood (Gianotti–Crosti syndrome) (Dienstag, 1981; Trepo and Guillevin ., 2001). The serum-sickness–like syndrome occurs in the setting of acute hepatitis B, often preceding the onset of jaundice (Alpert *et al* ., 1971).

The clinical features are fever, skin rash, and polyarteritis. The symptoms often subside shortly after the onset of jaundice but can persist throughout the duration of acute hepatitis B (Liang ., 2009). About 30–50% of people with acute necrotizing vasculitis (polyarteritisnodosa) are HBV carriers (Gocke *etal* ., 1970). HBV-associated nephropathy has been described in adults but is more common in children (Takekoshi *et al* ., 1978).

Membranouse glomerulonephritis is the most common form other immune mediated hematological disorder such as essential mixed cryogolobuline emia and aplastic anemia have been described as apart of extra hepatic manifestation of HBV infection , but their association is not as well defined ; therefore , they probably should not be considered etiologically linked to HBV (liang ., 2009).

2.8. Pathogenesis of HBV infection:

Hepatitis B virus, after entering the blood, infects the hepatocytes in the liver with the expression of viral antigen on the surface of infected cells.Cytotoxic T cells, such as activated CD4 and CD8 lymphocytes, recognize various HBV-derived proteins present on the surface of hepatocytes resulting in an immunological reaction   (Subhash *et al* .,   2012).

The virus by itself does not cause any cytopathic effect in the infected liver cells.The injury or cytopathic effects most probably occur as a result of cell-mediated injury. The formation of antigen–antibody complexes is responsible for some of the symptoms, such as arthralgia, arthritis seen during early stage of the disease. The immune complex is also responsible for some of the complications associated with chronic hepatitis, such as immune complex glomerulonephritis, cryoglobulinemia, and vasculitis. A restricted T-cell-mediated lymphocyte response occurs against the HBV-

infected hepatitis , A chronic carrier stage with HBV infection is an important event in the pathogenesis of HBV infection. A person with chronic carrier stage has HBsAg persisting in the blood for at least 6 months. This stage is caused by a persistent infection of the hepatocytes that leads to the presence of HBV and HBsAg in the blood,This chronic carrier stage occurs in about 5% of patients with HBV infection in contrast to no chronic carrier stage in patients with HAV infection In an infected host whether the person (Subhash *et al .,* 2012).

will become a chronic carrier state or will be free of infection depends on the cytotoxic T-cell response. If the cytotoxic T-cell response is strong, the infection is cleared in the person but if the response is inadequate, the person becomes a chronic carrier. During the chronic stage, the HBV DNA is present in episome in the cytoplasm of persistently infected cells, and in some cells the viral DNA is integrated with cellular DNA. (Subhash *et al .,* 2012).

Chronic carrier state is more likely to occur when infection occurs in a newborn than in adult. It has been observed that approximately 90% of the infected neonates become chronic carriers. Approximately 20% of HBsAg carriers, nearly 1% of all adult patients infected with HBV, and high percentage of neonates infected with the virus progress to develop hepatocellular carcinoma or cirrhosis. (Subhash *et al.,* 2012).

The hepatocellular carcinoma appears to be the result of persistent cellular regeneration that tends to replace the dead hepatocytes. Also it is suggested that the integration of HBV DNA with hepatocytes DNA could activate a cellular oncogene, resulting in loss of control of the growth of hepatocytes.

However, the HBV genome has no oncogene which can be responsible directly for causing hepatocellular carcinoma (Subhash *et al .,* 2012).

2.9 Mechanisms of host immuune response:

Hepatitis B virus primarily interferes with the functions of the liver by replicating in hepatocytes. A functional receptor is NTCP (Yan *et al*, 2012). There is evidence that the receptor in the closely related duck hepatitis B virus is carboxypeptidase D (Tong *et al*, 1999; Glebe and Urban ., 2007).

The virions bind to the host cell via the pre S domain of the viral surface antigen and are subsequently internalized by endocytosis. HBV-preS-specific receptors are expressed primarily on hepatocytes; however, viral DNA and proteins have also been detected in extrahepatic sites, suggesting that cellular receptors for HBV may also exist on extrahepatic cells (Coffin *et al .,* 2011).

During HBV infection, the host immune response causes both hepatocellular damage and viral clearance. Although the innate immune response does not play a significant role in these processes, the adaptive immune response, in particular virus-specific cytotoxic T lymphocytes (CTLs), contributes to most of the liver injury associated with HBV infection. CTLs eliminate HBV infection by killing infected cells and producing antiviral cytokines, which are then used to purge HBV from viable hepatocytes (Iannacone *et al* ., 2007). Although liver damage is initiated and mediated by the CTLs, antigen-nonspecific inflammatory cells can worsen CTL-induced immunopathology, and platelets activated at the site of infection may facilitate the accumulation of CTLs in the liver (Iannacone *et al .,* 2005).

2.10 Diagnosis:

The tests, called assays, for detection of hepatitis B virus infection involve serum or blood tests that detect either viral antigens (proteins produced by the virus) or antibodies produced by the host. Interpretation of these assays is complex (Bonino *et al*., 1988).

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 the core protein, alternatively known as hepatitis Bcore 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-HBcIgM*) may be the only serological evidence of disease. Therefore, most hepatitis B diagnostic panels contain HBsAg and total anti-HBc (both IgM and IgG) (Mahy and Van., 2010).

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 (Liaw *et al*., 2010).

 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 HBcIgG*) (Zuckerman ., 1996).

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.

Individuals who remain HBsAg positive for at least six months are considered to be hepatitis B carriers (Lok and McMahon ., 2007).

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 (Chu and Liaw ., 2007). However, it is possible for individuals to enter an "immune escape" with HBeAg-negative hepatitis.

PCR tests have been developed to detect and measure the amount of HBV DNA, called the viral load, in clinical specimens. These tests are used to assess a person's infection status and to monitor treatment (Zoulim ., 2006).

Individuals with high viral loads, characteristically have ground glass hepatocytes on biopsy.

2.11. Treatment:

Acute hepatitis B infection does not usually require treatment and most adults clear the infection spontaneously (Hollinger and Lau, 2006; Wood *et al* ., 1993).

Early antiviral treatment may be required in fewer than 1% of people, whose infection takes a very aggressive course (fulminant hepatitis) or who are immunocompromised. On the other hand, treatment of chronic infection may be necessary to reduce the risk of cirrhosis and liver cancer. Chronically infected individuals with persistently elevated serum alanine aminotransferase, a marker of liver damage, and HBV DNA levels are candidates for therapy (Lai and Yuen ., 2007).

Treatment lasts from six months to a year, depending on medication and genotype (Alberti and Caporaso ., 2011). Treatment duration when medication is taken by mouth, however, is more variable and usually longer than one year (Terrault *et al* ., 2016).

Although none of the available drugs can clear the infection, they can stop the virus from replicating, thus minimizing liver damage. As of 2008, there are seven medications licensed for the treatment of hepatitis B in the united state.
include antiviral drugs lamivudine (Epivir), adefovir (Hepsera), tenofovir (Viread), telbivudine (Tyzeka)   and entecavir (Baraclude),   and   the two immune   system modulators interferon   alpha-2a and PEGylated interferon alpha-2a (Pegasys). In 2015 the World Health Organization

recommended tenofovir or entecavir as first-line agents. Those with current cirrhosis are in most need of treatment (WHO ., 2015).

The use of interferon, which requires injections daily or thrice weekly, has been supplanted by long-acting PEGylated interferon, which is injected only once weekly (Dienstag ., 2008).

However, some individuals are much more likely to respond than others, and this might be because of the genotype of the infecting virus or the person's heredity. The treatment reduces viral replication in the liver, thereby reducing the viral load (the amount of virus particles as measured in the blood) (Pramoolsinsup ., 2002).

Response to treatment differs between the genotypes. Interferon treatment may produce an e antigen seroconversion rate of 37% in genotype A but only a 6% seroconversion in type D. Genotype B has similar seroconversion rates to type A while type C seroconverts only in 15% of cases. Sustained e antigen loss after treatment is ~45% in types A and B but only 25–30% in types C and D (Cao ., 2009).

2.12 Prognosis:

Hepatitis B virus infection may be either acute (self-limiting) or chronic (long-standing). Persons with self-limiting infection clear the infection spontaneously within weeks to months.

Children are less likely than adults to clear the infection. More than 95% of people who become infected as adults or older children will stage a full recovery and develop protective immunity to the virus. However, these drops to 30% for younger children, and only 5% of newborns that acquire

the infection from their mother at birth will clear the infection (Bell and Nguyen ., 2009).

This population has a 40% lifetime risk of death from cirrhosis or hepatocellular carcinoma (Dienstag ., 2008). Of those infected between the age of one to six, 70% will clear the infection (Kerkar ., 2005).

Hepatitis D (HDV) can occur only with a concomitant hepatitis B infection, because HDV uses the HBV surface antigen to form a capsid (Taylor ., 2006). Co-infection with hepatitis D increases the risk of liver cirrhosis and liver cancer (Oliveri *et al* ., 1991).

2.13 Prevention:

2.13.1 Vaccine:

Vaccines for the prevention of hepatitis B have been routinely recommended for babies since 1991 in the United States (Schillie *et al* ., 2013). The first dose is generally recommended within a day of birth (committee on infectious disease ., 2017).

Most vaccines are given in three doses over a course of months. A protective response to the vaccine is defined as an anti-HBs antibody concentration of at least 10 mIU/ml in the recipient's serum . The vaccine is more effective in children and 95 percent of those vaccinated have protective levels of antibody. This drops to around 90% at 40 years of age and to around 75 percent in those over 60 years. The protection afforded by vaccination is long lasting even after antibody levels fall below 10 mIU/ml . For newborns of HBsAg-positive mothers: hepatitis B vaccine alone, hepatitis B immunoglobulin alone, or the combination of vaccine plus hepatitis B

immunoglobulin, all prevent hepatitis B occurrence. Furthermore, the combination of vaccine plus hepatitis B immunoglobulin is superior to vaccine alone (Lee ., 2006). This combination prevents HBV transmission around the time of birth in 86% to 99% of cases (Wong *et al* ., 2014).

Tenofovir given in the second or third trimester can reduce the risk of mother to child transmission by 77% when combined with hepatitis B immunoglobulin and the hepatitis B vaccine, especially for pregnant women with high hepatitis B virus DNA levels (Hyun *et al* ., 2017). However, there is no sufficient evidence that the administration of hepatitis B immunoglobulin alone during pregnancy, might reduce transmission rates to the newborn infant (Eke *et al* ., 2010). No randomized control trial has been conducted to assess the effects of hepatitis B vaccine during pregnancy for preventing infant infection (Sangkomkamhang *et al* ., 2011).

All those with a risk of exposure to body fluids such as blood should be vaccinated, if not already. Testing to verify effective immunization is recommended and further doses of vaccine are given to those who are not sufficiently immunized (Schillie *et al* ., 2013).

Both types of the hepatitis B vaccine, the plasma-derived vaccine (PDV) and recombinant vaccine (RV) are of similar effectiveness in preventing the infection in both healthcare workers and chronic renal failure groups (Chen and Gluud ., 2005).

With one difference noticed among health worker group, that the RV intramuscular route is significantly more effective compared with RV intradermal route of administration (Chen and Gluud ., 2005).

In assisted reproductive technology, sperm washing is not necessary for males with hepatitis B to prevent transmission, unless the female partner has not been effectively vaccinated. In females with hepatitis B, the risk of transmission from mother to child with IVF is no different from the risk in spontaneous conception (Lutgens, etal ., 2009).

Those at high risk of infection should be tested as there is effective treatment for those who have the disease (LeFevre ., 2014). Groups that screening is recommended for include those who have not been vaccinated and one of the following: people from areas of the world where hepatitis B occurs in more than 2%, those with HIV, intravenous drug users, men who have sex with men, and those who live with someone with hepatitis B (LeFevre ., 2014).

The Vaccine is indicated for prevention of infection caused by all known subtypes of hepatitis B virus. Recombivax HB is approved for use in individuals of all ages. Hepatitis B Vaccine is available under the following different brand names: Energix B, and Recombivax HB.

2.13.2. Dosages of Hepatitis B Vaccine:

Intramuscular suspension (adult formulation).

10 mcg/ml (Recombivax HB).

20 mcg/ml (Engerix B).

40 mcg/ml (Recombivax HB [dialysis formulation]).

Intramuscular suspension (pediatric/adolescent formulation).

5 mcg/0.5 ml (Recombivax HB).

10 mcg/0.5 mg (Engerix B).

Dosage Considerations – Should be Given as Follows for adults:

Engerix B: 1 mL (20 mcg) intramuscularly at 0, 1, and 6 months.

Recombivax HB: 1 mL (10 mcg) intramuscularly at 0, 1, and 6 months.

Adults receiving dialysis or other immunocompromising conditions.

Recombivax HB (40 mcg/mL): 40 mcg intramuscularly at 0, 1, and 6 months.

Engerix-B (20 mcg/mL): 40 mcg intramuscularly at 0, 1, and 6 months.

2.13.3. Routine vaccination:

First dose: Administer first dose to all newborns before hospital discharge.

Infants born to HBsAg-positive mothers: 0.5 mL intramuscularly within 12 hours of birth PLUS hepatitis B immune globulin (HBIG); test for HBsAg and antibody to HBsAg (anti-HBs) 1-2 months after completion of hepatitis B vaccination series, at age 9 through 18 months.

Mother's HBsAg status unknown: 0.5 mL intramuscularly within 12 hours of birth PLUS give HBIG if newborn weight under 2 kg; determine mother's HBsAg status as soon as possible and, if she is HBsAg-positive, also administer HBIG for infants weighing 2 kg or more (no later than age 1 week).

Second dose: Administered at age 1-2 months Monovalent Hepatitis B vaccine should be used for doses administered before age 6 weeks.(rxlist ., 2017).

Infants who did not receive a birth dose should receive 3 doses of a Hepatitis B-containing vaccine on a schedule of 0, 1 to 2 months, and 6 months starting as soon as feasible.

Minimum interval between dose 1 and dose 2 is 4 weeks, and between dose 2 and 3 is 8 weeks.

Final (3rd or 4th) dose in the Hepatitis B vaccine series should be administered no earlier than age 24 weeks and at least 16 weeks after the first dose.

A total of 4 doses of Hepatitis B vaccine is recommended when a combination vaccine containing Hepatitis B is administered after the birth dose (rxlist ., 2017).

2.13.4. Catch-up vaccination

Unvaccinated children should complete a 3-dose series.

Children aged 11-15 years: 2-dose series (doses separated by at least 4 months) of adult formulation Recombivax HB is licensed for use in children aged 11 through 15 years.

2.13.5. Dosing Considerations

Routine immunization against hepatitis B; also protects against hepatitis D which always occurs in the presence of hepatitis B

Targeted groups that should receive Hepatitis B vaccination series include:

Sexually active persons who are not in a long-term, mutually monogamous relationship persons seeking evaluation or treatment for a sexually transmitted disease (STD); current or recent injection-drug users; and men who have sex with men.

Healthcare personnel and public-safety workers who are potentially exposed to blood or other infectious body fluids.

Persons with diabetes.

Persons with end-stage renal disease, including patients receiving hemodialysis; persons with HIV infection; and persons with chronic liver disease.

Household contacts and sex partners of hepatitis B surface antigen-positive persons; clients and staff members of institutions for persons with developmental disabilities; and international travelers to countries with high or intermediate prevalence of chronic HBV infection.

All adults in the following settings: STD treatment facilities; HIV testing and treatment facilities; facilities providing drug-abuse treatment and prevention services; health-care settings targeting services to injection-drug users or men who have sex with men; correctional facilities; end-stage renal disease programs and facilities for chronic hemodialysis patients; and institutions and nonresidential daycare facilities for persons with developmental disabilities (Rxlist ., 2017).

2.13.6. Administration of vaccine:

Adult: Administer intramuscularly in deltoid muscle, do not give IV/intradermal.

Pediatric: Administer in deltoid muscle for older children and adolescents; anterolateral thigh preferred for neonates/infants/small children, do not give intravenously/intradermal.

2.13.7. Side Effects Associated with Using Hepatitis B Vaccine:

Pain.

Severe itching.

Reddening of the skin.

Weakness.

Feeling unwell (malaise).

It is not known if hepatitis b vaccine is excreted in breast milk. Consult your doctor before breastfeeding (rxlist ., 2017).

2.14. Previous studies:

Study conducted in Dr Sampurnanand Medical College is one of the popular medical colleges of Rajasthanin which 49.6% HCWs were vaccinated, 46.1% were unvaccinated and 4.3% were partially vaccinated. The study revealed that Healthcare workers (HCWs) are at high risk for hepatitis B virus (HBV) infection Of the vaccinated HCWs, 30% had anti-HBs titer

<10 mIU/mL, 10.8% between 10-100 mIU/mL, and 59.2% >100 mIU/mL (Batra *et al* ., 2015).

Study done to Durability of immunity by hepatitis B vaccine in Japanese health care workers depends on primary response titers and durations found that From 2001 to 2012, data of 264 HCWs with a median age of 25.3 were collected. The rate of anti-HBs positivity after primary vaccination were 93.0% after three doses (n = 229), 54.5% after two doses (n = 11), and 4.2% after a single dose (n = 24). Of 213 primary responders, the anti-HBs levels of 95 participants (44.6%) fell below the protective levels, including 46 short responders and 49 long responders. (Yoshioka *etal* ., 2017).

Study conducted in Sudan covered vaccinated laboratory workers at Al-Neelain University, revealed that out of 61 heath laboratory workers 54 (88.5%) were found responders and 7 (11.5%) were found non-responders to hepatitis B surface antigen vaccine (Elsir *et al* ., 2018).

Study conducted in in Bahri Teaching Hospital, Khartoum state, Sudan, ninty sample collected , A total of 49 (54.4%) participants showed antibody titers above 10 IU/mL, 14 (15.6%) of them revealed strong response with anti-HBs Ab titers >100 IU/mL while HBsAb titers less than 10 IU/mL was detected in 41(45.6%) participants, Sixty-six (66; 73.33%) of the entire participants were females and 24 (26.67%) were males, out of which 28(42.2%) and 13(54.2%) of male and female, respectively fail to develop anti-HBsAb titer 10 IU/mL.(Eltayib Ahmed ., 2014).

Study conducted at a national hospital in Tanzania A total of 348 HCWs were interviewed, of whom 198 (56.9%) had received at least one dose of hepatitis B vaccination, while only 117 (33.6%) were fully vaccinated.

About half of the 81 HCWs with partial vaccination (49.4%) had missed their subsequent vaccination appointments. Among unvaccinated HCWs, 14 (9.3%) had either HBV infection or antibodies against HBV infection upon pre-vaccination screening. Nearly all respondents (347, 99.3%) had heard about the hepatitis B viral vaccine. The following reasons for non-vaccination were given: 98 (65.3%) reported that they had not been offered the vaccine; 70 (46.7%) observed standard precautions to ensure infection prevention and 60 (41.3%) blamed a low level of awareness regarding the availability of the hepatitis B vaccine (Dotto *et al.*, 2017).

CHAPTER THREE

MATERIALS and METHODS

3.1. Study design:

The study is observational descriptive cross sectional hospital base study conducted in period from April to July 2018.

3.2. Study area:

This study is conducted in Sudan cardiac center which is institute fully dedicated to cardiovascular diseases; the center is located in Khartoum city, Africa Street and founded in 2000.

Sudan cardiac center is consisting of six units: coronary care unit, intensive care unit, theater, catheter laboratory, emergency unit and ward.

3.3. Study Population:

Medical field professionals who were vaccinated by hepatitis B vaccine.

3.4. Inclusion criteria:

All medical care professionals who were vaccinated by hepatitis B vaccine had no history of HBV infection or received hepatitis B immunoglobulin prophylaxis were included and still work in hospital or clinic.

3.5. Exclusion criteria:

Medical care professionals who were not vaccinated or who were infected by acute or chronic hepatitis B virus, and who received hepatitis B immunoglobulin prophylaxis were excluded.

3.6. Ethical considerations:

The ethical consideration of this study was approved by ethics committee, faculty of graduate studies, Shendi University, Sudan. The participants were informed about the purpose of the research before sample collection, and verbal consent obtained from them. Privacy and confidentiality of participants were ensured.

1.7.Sample size:

Convenience sampling techniques were used ; 89 medical field professionals agreed to participate in this study.

3.8. Data collection:

An interview with structured questionnaire was done for all participants in this study for obtaining the clinical data.

3.9. Data analysis:

Data were analyzed by statistical package for social sciences (SPSS) version 22.

3.10. Study procedure:

3.9.1. Blood Sample Collection:

Sample of venous blood was collected in plain container from each individual included in this study; the serum was harvested by centrifugation at (2000 rpm) for 5 minutes.

3.9.2 Principle of sandwich ELISA Assay:

Known antigen is attached to the inside surface of the well and serum of patient is added .After incubation and washing, enzyme labelled anti human globulin is reacted with antibody that has attached to antigen. The uncombined labeled enzyme is washed from the well and substrate is then added .the presence and concentration of antibody that has reacted with the antigen is shown by a change in color of substrate .The more intense the color, the higher the concentration of antibody in the serum.

3.9.3. Procedure:
o 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.
o The stock wash buffer was diluted 20 times with distilled.
o 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).
o Adding Sample: 50 μl calibration curve standards and 50 μl of specimen were added into their respective wells.

o Adding HRP-Conjugate: 50 µl of HRP-Conjugate Reagent was added into each well except the blank, and mixed gently.

o Incubation : The plate was covered with the plate sealer and incubated for 60 min. at 37°C

o 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.

o Colouring : 50µl of Chromogen A and 50µl Chromogen B solution were Dispensed into each well included the Blank, coverd the plate with plate sealer and mixed gently by tapping the plate. The plate was Incubated at 37oC 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 450nm.

3.9.4. 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).

CHAPTER FOUR

RESULTS

In this study a total of 89 participants were included the majority of them 59.7% were females and 40.3% were males **Table 4.1.**

The age of study participants ranged from 21 to 60 years, more than one half were within the age group **25 – 30** years , **Table 4.2 .**

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) **(Table 4.3).**

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) **(Table 4.4).**

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 **(Table 4.5).**

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 **(Table 4.6).**

Table (4.1). Shows distribution of study population according to gender.

Gender	Frequency	Percentage
Male	36	40.3%
Female	53	59.7%
Total	89	100

Table (4.2). Shows distribution of study population according to age group.

Age group	Frequency	Percentage
> 25Year	19	21%
25 - 30Year	54	61%
<30Year	16	18%
Total	89	100%

Table (4.3). Shows the degree of immune response against HBV vaccine among study participants.

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

Table (4.4). 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 (4.5). 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	%	
< 25Year (n=19)	1	5.3%	5	26.3%	13	68.4%	0.169
25 -30Year (n=54)	2	3.8%	21	38.8%	31	57.4%	
>30Year (n=16)	1	6.2%	1	6.2%	14	87.6%	

Table (4.6). 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 -2Year (n=40)	2	5%	11	27.5%	27	67.5%	
<2Year (n=26)	2	7.7%	11	42.3%	13	50%	

CHAPTER FIVE

DISCUSSION, CONCLUSSION and RECOMMENDATIONS

5.1. DISCUSSION

The present study involved 89 Sudanese Health Care professionals vaccinated against HBV infection and subjected to the determination of anti-HBsAb titers.

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 male non responders were more frequent (18%) than female (8%),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).

5.2. Conclusion:

- 65.2 % of study participants show strong immune response to HBV vaccine.
- Only 4.5% of study participants show no response to HBV vaccine.
- 2.7% of male participants failed to respond to HBV vaccine and 5.6% of female participants failed to respond to HBV vaccine.
- Study participants of age group >30Years show the highest percentage of strong immune response to HBV vaccine.

5.3. Recommendations:

1. More studies should be conducted on different age groups including children less than five years   to estimate the titer of HBs antibody.
2. HBV vaccine should be recommended to all health care workers to protect them.
3. Vaccinated people should be tested for estimation of Anti-HBs antibodies titer to insure Immunity.
4. High titer antibody should checked to HBV antigen .
5. Participant how vaccinated more than 10 year should be checked to estimate amount of antibody titer .

REFERENCES

Abe, M., S. Abkar and M. Onji, (2006). Zinc and hepatitis B virus immunization. Hepatol. Res., 35(1): 1-2.

Abubakar, I.I., Tillmann, T. and Banerjee, A., (2015). Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*, *385*(9963), pp.117-171.

Alberti, A. and Caporaso, N., (2011). HBV therapy: guidelines and open issues. *Digestive and Liver Disease*, *43*, pp.S57-S63.

Alpert, E., Isselbacher, K.J. and Schur, P.H., 1971. The pathogenesis of arthritis associated with viral hepatitis: Complement-component studies. *New England Journal of Medicine*, *285*(4), pp.185-189.

Alter, M.J.,(2003). Epidemiology and prevention of hepatitis B.In *Seminars in liver disease* (Vol. 23, No. 01, pp. 039-046).Copyright© 2002 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA. Tel.:+ 1 (212) 584-4662.

Araujo, N.M., (2015). Hepatitis B virus intergenotypic recombinants worldwide: an overview. *Infection, Genetics and Evolution*, *36*, pp.500-510.

Barker, L.F., Shulman, N.R., Murray, R., Hirschman, R.J., Ratner, F., Diefenbach, W.C. and Geller, H.M., (1996).Transmission of serum hepatitis. *JAMA*, *276*(10), pp.841-844.

Bar- On, E.S., Goldberg, E., Hellmann, S. and Leibovici, L., (2012).Combined DTP- HBV- HIB vaccine versus separately administered DTP- HBV and HIB vaccines for primary prevention of diphtheria, tetanus, pertussis, hepatitis B and Haemophilusinfluenzae B (HIB). *The Cochrane Library*.

Batra, V., Goswami, A., Dadhich, S., Kothari, D. and Bhargava, N., (2015). Hepatitis B immunization in healthcare workers. *Annals of Gastroenterology: Quarterly Publication of the Hellenic Society of Gastroenterology*, *28*(2), p.276.

Beck, J. and Nassal, M.,(2007).Hepatitis B virus replication. *World journal of gastroenterology: WJG*, *13*(1), p.48.

Bell, S.J. and Nguyen, T.,(2009).The management of hepatitis B. *Australian Prescriber*, *32*(4), pp.99-104.

Bonino, F., Chiaberge, E., Maran, E. and Piantino, P., (1988). SEROLOGICAL MARKERS OF HBV INFECTIVE Y. *Ann. Ist. Super. Sanita*, *24*(2), pp.217-224.

Borch, A., Kolster, C., Gluud, C. and Gluud, L.L.,(2017). Vaccines for preventing hepatitis B in healthcare workers (an updated protocol). *The Cochrane Library*.

Buddeberg, F., Schimmer, B.B. and Spahn, D.R., (2008).Transfusion-transmissible infections and transfusion-related immunomodulation. *Best practice & research Clinical anaesthesiology*, *22*(3), pp.503-517.

Burton, A., Monasch, R., Lautenbach, B., Gacic-Dobo, M., Neill, M., Karimov, R., Wolfson, L., Jones, G. and Birmingham, M., (2009). WHO

and UNICEF estimates of national infant immunization coverage: methods and processes. *Bulletin of the World Health Organization*, *87*(7), pp.535-541.

Busch, K. and Thimme, R.,(2015). Natural history of chronic hepatitis B virus infection. *Medical microbiology and immunology*, *204*(1), pp.5-10.

Buti, M., Rodriguez-Frias, F., Jardi, R. and Esteban, R., (2005). Hepatitis B virus genome variability and disease progression: the impact of pre-core mutants and HBV genotypes. *Journal of clinical virology*, *34*, pp.S79-S82.

Cao, G.W., (2009). Clinical relevance and public health significance of hepatitis B virus genomic variations. *World journal of gastroenterology: WJG*, *15*(46), p.5761.

Cardell, K., Åkerlind, B., Sällberg, M. and Frydén, A., (2008).Excellent response rate to a double dose of the combined hepatitis A and B vaccine in previous nonresponders to hepatitis B vaccine. *The Journal of infectious diseases*, *198*(3), pp.299-304.

Chang, M.H.,(2007), June. Hepatitis B virus infection.In *Seminars in fetal and neonatal medicine* (Vol. 12, No. 3, pp. 160-167).Elsevier.

Chang, M.H., Chen, C.J., Lai, M.S., Hsu, H.M., Wu, T.C., Kong, M.S., **Liang, D.C., Shau, W.Y. and Chen, D.S.,** (1997). Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. *New England Journal of Medicine*, *336*(26), pp.1855-1859.

Chen, W. and Gluud, C., (2005).Vaccines for preventing hepatitis B in health- care workers. *The Cochrane Library*.

Chu, C.M. and Liaw, Y.F.,(2007). Predictive factors for reactivation of hepatitis B following hepatitis B e antigen seroconversion in chronic hepatitis B. *Gastroenterology*, *133*(5), pp.1458-1465.

Coffin, C.S., Mulrooney- Cousins, P.M., van Marle, G., Roberts, J.P., Michalak, T.I. and Terrault, N.A.,(2011). Hepatitis B virus quasispecies in hepatic and extrahepatic viral reservoirs in liver transplant recipients on prophylactic therapy. *Liver Transplantation*, *17*(8), pp.955-962.

Committee on infectious disease,(2017). Elimination of Perinatal Hepatitis B: Providing the First Vaccine Dose Within 24 Hours of Birth. *Pediatrics*, p.e20171870.

Cowan, S.A. and Qureshi, K.M.,(2007). Hepatitis B immunization for newborn infants of hepatitis B surface antigen-positive mothers. *Ugeskrift for laeger*, *169*(41), pp.3471-3474.

Custer, B., Sullivan, S.D., Hazlet, T.K., Iloeje, U., Veenstra, D.L. and Kowdley, K.V.,(2004).Global epidemiology of hepatitis B virus.*Journal of clinical gastroenterology*, *38*(10), pp.S158-S168.

Davila, J.A., Henderson, L., Kramer, J.R., Kanwal, F., Richardson, P.A., Duan, Z. and El-Serag, H.B.,(2011).Utilization of surveillance for hepatocellular carcinoma among hepatitis C virus–infected veterans in the United States. *Annals of internal medicine*, *154*(2), pp.85-93.

Dotto Aaron†, Tumaini J. John Rwegasha and Ewaldo Komba (2017).

Dienstag, J.L., (1981), February. Hepatitis B as an immune complex disease.In *Seminars in liver disease* (Vol. 1, No. 1, pp. 45-57).

Dienstag, J.L., (2008). Hepatitis B virus infection.*New England Journal of Medicine*, *359*(14), pp.1486-1500.

Eke, A.C., Eke, U.A. and Uchenna, E.,(2010). Hepatitis B immunoglobulin during pregnancy for the prevention of mother to child transmission of hepatitis B virus. *Protocol) Cochrane collaboration*.

El–Serag, H.B. and Rudolph, K.L., (2007). Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology*, *132*(7), pp.2557-2576.

Elsir et al., (2018) Apr 20 */evaluation-of-immune-response-to-hepatitis-b-vaccine...*, 2018 - Research Article. *Clinical Infectious Diseases*.

Eltayib Ahmed (2014) on 10 July ,antibody titer following hepatitis B vaccination in health care warker in khartoum state sudan .

Forton, D.M., Taylor- Robinson, S.D. and Thomas, H.C., (2003). Cerebral dysfunction in chronic hepatitis C infection. *Journal of Viral Hepatitis*, *10*(2), pp.81-86.

Gabbuti, A., Romanò, L., Blanc, P., Meacci, F., Amendola, A., Mele, A., Mazzotta, F. and Zanetti, A.R., (2007).Long-term immunogenicity of hepatitis B vaccination in a cohort of Italian healthy adolescents. *Vaccine*, *25*(16), pp.3129-3132.

Gan, S.I., Devlin, S.M., Scott-Douglas, N.W. and Burak, K.W., (2005).Lamivudine for the treatment of membranous glomerulopathy secondary to chronic Hepatitis B infection. *Canadian Journal of Gastroenterology and Hepatology*, *19*(10), pp.625-629.

Ge**, G., Wang, S., Han, Y., Zhang, C., Lu, S. and Huang, Z.,** (2012). Removing N-terminal sequences in pre-S1 domain enhanced antibody and B-cell responses by an HBV large surface antigen DNA vaccine. *PloS one*, *7*(7), p.e41573.

Glebe, D. and Urban, S., (2007). Viral and cellular determinants involved in hepadnaviral entry. *World journal of gastroenterology: WJG*, *13*(1), p.22.

Gocke, D., Hsu, K., Morgan, C., Bombardieri, S., Lockshin, M. and Christian, C., (1970).Association between polyarteritis and Australia antigen. *The Lancet*, *296*(7684), pp.1149-1153..

Hay, S.I., Jayaraman, S.P., Truelsen, T., Sorensen, R.J., Millear, A., Giussani, G. and Beghi, E., (2017).GBD 2015 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015 (vol 388, pg 1545, 2016). *Lancet*, *389*(10064), pp.E1-E1.

https://www.rxlist.com/consumer_hepatitis_b_vaccine_energix_recombivax/drugs-condition.htm

Hollinger, F.B. and Lau, D.T.Y., (2006). Hepatitis B: the pathway to recovery through treatment. *Gastroenterology Clinics*, *35*(2), pp.425-461.

Hope, V.D., Eramova, I., Capurro, D. and Donoghoe, M.C., 2014. Prevalence and estimation of hepatitis B and C infections in the WHO European Region: a review of data focusing on the countries outside the European Union and the European Free Trade Association. *Epidemiology & Infection*, *142*(2), pp.270-286.

Howard, C.R., (1986). The biology of hepadnaviruses. *Journal of general virology*, *67*(7), pp.1215-1235.

Hughes, R.A., (2000). Drug injectors and the cleaning of needles and syringes. *European addiction research*, *6*(1), pp.20-30.

Hosseini, S.M., R. Ranjbar, A. Hassan and T. Mohammed, (2009). Evaluation of the level of HBV antibody titer after HBV vaccination among children in Tehran, Iran. Hepat. Mon., 9(2): 150-153.

Hyun, M.H., Lee, Y.S., Kim, J.H., Je, J.H., Yoo, Y.J., Yeon, J.E. and Byun, K.S., (2017). Systematic review with meta- analysis: the efficacy and safety of tenofovir to prevent mother- to- child transmission of hepatitis B virus. *Alimentary pharmacology & therapeutics*, *45*(12), pp.1493-1505.

Iannacone, M., Sitia, G., Isogawa, M., Marchese, P., Castro, M.G., Lowenstein, P.R., Chisari, F.V., Ruggeri, Z.M. and Guidotti, L.G., (2005). Platelets mediate cytotoxic T lymphocyte–induced liver damage. *Nature medicine*, *11*(11), p.1167.

Iannacone, M., Sitia, G., Ruggeri, Z.M. and Guidotti, L.G., (2007). HBV pathogenesis in animal models: recent advances on the role of platelets. *Journal of hepatology*, *46*(4), pp.719-726.

Israr, M., Ali, F., Muhammad, M. and Bahadar, N., (2017).Seroprevalence and risk factors of hepatitis B virus among blood donors in district Charsadda Khyber Pakhtunkhwa Pakistan. *Pure and Applied Biology*, *6*(2), p.669.

Kane, M., Banatvala, J., DaVilla, G., Esteban, R., Franco, E., Goudeau, A., Grob, P., Jilg, W., Rizzetto, M., Van Damme, P. and Van Hattum, J.,

(2000). Are booster immunizations needed for lifelong hepatits B immunity (Consensus statement). *Lancet*, *355*, pp.561-65.

Kay, A. and Zoulim, F., (2007).Hepatitis B virus genetic variability and evolution. *Virus research*, *127*(2), pp.164-176.

Kane, A., J. Lloyd, M. Zaffran, L. Simonsen and M. Kane, (1999). Transmission of hepatitis B, hepatitis C and human immunodeficiency viruses through unsafe injections in the developing world: Model-based regional estimates. Bull World Health Organ., 77(10): 801–807.

Kerkar, N.,(2005). Hepatitis B in children: complexities in management. *Pediatric transplantation*, *9*(5), pp.685-691.

King, J.W., Taylor, E.M., Crow, S.D., White, M.C., Todd, J.R., Poe, M.B., Conrad, S.A. and Gelder, F.B., (1990). Comparison of the immunogenicity of hepatitis B vaccine administered intradermally and intramuscularly. *Reviews of infectious diseases*, *12*(6), pp.1035-1043.

Kramvis, A., Kew, M. and François, G., (2005) Hepatitis B virus genotypes. *Vaccine*, *23*(19), pp.2409-2423.

Krugman, S.A.U.L. and Davidson, M., (1987). Hepatitis B vaccine: prospects for duration of immunity. *The Yale journal of biology and medicine*, *60*(4), p.333.

Komas, N.P., Vickos, U., Hübschen, J.M., Béré, A., Manirakiza, A., Muller, C.P. and Le Faou, A., (2013). Cross-sectional study of hepatitis B virus infection in rural communities, Central African Republic.*BMC infectious diseases*, *13*(1), p.286.

Kuschner**, R.A., Russell, K.L., Abuja, M., Bauer, K.M., Faix, D.J., Hait, H., Henrick, J., Jacobs, M., Liss, A., Lynch, J.A. and Liu, Q.,**(2013). A pha)se 3, randomized, double-blind, placebo-controlled study of the safety and efficacy of the live, oral adenovirus type 4 and type 7 vaccine, in US military recruits. *Vaccine*, *31*(28), pp.2963-2971.

Lai, C.L. and Yuen, M.F., (2007) The natural history and treatment of chronic hepatitis B: a critical evaluation of standard treatment criteria and end points. *Annals of Internal Medicine*, *147*(1), pp.58-61.

Lai, K.N., Li, P.K., Lui, S.F., Au, T.C., Tam, J.S., Tong, K.L. and Lai, F.M.M., (1991). Membranous nephropathy related to hepatitis B virus in adults. *New England Journal of Medicine*, *324*(21), pp.1457-1463.

Laing, R., Waning, B., Gray, A., Ford, N. and Hoen, E.T.,(2003). 25 years of the WHO essential medicines lists: progress and challenges. *The Lancet*, *361*(9370), pp.1723-1729.

Lee, C.F., (2006). Pg11 hepatitis B immunization for new borns of hepatitis B surface antigen positive mothers : acochrane hepato biliary group systemiatic review and meta analysis. *Value in Health*, *9*(3), p.A43.

LeFevre, M.L., (2014). Screening for hepatitis B virus infection in nonpregnant adolescents and adults: US Preventive Services Task Force recommendation statement. *Annals of internal medicine*, *161*(1), pp.58-66.

Li**, W., Miao, X., Qi, Z., Zeng, W., Liang, J. and Liang, Z.,** (2010).Hepatitis B virus X protein upregulates HSP90alpha expression via

activation of c-Myc in human hepatocarcinoma cell line, HepG2. *Virology journal*, *7*(1), p.45.

Liang, T.J., (2009). Hepatitis B: the virus and disease. *Hepatology*, *49*(S5).

Liao, X. and Liang, Z., (2015).Strategy vaccination against Hepatitis B in China. Human vaccines &immunotherapeutics, 11(6), pp.1534-1539.

Liaw, Y.F., Brunetto, M.R. and Hadziyannis, S., (2010). The natural history of chronic HBV infection and geographical differences. *Antiviral therapy*, *15*(3), p.25.

Lok, A.S. and McMahon, B.J., (2007). Chronic hepatitis B. *Hepatology*, *45*(2), pp.507-539.

Lutgens, S.P., Nelissen, E.C., van Loo, I.H., Koek, G.H., Derhaag, J.G. and Dunselman, G.A.,(2009). To do or not to do: IVF and ICSI in chronic hepatitis B virus carriers. *Human reproduction*, *24*(11), pp.2676-2678.

Magnius, L.O. and Norder, H., (1995). Subtypes, genotypes and molecular epidemiology of the hepatitis B virus as reflected by sequence variability of the S-gene. *Intervirology*, *38*(1-2), pp.24-34.

Mahy, B.W. and Van Regenmortel, M.H. eds.,(2010). *Desk encyclopedia of human and medical virology*.Academic Press.

Martínez-Sernández, V. and Figueiras, A.,(2013). Central nervous system demyelinating diseases and recombinant hepatitis B vaccination: a critical systematic review of scientific production. Journal of neurology, 260(8), pp.1951-1959.

Norder, H., Couroucé, A.M. and Magnius, L.O., (1994). Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. *Virology*, *198*(2), pp.489-503.

Oliveri, F., Brunetto, M.R., Actis, G.C. and Bonino, F., (1991).Pathobiology of chronic hepatitis virus infection and hepatocellular carcinoma (HCC).*The Italian journal of gastroenterology*, *23*(8), pp.498-502.

Petersen, K.M., Bulkow, L.R., McMahon, B.J., Zanis, C., Getty, M., Peters, H. and Parkinson, A.J., (2004).Duration of hepatitis B immunity in low risk children receiving hepatitis B vaccinations from birth. *The Pediatric infectious disease journal*, *23*(7), pp.650-655.

 Pramoolsinsup, C (2002). Management of viral hepatitis B. *Journal of gastroenterology and hepatology*, *17*(s1).

Pungpapong, S., Kim, W.R. and Poterucha, J.J., (2007), August. Natural history of hepatitis B virus infection: an update for clinicians. In *Mayo clinic proceedings* (Vol. 82, No. 8, pp. 967-975).

Platkov, E., E. Shlyakhov, Y. Glick, S. Khalemsky and A. Fischbein, (2003). Immunologic evaluation of hepatitis B vaccine application in hospital staff. Int. J. Occup. Med. Environ. Health, 16(3): 249-253.

Redd, J.T., Baumbach, J., Kohn, W., Nainan, O., Khristova, M. and Williams, I., (2007). Patient-to-patient transmission of hepatitis B virus associated with oral surgery. *The Journal of infectious diseases*, *195*(9), pp.1311-1314.

Sangkomkamhang, U.S., Lumbiganon, P. and Laopaiboon, M., (2011).Hepatitis B vaccination during pregnancy for preventing infant infection. *Cochrane Database Syst Rev*, *3*.

Schaefer, S., (2007).Hepatitis B virus taxonomy and hepatitis B virus genotypes. *World journal of gastroenterology: WJG*, *13*(1), p.14.

Schillie, S.F., Murphy, T.V., Sawyer, M., Ly, K., Hughes, E., Jiles, R., de Perio, M.A., Reilly, M., Byrd, K. and Ward, J.W., (2013).CDC guidance for evaluating health-care personnel for hepatitis B virus protection and for administering postexposure management.

Schilsky, M.L., (2013), April. Hepatitis B "360".In *Transplantation proceedings* (Vol. 45, No. 3, pp. 982-985).

Shapiro, C.N., (1993). Epidemiology of hepatitis B. *The Pediatric infectious disease journal*, *12*(5), pp.433-437.

Shepard, C.W., Simard, E.P., Finelli, L., Fiore, A.E. and Bell, B.P., (2006). Hepatitis B virus infection: epidemiology and vaccination. *Epidemiologic reviews*, *28*(1), pp.112-125.

Shibayama, T., Masuda, G., Ajisawa, A., Hiruma, K., Tsuda, F., Nishizawa, T., Takahashi, M. and Okamoto, H., (2005). Characterization of seven genotypes (A to E, G and H) of hepatitis B virus recovered from Japanese patients infected with human immunodeficiency virus type 1. *Journal of medical virology*, *76*(1), pp.24-32.

Sood, E., Deb, V.K., Kumar, V., Mahato, B., Sharma, N., Kumar, A. and Verma, U., (2018). Hepatitis B Virus (HBV) DNA Quantification by Real Time PCR. *Int J Pharma Res Health Sci*, *6*(2), pp.2468-71.

Takekoshi, Y., Shida, N., Saheki, Y., Tanaka, M., Satake, Y. and Matsumoto, S., (1978). Strong association between membranous nephropathy and hepatitis-B surface antigenaemia in Japanese children. *The Lancet*, *312*(8099), pp.1065-1068.

Taylor, J.M., (2006). Hepatitis delta virus.*Virology*, *344*(1), pp.71-76.

Terrault, N., Roche, B. and Samuel, D., (2005). Management of the hepatitis B virus in the liver transplantation setting: a European and an American perspective. *Liver transplantation*, *11*(7), pp.716-732.

Tohme, R.A., D. Awosika-Olumo, C. Nielsen, S. Khuwaja, J. Scott, J. Xing, J. Drobeniuc, D. Hu, C. Turner, T. Wafeeg, U. Sharapov and P. Spradling, (2011). Evaluation of hepatitis B vaccine immunogenicity among older adults during an outbreak response in assisted living facilities. Vaccine, 29(50): 9316-

Terrault, N.A., Bzowej, N.H., Chang, K.M., Hwang, J.P., Jonas, M.M. and Murad, M.H., (2016).AASLD guidelines for treatment of chronic hepatitis B. *Hepatology*, *63*(1), pp.261-283.

 Subhash , Chandra Parija, (2012), Textbook of microbiology and immunology, 2nd Edition, page551-552.

Thomas, B., Van Deynze, A. and Bradford, K., (2002). *Production of therapeutic proteins in plants*.UCANR Publications.

Thun, M.J., DeLancey, J.O., Center, M.M., Jemal, A. and Ward, E.M., (2009). The global burden of cancer: priorities for prevention. Carcinogenesis, 31(1), pp.100-110.

Tong, S., Li, J. and Wands, J.R., (1999). Carboxypeptidase D is an avian hepatitis B virus receptor. *Journal of virology*, *73*(10), pp.8696-8702.

Van Damme, P. and Van Herck, K., (2007). A review of the long-term protection after hepatitis A and B vaccination. *Travel medicine and infectious disease*, *5*(2), pp.79-84.

Vincent, C.C., (2016) In-Patients' Satisfaction with Food Served in Imo State University Teaching Hospital, Orlu. *Contemp Econ Policy*, *14*, pp.112-24.

Vos, T., Barber, R.M., Bell, B., Bertozzi-Villa, A., Biryukov, S., Bolliger, I., Charlson, F., Davis, A., Degenhardt, L., Dicker, D. and Duan, L., (2015). Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *The Lancet*, *386*(9995), pp.743-800.

Williams, R., (2006). Global challenges in liver disease. *Hepatology*, *44*(3), pp.521-526.

Wong, F., Pa, R., Van Schalkwyk, J. and Yoshida, E.M., (2014). Hepatitis B in pregnancy: a concise review of neonatal vertical transmission and antiviral prophylaxis. *Annals of Hepatology: Official Journal of the Mexican Association of Hepatology*, *13*(2).

Wood, R.C., MacDonald, K.L., White, K.E., Hedberg, C.W., Hanson, M. and Osterholm, M.T., (1993).Risk factors for lack of detectable antibody following hepatitis B vaccination of Minnesota health care workers.*Jama*, *270*(24), pp.2935-2939.

World Health Organization,(2015).*Guidelines for the Prevention Care and Treatment of Persons with Chronic Hepatitis B Infection: Mar-15*. World Health Organization.

World Health Organization, (2017). *Global hepatitis report 2017*. World Health Organization.

www.Fortressdiagnostics.com.

Xiao-wen, H., S. Shu-han, H. Zhen-lin, L. Jun, J. Lei, Z. Feng-juan, Z. Ya-nan and G. Ying-jun,(2005). Augmented humoral and cellular immune responses of a hepatitis B DNA vaccine encoding HbsAg by protein boosting. Vaccine, 23(14): 1649-1656.

Yan, H., Zhong, G., Xu, G., He, W., Jing, Z., Gao, Z., Huang, Y., Qi, Y., Peng, B., Wang, H. and Fu, L., (2012). Sodium taurocholatecotransporting polypeptide is a functional receptor for human hepatitis B and D virus. *elife*, *1*.

Yoshioka, N., Deguchi, M., Hagiya, H., Kagita, M., Tsukamoto, H., Takao, M., Yoshida, H., Yamamoto, N., Akeda, Y., Nabetani, Y. and Maeda, I., (2017). Durability of immunity by hepatitis B vaccine in Japanese health care workers depends on primary response titers and durations. *PloS one*, *12*(11),

p.e0187661.

Zeeshan, M., K. Jabeen, N.A. Ali, A.W. Ali, S.Z. Farooqui, V. Mehraj and A. Zafa, (2007). Evaluation of immune response to hepatitis B vaccine in health care workers at a tertiary care hospital in Pakistan: An observational prospective study. BMC Infect. Dis., 2007(7): 120.

Zoulim, F., (2006), November.New nucleic acid diagnostic tests in viral hepatitis.In *Seminars in liver disease* (Vol. 26, No. 04, pp. 309-317).

Rezaei H, Hossein talaei M, pakzd I, and Maleki F, (2017). Evaluation of hepatitis B vaccination efficency among health care wokers in west of iran, Der pharmacia lettre, 9(5), 29 -43.

APPENDIX I

Estimation of Hepatitis B Antibody Titer due to Vaccine among Medical field professionals

Questionnaire

NO: ....

Age in years: ……………………………

Gender: Male (   )   Female (   )

Completed three doses   ?   Yes………   No ………..

Date of vaccination?   ………………………

Did you have a booster dose?   Yes………   No………

Date of booster dose? ....

Number of booster dose? ....

Chronic disease:

- ✓ Diabetes mellitus   (   )
- ✓ Renal failure   (   )
- ✓ Hypertension   (   )
- ✓ Liver disease   (   )
- ✓ Others   (   )

APPENDIX II

ELISA kit components

APPENDIX III

ELISA washer apparatus

APPENDIX IV

ELISA reader apparatus

APPENDIX V

The titers HBs antibody

APPENDIX VI

Fortress diagnostics HBsAb ELISA kit