Sudan University of Science and Technology

College of Graduate studies

Immune Response to Hepatitis B Vaccination among Medical Laboratory students in Omdurman Islamic University

الاستجابة المناعية للتحصين ضد مرض فيروس الكبد الوبائي (ب) وسط طلاب المختبرات الطبية بجامعة ام درمان الاسلامية

A thesis submitted in Partial fulfillment for the requirement of M.Sc degree in Medical Laboratory Sciences (Microbiology)

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الآية

قال تعالى :

(قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ)

صدق الله العظيم

سورة البقرة الاية (32)

Dedication

To my father who gave me peace of life

To my mother who was the perfumes of existence ...

To my friends ...

Acknowledgments

Thanks first to ALLAH for helping and blessing me in doing this work, then I would like to express my deepest thanks and gratitude's to my supervisor Prof. Yousif Fadlalla Hamed Elnil for his great efforts, help and patience I received from him during my work and I am asking ALLAH to bless him as well as good things he does in his life.

I would like to thank every student of Medical College , asking ALLAH to cure and bless them.

Thanks to my colleagues, Baderldin, Sayed, Hanaa, my teacher Wafaa, and the rest of my colleagues who supported me in all steps of this research.

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Abstract

Hepatitis B Virus infection is a major public health problem and causes majority of primary liver cancer. This was descriptive and cross sectional study aimed to evaluate the immune response after hepatitis B Vaccination in Medical laboratory Students. Study was conducted among Medical laboratory students in Omdurman state (Omdurman Islamic University) from April (2015) to May (2016).

The blood specimens were collected and sera were separated and stored at -20ºC until used, sera were tested using ELISA for qualititative detection of anti HBVs Ag. Of the total 90 students, with mean age of 25 years included 70 vaccinated students, 62/70 (88.5 %) were responded to HBV vaccine and 8/70 (11.4%) were non responded, of the 20 unvaccinated students only 1/20 (5%) was reactive.

Out of seventy vaccinated students 35 were females and 35 were males, females immunologic response were significantly higher than males (p.value :0.000). Responsiveness was significantly lower in smokers than non-smokers. Diabetes was a risk factor it was found to be significantly correlated (p.value:0.00) with hepatitis B vaccination irresponsiveness.

مستخلص الاطروحة

فيروس التهاب الكبدالوبائي (ب) مشكلة صحية عامة رئيسية ويسبب الأغلبية من سرطان الكبد الأولى. تعتبر هذه الدراسه وصفيه مقطعيه وهدفت هذه الدراسة إلى تقييم الاستجابة المناعية بعد تطعيم التهاب الكبد(ب) في طلاب كليات المختبرات الطبية. وأجريت الدراسة بين طلاب كليات المختبرات الطبية في ولاية أم درمان (جامعة أم درمان الإسلامية) من أبريل (2015) إلى مايو (2016). وجمعت عينات الدم وفصلت الأمصال وتم تخزينها في $20^{o}C$- حتى تم استخدامها، تم اختبار الأمصال بواسطة اختبار تفاعل الانزيم التسلسلي للكشف النوعي لوجود المضاد لمستضد فيروس التهاب الكبد(ب).

من مجموع 90 طالب, متوسط اعمارهم 25 عام شملوا70 طلاب محصنين منهم 70/26 (88.5٪) استجابو للقاح الالتهاب الكبدي الوبائي و70/8 (11.4٪) من غير استجابة ، من 20 غير المحصنين 20/1 (5٪) كان مستجيب. من اصل سبعين طالب محصنين 35 كانو من الاناث و 35 كانو من الذكور, وكانت الاستجابة المناعية للاناث اعلى نسبيا من الذكور. كانت الاستجابة اقل نسبيا لدى المدخنين مقارنة بغير المدخنين . وجد ان مرض السكر من العوامل المثبطه للاستجابة للقاح التهاب الكبد الوبائي.

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List of Abbreviations

Abbreviation	Complete Word
Ab	Antibody
Ag	Antigen
HBV	Hepatitis B virus
HBs Ag	Hepatitis B surface antigen
HBc Ag	Hepatitis B core antigen
HBe Ag	Hepatitis B e antigen
WHV	Woodchuck hepatitis virus
DHBV	Duck hepatitis B virus
HCWs	Health care workers
CDC	Centers for Disease Control and Prevention
MHC class11	Major Histocompatibility Complex
HCC	Hepatocellular carcinoma
DNA	Deoxy Ribonucleic Acid
ELISA	Enzyme linked immunosorbent assay
PCR	Polymerase Chain Reaction
EIA	Enzyme immunoassay
ALT	Serum amino alanine transferases
AST	Serum aspartate aminotransferase
GGT	Serum gamma glutamyltransferase

ICT	Immuno Chromatography Test
IgG	Immunoglobulin G
IgM	Immunoglobulin M
WHO	World Health Organization

CHAPTER ONE

Chapter One

1. Introduction

Hepatitis B virus (HBV) is a causative agent of hepatitis infection, which is asymptomatic in most individuals, but it can show features of fulminant, acute, or chronic hepatitis. The acute type produces serious illness and approximately 0.5% of cases are fatal. Chronic infection is often lifelong, and can lead to liver failure and hepatocellular carcinoma (Seeger and Mason, 2000).

Transmission of HBV is through the parenteral route, blood transfusion products and sexual intercourse and vertically from infected mothers to neonates. The virus is found in body fluids such as urine, saliva, nasopharyngeal fluids, semen and menstrual fluids, and can be transmitted through contact with these fluids (Mahony, 1999).

Hepatitis B virus is the most commonly transmitted bloodborne virus in the health-care setting. Transmission generally occurs from patient to patient or from patients to health-care personnel via contaminated instruments or accidental needle-stick or sharps injuries. The virus can be transmitted directly through body fluids to mucous membranes, cutaneous scratches, abrasions, burns or other lesions. Indirect transmission can occur from surfaces contaminated with blood or body fluids to mucous membranes. HBV has been shown to survive in dried blood on surfaces at room temperature for at least a week (Bond,1981). In terms of public health, the best strategy to eradicate HBV infection is a universal vaccination program as recommended by WHO. Implementation of a universal vaccination program is highly desirable, particularly among high-risk situations that might not be adequately addressed by mass vaccination programs such as person who perform tasks involving contact with blood, blood-contaminated body fluids, other body fluids, and sharps (Pruss-Ustun *et al*., 2005 ; Fed, 1991). Hepatitis B vaccine

can be administered at the same time as other vaccines with no interference of antibody response to other vaccines (Pruss-Ustun *et al.*, 2005). About 5-10% of those vaccinated against HBV fail to respond with development of antibody to the HBV (Abe *et al.*, 2006; Xiao-wen *et al.*, 2005).

A vaccine for hepatitis B has been available since 1982. The initial vaccine was prepared by purifying HBsAg associated with the 22 nm particles from healthy HBsAg-positive carriers and treating the particles with virus-inactivating agents (Roome *et al.*, 1993). Currently, recombinant HBsAg is used for HBV vaccination, and the development of antibody to HBsAg is typically associated with protective immunity. The core open reading frame encodes a polypeptide that is expressed as either the hepatitis B e antigen (Hbe Ag) or the viral capsid protein (HBcAg) (Krajden *et al*., 2005). These vaccines are administered by the intramuscular route in the deltoid muscle and are highly immunogenic, inducing a protective anti-HBs antibody titer (>10 IU per mL) in more than 95% of healthy children or young adults (Alter, 2003). Two schedules of administration are approved:

(a) Three initial injections at 1-month intervals and a booster at 12 months.

(b) Two initial injections 1 month apart, followed by a booster at 6 months.

The HBV vaccine is associated with rare side effects, most commonly pain or soreness at the injection site. Neurologic disorders such as multiple sclerosis and transverse myelitis have not been causally linked to the HBV vaccine (Elduma and Saeed, 2011).

Sudan is considered highly endemic for HBsAg, with prevalence about 16%–20% in the general population. In a study conducted in Omdurman state among adults with acute hepatitis, HBV infection was 12.6% (Elduma and Saeed, 2011). Research findings have indicated that 10%–30% of health- care workers show serologic evidence of past or present HBV infection (Kunches, 1983).

1.1 Rationale

Medical students during their clinical training years should be considered as having the same high risk as health care workers for catching HBV infection, and should be protected before starting their clinical years.

Prevention of active infection by vaccination is an important strategy to decrease the risk of active HBV infection and of its subsequent complications. Antibody response to HBV surface antigen (Anti HBs) is an important serological marker for vaccine induces immunity to HBV. Multiple different factors include: gender, age, diabetes, and smoking can influence the immunologic response.

The research is an attempt to determine the immune status of the medical laboratory students at Omdurman Islamic University and to determine the possible host factors related to the poor response to previously administered hepatitis B vaccine.

1.2 Objectives

1.2.1 General objective

To assess the immune response following hepatitis B vaccination among medical laboratory students.

1.2.2 Specific objectives

1. To determine IgG anti-hepatitis B surface post vaccination in medical laboratory students.

2.To determine risk factors affecting the vaccination which include: gender, age, smoking and diabetes.

CHAPTER TWO

Chapter Two

2. Literature Review

2.1 Hepatitis B virus

Hepatitis B is a contagious liver disease that results from infection with the Hepatitis B virus. When first infected, a person can develop an “acute” infection, which can range in severity from a very mild illness with few or no symptoms to a serious condition requiring hospitalization. Acute Hepatitis B refers to the first 6 months after someone is exposed to the Hepatitis B virus. Some people are able to fight the infection and clear the virus. For others, the infection remains and leads to a “chronic,” or lifelong, illness. Chronic Hepatitis B refers to the illness that occurs when the Hepatitis B virus remains in a person’s body. Over time, the infection can cause serious health problems (CDC, 2010).

2.1.1 Taxonomy and Classification

Hepatitis B is the prototype virus of the family *Hepadnaviridae,* a name that signifies the hepatotropism and DNA nature of the genome of its members. There are two genera within the family. The genus *Orthohepadnavirus* contains members that infect mammals, and, other than HBV, includes hepadnaviruses that infect rodents such as woodchucks (woodchuck hepatitis virus, WHV) and squirrels (70% nucleotide identity). In recent years, HBV like isolates have also been obtained from primates such as chimpanzees, gibbons, gorillas, orangutans, and woolly monkeys. These are more closely related to HBV and may in fact represent progenitors of the human viruses . The *Avihepadnavirus* genus on the other hand contains members that infect birds such as ducks (duck hepatitis B virus, DHBV), herons, storks, and geese. Over the years, the woodchuck and duck animal models, as well as chimpanzees, which are susceptible to infection with human HBV isolates,

have proved invaluable in the study of the replication of these viruses, the natural history of infection, and the testing for efficacy of vaccines and antiviral drugs (Mahy and Van, 2010).

2.1.2 Structure

Three different particles can be seen in HBV infection Fig (2.1). The predominant form is a small, spherical particle with a diameter of 22 nm. Filaments are also present with a diameter of about 22 nm. Both types of particles are composed of lipid, protein and carbohydrate; they are infectious and consist solely of surplus virion envelope. The particles cary the hepatitis B surface antigen (HBsAg). The third type of particle, the virion or *Dane particle*, has a diameter of 42 nm; enclosed within the envelope is the core (72 nm), a shell composed of hepatitis B core antigen (HBcAg). There may be as many as of the small particles and filaments per milliliter. The virions are present in much smaller numbers, usually by a factor of 10 or more, and the proportion varies considerably in different stages of the disease. The viral DNA is about 3200 nucleotides long and is circular. The long strand is complete, but there is a gap of variable length of about 1000 nucleotides in the complementary strand; this can be closed via the action of the virion polymerase when virus replication starts (Greenwood *et al.*, 2007).

There are four open reading frames that encode seven polypeptides. These include structural proteins of the virion surface and core, a small transcriptional transactivator (X), and a large polymerase (P) protein that includes DNA polymerase, reverse transcriptase, and RNase H activities. The S gene has three in-frame initiation codons and encodes the major HBsAg, as well as polypeptides containing in addition pre-S2 or pre-S1 and pre-S2 sequences. The C gene has two in-frame initiation codons and encodes HBcAg plus the HBe protein, which is processed to produce soluble HBeAg. The particles containing HBsAg are antigenically complex. Each contains a group-specific antigen, a, in addition to two pairs of mutually exclusive

subdeterminants, d/y and w/r. Thus, four phenotypes of HBsAg have been observed: adw, ayw, adr, and ayr. In the United States, adw is the predominant subtype. These virus-specific markers are useful in epidemiologic investigations, as secondary cases have the same subtype as the index case (Brooks *et al*., 2010).

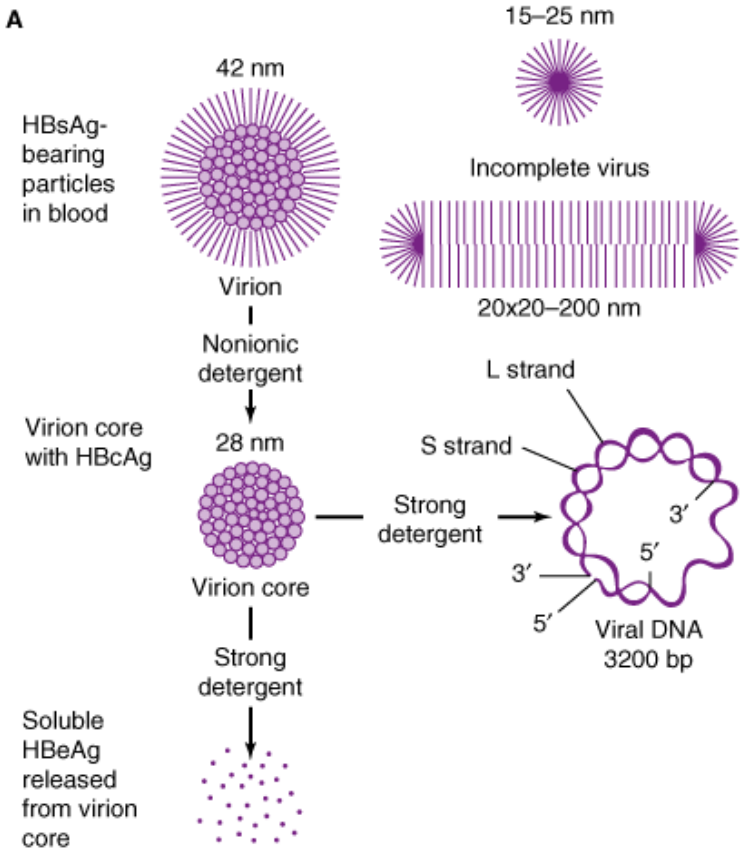

Fig (2.1) Structure of HBV. Available at : http: //www.accessmedicine.com

2.1.3 Function of the gene products

(a) Surface proteins :

The HBV surface protein: small (SHB), medium (MHB) and large (LHB), together with cellular lipid material, form the viral envelope (Kann, 2002). SHB antigen which represents 85% of hepatitis B surface antigen (HBsAg), is highly immunogenic and provokes the host's immune response to HBV. Excess surface protein circulating in subviral particles is thought to dilute the

host's immunological response to the virus. LHB, in contrast to MHB, is essential for infection and viral morphogenesis. It represents 10–30% of the HBsAg of virions and filaments. LHB plays a role in viral entry into hepatocytes, although SHB may also be needed in this process (Kann, 2002).

(b) Core protein and 'e' antigen

Core protein (C) :

Is the major structural component of the nucleocapsid. The preC/C ORF is transcribed into a precore/core fusion protein. During entry into the endoplasmic reticulum, 19 amino acids are cleaved from the N-terminal end of the precore protein by a signal peptidase. When transported into the Golgi compartment, additional amino acids are removed from the C-terminal end by intra-Golgi proteases to form HBe antigen. This antigen is secreted into the serum. The biological function of HBe remains unsolved (Kann, 2002).

(c) Polymerase protein :

Polymerase (P) has four domains: a terminal domain, which serves as a protein primer for reverse transcription of pregenomic viral RNA; a spacer region without apparent function; the polymerase domain, which has reverse transcription activity; and the RNase H domain, which is responsible for the degradation of the RNA template during reverse transcription (Kann, 2002).

(d) X protein :

The X protein (HBx) has been shown to be a promiscuous regulator of transcription that is essential for viral replication. Although not binding itself to DNA, it regulates transcription from HBV enhancers/promoters, and from the promoters of cellular genes, including oncogenes, cytokines, growth factors, and several genes involved in cell-cycle control and progression, DNA repair, apoptotic cell death, and cellular adhesion. HBx also forms complexes with several signal transduction proteins and regulators of cell growth and survival (Murakami, 1999; Feitelson., 2005; Benhenda *et al.*, 2009) Fig (2.2) .

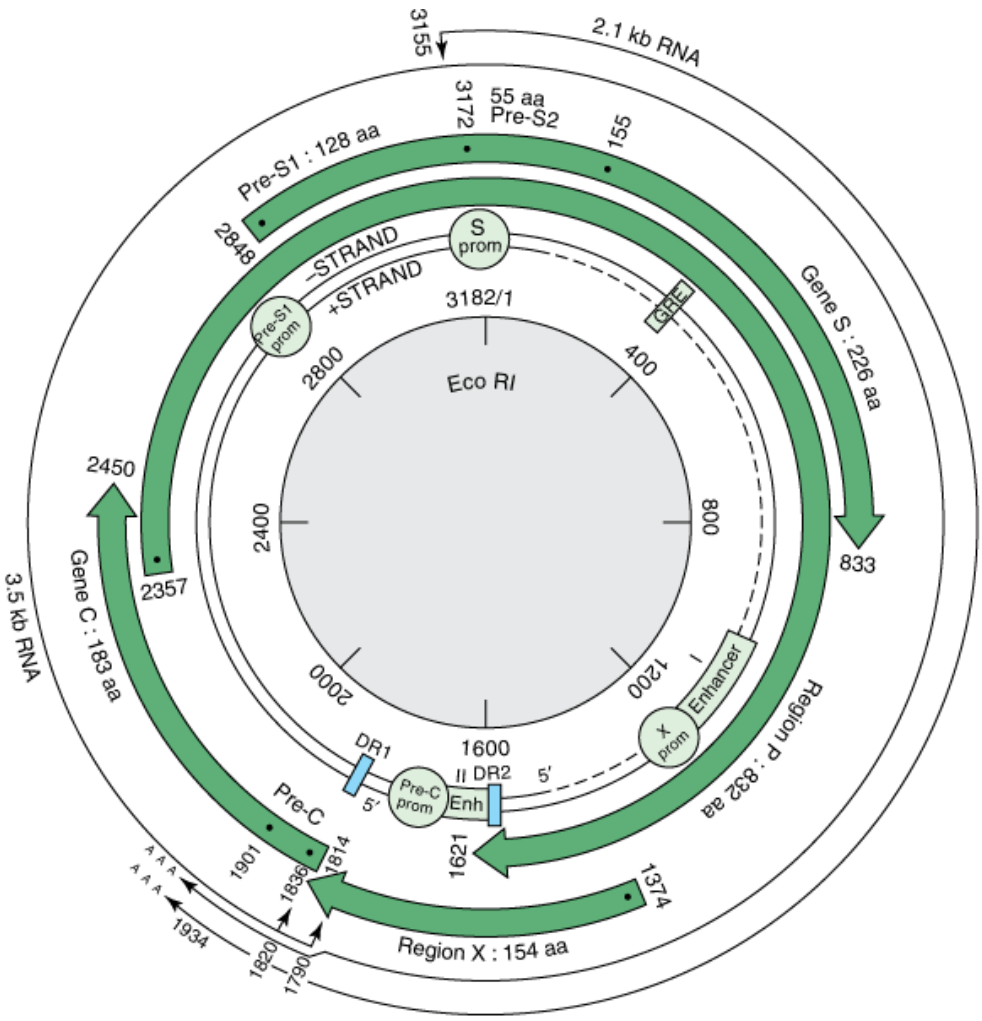

Fig (2.2) Hepatitis B virus genome. Available at: World J Gastroenterology

2.1.4 Replication Cycle

The replication of HBV involves a reverse transcription step, and as such is unique among DNA viruses. HBV has a specific tropism for the liver. However, the receptor for HBV and the mechanism of viral entry are not known. The attachment or adsorption of HBV to hepatocytes (liver cells) is mediated by the envelope protein (HBsAg) of the virus, probably by binding of HBsAg with polymerized human serum albumin or other serum proteins. After viral entry, the partial double-stranded DNA (incomplete) is transported to the nucleus. The double-stranded DNA is organized as two strands. One, a short strand, is associated with the viral DNA polymerase and is of positive

polarity. The complete or long strand is complementary and thus of negative polarity.The partial incomplete strand is formed into a complete double-stranded circular DNA, which is essential before transcription can take place. Host RNA polymerase directs the transcription of viral mRNAs to encode early proteins, including HBcAg, HBeAg, and viral DNA polymerase as well as full-length RNA (pre-genomic RNA). HBsAg is encoded later and associates with the membranes of endoplasmic reticulum or Golgi apparatus. HBcAg forms the core by enclosing the full-length positive-sense viral pre-genomic RNA along with viral DNA polymerase into maturing core particles late in the replication cycle. These full-length RNA strands form a template for a reverse transcription step in which negatively stranded DNA is synthesized. The RNA template strands are then degraded by ribonuclease H activity. A positive-stranded DNA is then synthesized, although this is not completed before virus maturation in which HBsAg-containing membranes of the endoplasmic reticulum or Golgi apparatus are wrapped over the nucleocapsid core, resulting in the variable-length, short, positive DNA strands found in the virions. The virions are released by exocytosis.

Unique replication using a reverse transcriptase step HBV DNA has also been found to integrate into the host chromosomes, especially in HBV-infected patients with hepatocellular carcinoma (HCC). However, the significance of integrated HBV DNA in viral replication is not known. Despite extensive attempts, HBV has not been successfully propagated in the laboratory. Humans appear to be the major host; however, as with hepatitis A, infection of subhuman primates has been accomplished experimentally, viral integration. Humans are the major hosts (Ryan *et al.*, 2010).

2.1.5 Stability

The stability of HBsAg does not always coincide with that of the infectious agent. However, both are stable at –20°C for over 20 years and stable to repeated freezing and thawing. The virus also is stable at 37°C for 60 minutes and remains viable after being dried and stored at 25°C for at least 1 week. HBV (but not HBsAg) is sensitive to higher temperatures (100°C for 1 minute) or to longer incubation periods (60°C for 10 hours). HBsAg is stable at pH 2.4 for up to 6 hours, but HBV infectivity is lost. Sodium hypochlorite, 0.5% (eg, 1:10 chlorine bleach), destroys antigenicity within 3 minutes at low protein concentrations, but undiluted serum specimens require higher concentrations (5%). HBsAg is not destroyed by ultraviolet irradiation of plasma or other blood products, and viral infectivity may also resist such treatment (Brooks *et al*., 2010).

2.1.6 Epidemiology

HBV infection is a formidable immense worldwide problem. More than 200 million people are chronically infected. The prevalence is highly variable in the Far East, and in Mediterranean and Eastern European countries, whereas in sub-Saharan Africa the endemic rates are highest, with as many as 20% of the population being infected. In North America and Western Europe the infection is not common (0.1–0.2%). The major route of infection in high endemic areas is perinatal. In countries of low endemicity, the major routes of infection are sexual and shared needles amongst intravenous drug users. The latter group is notoriously difficult to target by vaccination. However, universal or extensive vaccination may be the only practical means of achieving a significant reduction of HBV prevalence (Haaheim *et al*., 2002).

2.1.7 Epidemiology in Sudan

Hepatitis virus infections are the most common cause of liver disease worldwide. Sudan is classified among the countries with high hepatitis B virus seroprevalence. Hepatitis B Virus (HBV) infection is a global public health problem. It is estimated that there are more than 350 million HBV carriers in the world, of whom one million die annually from HBV-related liver disease (Hwang and Cheung, 2011).

likewise recent studies showed that the seroprevalence of HBV was 5.1 and 5.6% among blood donors in northern and central Sudan, respectively (Nagi *et al*., 2007; Elsheikh *et al*., 2007).

In study was conducted at Kassala, Eastern Sudan to determine the seroprevalence and epidemiological risk factors of hepatitis B virus (HBV) infection among healthy people, the seroprevalence revealed from ELISA was (8.2%). Among the epidemiological and risk factors, the seropositivity of HBV varied with residence, ethnicity and gender distribution (Abdallah *et al*., 2011).

2.1.8 History

In 1963 Blumberg , a geneticist investigating hereditary factors in the sera of isolated racial groups, discovered an antigen in the serum of an Australian aborigine that reacted with sera from multiply transfused American hemophiliacs . In due course the antigen was demonstrated to be present on the surface of particles with three different morphologic forms and to be associated with the disease serum hepatitis , now known as hepatitis B. The 22 nm particles of ″Australia antigen ‶, subsequently renamed HBs Ag, for hepatitis B surface antigen, were found to be noninfectious, but the 42nm particles were shown to be infectious virion capable of transmitting hepatitis to chimpanzees. The unique characteristics of these viruses led to their classification within a new family, named hepadnaviridae to reflect

association with hepatitis and the DNA genome. The very small genome replicates via a unique mechanism. Hepatitis B is one of the world's major unconquered diseases. Some 300 million people are chronic carrier of the virus and a significant minority go on to develop cirrhosis or cancer of the liver from which over 1 million die every year. Although Hepatitis B virus (HBV) has yel to be cultivated reproducibly in vitro, reliable diagnostic procedures and a much- needed vaccine are available (White and Fenner, 1994) .

2.1.9 Pathogenesis & Immunity

After entering the blood, the virus infects hepatocytes, and viral antigens are displayed on the surface of the cells. Cytotoxic T cells mediate an immune attack against the viral antigens, and inflammation and necrosis occur. Immune attack against viral antigens on infected hepatocytes is mediated by cytotoxic T cells. The pathogenesis of hepatitis B is probably the result of this cell-mediated immune injury, because HBV itself does not cause a cytopathic effect. Antigen–antibody complexes cause some of the early symptoms, e.g., arthralgias, arthritis, and urticaria, and some of the complications in chronic hepatitis, e.g., glomerulonephritis, cryoglobulinemia, and vasculitis (Levinson, 2010).

About 5% of patients with HBV infection become chronic carriers; in contrast, there is no prolonged carrier state in patients with HAV infection. A chronic carrier is someone who has HBsAg persisting in their blood for at least 6 months. The chronic carrier state is attributed to a persistent infection of the hepatocytes, which results in the prolonged presence of HBV and HBsAg in the blood. The main determinant of whether a person clears the infection or becomes a chronic carrier is the adequacy of the cytotoxic T-cell response. HBV DNA exists primarily as an episome in the cytoplasm of persistently infected cells; a small number of copies of HBV DNA are integrated into cell DNA (Levinson, 2010).

A high rate of hepatocellular carcinoma occurs in chronic carriers. The HBV genome has no oncogene, and hepatocellular carcinoma appears to be the result of persistent cellular regeneration that attempts to replace the dead hepatocytes. Alternatively, malignant transformation could be the result of insertional mutagenesis, which could occur when the HBV genome integrates into the hepatocyte DNA. Integration of the HBV DNA could activate a cellular oncogene, leading to a loss of growth control.

Chronic carriage is more likely to occur when infection occurs in a newborn than in an adult, probably because a newborn's immune system is less competent than that of an adult's. Approximately 90% of infected neonates become chronic carriers. Chronic carriage resulting from neonatal infection is associated with a high risk of hepatocellular carcinoma.

Lifelong immunity occurs after the natural infection and is mediated by humoral antibody against HBsAg. Antibody against HBsAg (HBsAb) is protective because it binds to surface antigen on the virion and prevents it from interacting with receptors on the hepatocyte. (HBsAb is said to neutralize the infectivity of HBV). Note that antibody against the core antigen (HBcAb) is not protective because the core antigen is inside the virion and the antibody cannot interact with it (Levinson, 2010).

2.1.10 Transmission:

Infectious HBV is present in all body fluids of an infected individual. Therefore, blood, semen, saliva, and mother’s milk, for example, serve as sources of infection. The titer of infectious virus in the blood of an acutely infected patient can be as high as 108 virus particles per ml, but generally is lower in other body fluids. In areas of high endemicity, for example, Southeast Asia, Africa, and the Middle East, the majority of the population becomes infected at or shortly after birth from a chronically infected mother or from infected siblings.

Individuals infected at this young age have a significant chance of becoming chronic carriers, maintaining the high prevalence of virus in the population. In the United States and other western countries, the carrier rate is much lower, and primary infection rarely occurs in newborns (Harvey *et al.*, 2007).

2.1.11 Natural history of the disease

Exposure to HBV may result in asymptomatic, acute icteric,or, in some instances, fulminant hepatitis (0.1–0.5%). Approximately 5% of adults and 95% of perinatally infected young children become persistently infected. The outcome depends on the age of the patient and genetic factors determining the efficiency of the host immune response. Genetic factors influencing outcome (in more than one study) include polymorphisms of the MHC class II glycoproteins, which influence presentation of viral peptides during induction of the cellular immune response, and mannin-binding lectins, which bind to mannose-terminated carbohydrate residues such as those present on the C-terminus of the Pre-S2 region of the middle envelope protein facilitating phagocytosis. The risk of chronicity in children decreases with increasing age. A small proportion of carriers each year may become HBsAg negative (0.05-2%, depending on age of infection), thus leading to resolution of the hepatitis (Mahy and Van, 2010).

2.1.12 Acute HBV Infection

The incubation period following exposure is 3–6 months. In the week before icterus appears, some patients develop serum sickness-like syndrome including arthralgia, fever, and urticarial. The clinical picture varies from asymptomatic anicteric infection to protracted icterus and, in some patients (<1%), liver failure (fulminant hepatitis). The acute infection is self-limiting and most patients recover within 1–2 months after the onset of icterus (Mahy and Van, 2010).

2.1.13 Chronic HBV Infection

This is defined as persistent viremia of more than 6 months duration and accompanied by hepatic inflammation. The latter is based on histological examination of liver biopsy material that is followed by assigning of scores for necroinflammatory activity (out of 18) and stage of fibrosis (out of 6), which are used to decide whether a patient needs therapy (Mahy and Van, 2010).

2.1.14 Laboratory diagnosis

The virology laboratory can test for a wide range of HBV antigens and antibodies, using radio-immuno-assays and enzyme-linked immunosorbent assays and (ELISAs), and for HBV DNA by PCR. The standard screening test is for HBsAg, which, if present in the serum, indicates that the patient is infected with HBV, either as a recent acute infection or as a carrier (Green wood, 2007).

In an acute infection the detection of HBsAg and IgM antibody to the nucleocapsid (HBc) is characteristic, followed by development of convalescent anti-HBs antibodies Fig (2.3). Chronic infection is indicated by the presence of HBsAg and absence of IgM anti-HBc. Viral replication occurs during the initial high replicative phase of infection and these patients are HBeAg and HBV-DNA (by a non-PCR method) positive Fig (2.4).

About 10% of patients annually will spontaneously develop into a low replication stage; becoming HBeAg and HBV-DNA (by a non-PCR method) negative and anti-HBe positive. Loss of HBsAg is extremely unusual in patients with chronic infection unless they have been treated with interferon (Haaheim *et al.*, 2002).

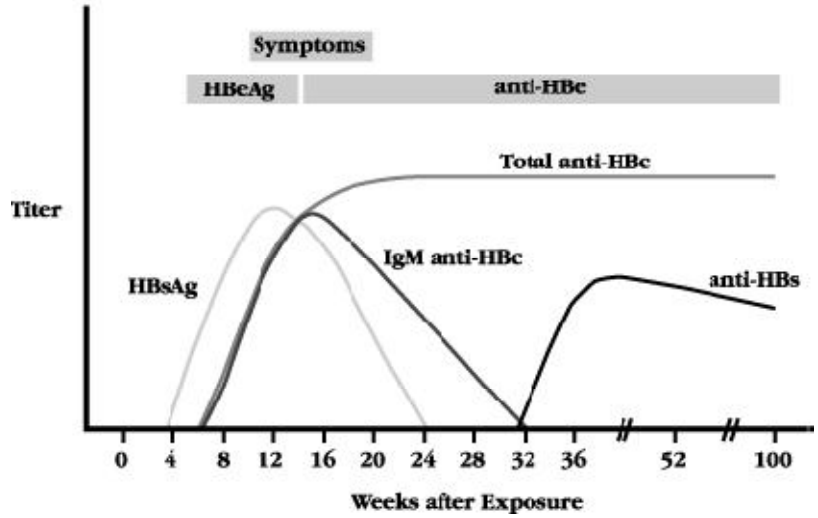

Fig (2.3) Acute hepatitis B virus infectionwith recovery typical serologic course. Reprinted from Centers for Disease Control and Prevention. Available at:http://www.cdc.gov/ncidod/diseases/hepatitis/slideset/hep b/hep b.pdf

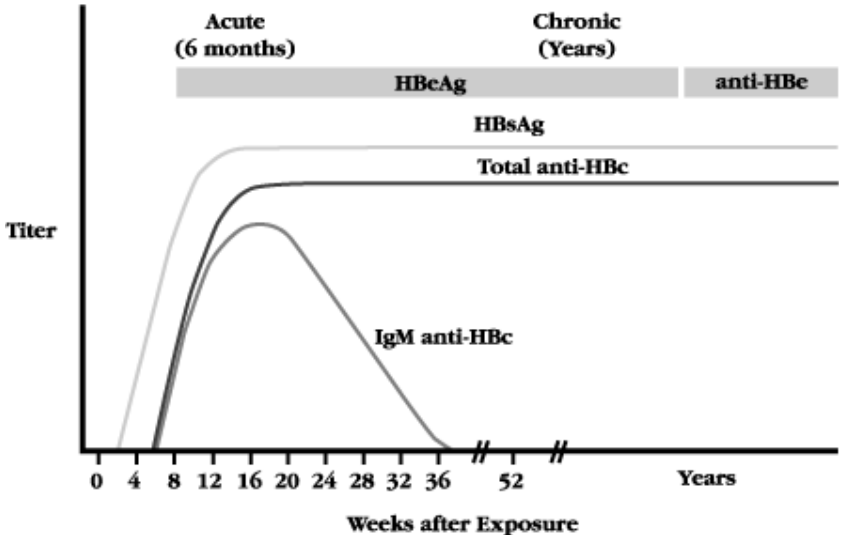

Fig (2.4) Progression to chronic hepatitis B virus infection typical serologic course. Reprinted from Centers for Disease Control and Prevention. Available at:http://www.cdc.gov/ncidod/diseases/hepatitis/slideset/hep_b/hep_b.pdf

2.1.14.1 Antigen detection

HBsAg tests

Testing for HBsAg is used to screen donor blood for HBV infectivity, and depending on resources, to test pregnant women for HBV carrier status.

HbsAg tests include ELISA (microtitration plate), rapid immunochromatographic (IC) tests and latex agglutination tests. Most of the IC tests are in cassette form. When screening blood, a sensitive test is required to ensure HBV infected blood is reliably detected, and also a test with adequate specificity to avoid false positive reactions resulting in blood being wasted unnecessarily. Most of the currently available HBsAg tests have high sensitivity and specificity. The rapid IC tests are particularly easy to perform and can be read visually.

Hepatitis B e antigen (HBeAg) Appears soon after HBsAg and persists for a short time, disappearing when recovery begins. Its presence is associated with increased infectivity. Persistence of e-antigen indicates chronic liver disease. Tests to detect HBeAg are usually performed only in specialist laboratories (Chessbrough, 2007).

2.1.14.2 Antibody markers of hepatitis B

– IgM antibody to hepatitis B core antigen (anti-HBc IgM) appears only in acute infection and is a useful marker of recent infection. The presence of IgG antibody to HBcAg (anti-HBc IgG) in the absence of IgM, indicates past infection (used in seroepidemiological studies).

– Antibody to HBsAg (anti-HBs) is the last serological marker to form, appearing in the convalescence stage. It indicates recent infection or past immunization.

– Antibody to HBeAg (anti-HBe) may be found in the convalescence stage and often in chronic hepatitis and the carrier state.

Note: Testing for HBV antibodies is usually performed only in specialist laboratories (Chessbrough, 2007).

2.1.14.3 Quantitative measurement of HBV DNA

The assays for HBV infectivity described above were indispensable for HBV research but are too expensive and laborious for the laboratory diagnosis of HB viremia. The best surrogate test is the sensitive and quantitative determination of the number of HBV DNA molecules in plasma or serum. In the early days, the endogenous DNA polymerase reaction developed by Robinson was the first feasible but relatively insensitive and laborious assay. Later this was replaced by various techniques of nucleic acid hybridization (e.g. dot blot) using cloned HBV DNA as labeled probes. These techniques were still of insufficient sensitivity and accuracy and could not detect the low viremia of healthy HBsAg carriers or occult HBV infections. They were, however, useful for distinction of high and low infectivity and for HBV monitoring in early therapy studies (Quint *et al*., 1995).

2.1.14.4 Liver function and liver enzyme assays:

Liver enzyme tests and other liver-related clinical assays are used to monitor liver function during acute or chronic hepatitis (NACB, 2000). The National Academy of Clinical Biochemistry (NACB) has recommended guidelines for serum tests to evaluate patients with either known or suspected liver disease, including HBV. The test panel includes the following: serum aspartate aminotransferase (AST), serum amino alanine transferases (ALT), alkaline phosphatase, serum gamma glutamyltransferase (GGT), total bilirubin, direct bilirubin, serum albumin, and prothrombin time. These assays, particularly ALT, provide simple biochemical tests to assess liver disease activity and to establish the severity of hepatitis in the individual (Lok and McMahon, 2001).

2.1.15 Prevention

The hepatitis B vaccine is the mainstay of hepatitis B prevention. WHO recommends that all infants receive the hepatitis B vaccine as soon as possible after birth, preferably within 24 hours. The birth dose should be followed by 2

or 3 doses to complete the primary series. In most cases, one of the following two options is considered appropriate:

- a 3 dose schedule of hepatitis B vaccine, with the first dose (monovalent) being given at birth and the second and third (monovalent or combined vaccine) given at the same time as the first and third doses of diphtheria, pertussis (whooping cough), and tetanus - (DTP) vaccine; or
- 4 doses, where a monovalent birth dose is followed by three monovalent or combined vaccine doses, usually given with other routine infant vaccines.

The complete vaccine series induces protective antibody levels in more than 95% of infants, children and young adults. Protection lasts at least 20 years and is probably lifelong.Thus, WHO does not recommend booster vaccination for persons who have completed the 3 dose vaccination schedule.

All children and adolescents younger than 18 years-old and not previously vaccinated should receive the vaccine if they live in countries where there is low or intermediate endemicity. In those settings it is possible that more people in high-risk groups may acquire the infection and they should also be vaccinated. They include:

- people who frequently require blood or blood products, dialysis patients, recipients of solid organ transplantations;
- people interned in prisons
- persons who inject drugs
- household and sexual contacts of people with chronic HBV infection
- people with multiple sexual partners
- health-care workers and others who may be exposed to blood and blood products through their work; and
- travellers who have not completed their hepatitis B vaccination series, who should be offered the vaccine before leaving for endemic areas.

The vaccine has an excellent record of safety and effectiveness. Since 1982, over 1 billion doses of hepatitis B vaccine have been used worldwide. In

many countries where 8–15% of children used to become chronically infected with the hepatitis B virus, vaccination has reduced the rate of chronic infection to less than 1% among immunized children.

As of 2013, 183 Member States vaccinate infants against hepatitis B as part of their vaccination schedules and 81% of children received the hepatitis B vaccine. This is a major increase compared with 31 countries in 1992, the year that the World Health Assembly passed a resolution to recommend global vaccination against hepatitis B. Furthermore, as of 2013, 93 Member States have introduced the hepatitis B birth dose vaccine.

In addition, implementing of blood safety strategies, including quality-assured screening of all donated blood and blood components used for transfusion, can prevent transmission of HBV. Safe injection practices, eliminating unnecessary and unsafe injections, can be effective strategies to protect against HBV transmission (WHO, 2015) .

2.1.16 Treatment

In the past, treatment for acute hepatitis was largely supportive and not directed toward inhibiting virus replication. Prolonged (months) treatment with interferon-α has succeeded in reducing or eliminating indicators of HBV replication in about one third of patients but, in some of these, recurrence of indications of the infection occurs after discontinuance of the therapy. Antiviral analogs used in HIV therapy and targeted especially against the reverse transcriptase have been tested and had some success. In clinical trials, lamivudine, an oral nucleoside analog, has been shown to be an effective treatment in patients with previously untreated chronic hepatitis B (Harvey *et al.*, 2007).

Treatment with lamivudine for one year was well tolerated. It decreased histologic liver abnormalities and increased the rate of HbeAg seroconversion (defined as the loss of HBeAg in serum, undetectable HBV DNA levels, and

the presence of antibodies against HBeAg). Most lamivudine-treated patients had undetectable serum HBV DNA during the one year of treatment, but four months after treatment was stopped, the median HBV DNA level had returned to about fifty percent of the pretreatment value. Initial reports show no greater benefits from combination therapy with interferon plus lamivudine than with lamivudine monotherapy (Harvey *et al*., 2007).

CHAPTER THREE

Chapter Three

3. Materials and Methods

3.1 Study design

This was a descriptive cross sectional study.

3.2 Study area

The study was conducted in Omdurman Islamic University in Medical Laboratory College.

3.3 Study population

Ninety medical students, 70 were vaccinated and 20 were not vaccinated but had no history of HBV infection.

3.4 Study duration

Study was carried out during 1 year period from April 2015 to May 2016.

3.5 Ethical consideration

Permission to carry out the study was obtained from the Collage of Graduate Studies, Sudan University of Science and Technology. All The participants were informed for the purpose of the study before collection of the samples and the verbal consent was taken from them.

3.6 Data collection

Data of some demographic factors such as gender, age, smoking, and diabetes, were collected using direct interview approach.

3.7 Sample collection

Under aseptic condition after wearing the gloves, alcohol antiseptic (70% ethanol) was used to clean the skin. Venous blood (3 ml) was obtained from medical students. Samples were collected by vein puncture and hemolysis was avoided. Blood was collected into plain containers and left to settle for 30 minutes at room temperature, and then was centrifuged at 3000 rpm for

5 minutes . Sera were collected and preserved at -20 ºC till used. Age group, history of diabetes and smoking were taken as risk factors.

3.8. Laboratory methods

3.8.1 Enzyme immunoassay for the qualitative detection and quantitative determination of specific antibodies to the hepatitis B surface antigen

3.8.1.1 Principle of the method

Enzygnost ® Anti-HBs II is a one-step assay based on the sandwich principle. Inactivated HBsAg of human origin containing subtypes ad and ay is used as solid phase and as conjugate antigen.

Peroxidase-labeled HBsAg binds to the HBs- specific antibodies contained in the sample. These bind to the HBsAg bound to the surface of microtitration plate (antigen sandwich).

The enzyme portion conjugate causes the chromogen working solution to turn blue. This reaction is stopped by the addition of stopping solution POD, which causes color change to yellow. The intensity of the yellow color produced proportional to the activity of specific antibody contained in the sample. Quantification in international Units is performed by calculation using α-method.

3.8.1.2 Procedure

Non-automated Test Procedure

Step 1 The necessary number of test plate well was given by the number of samples plus the number of determinations (n = 6) for Anti-HBs II Neg and Anti-HBs II REF P.

Step 2 Twenty five µl of Anti-HBs II Conj was added to each well. The controls and samples were pipetted immediately after completing the conjugate dispensing step.

Step 3 100 µl of Anti- HBs II Neg was dispensed into each of 4 wells (A1-D1), 100 µl of Anti- HBs II REF P into one well (E1) and 100 µl sample into each of the subsequent wells. At the end of series respectively test plate

fill one further well with 100 µl of Anti- HBs REF P.

Step 4 The plate was covered with foil and incubated for 60±2 minutes at 37±1 °C, then proceed immediately to the wash step.

Step 5 Foil was removed and all wells were aspirated . Each well was filled with approx. 300 µl diluted washing solution POD, the plate was aspirated and wash cycle was repeated three times.

Step 6 100 µl of TMB chromogen working solution was pipetted into each well and the microtitration plate was sealed with fresh foil.

Step 7 Immediately after the substrate dispensing step, the plate was incubated at 15 to 25°C for 30±2 minutes protected from light.

Step 8 foil was removed. 100 µl sulfuric acid stopping solution POD was added to each well, keeping to the same timing as during the substrate dispensing.

3.8.1.3 Reading of the result

The test plate was read at 450 nm within one hour. The recommended reference wavelength is 650nm, or where appropriate, between651 and 690 nm.

3.8.1.4 Internal quality control:

Validation criteria:

To evaluate the test the following criteria must be fulfilled:

Anti-HBs II Neg: $-0.010 \leq A \leq 0.120$

Anti-HBs II REF P: $A \geq 0.7$

3.8.1.5 Results:

Qualitative Evaluation:

The cut-off was calculated by the mean of the valid absorbance values of Anti-HBs II Neg and add a value of 0.08:

Ā Anti-HBs II Neg + 0.08 = cut-off

Based on the criteria of the test the samples were classified as follows:

Anti-HBs **negative** A sample < cut-off

Anti-HBs **positive** A sample ≥ cut-off

3.9 Data analysis

Statistical package of social science (SPSS version 15.0). Computer soft ware was used for data analysis .Significant level were set at ($P<0.05$).

Figures were performed by using Microsoft Office and excel soft ware program.

CHAPTER FOUR

Chapter Four

4. Results

A total of 90 medical students were included in this study. Of these, 70 (35 males, 35 females) were vaccinated against hepatitis B virus and 20 (10 males, 10 females) were unvaccinated. The majority of the students were within the age range of 20-30 years. Of the total 70 vaccinated students 62/70 (88.5 %) were responders to HBV vaccine and 8/70 (11.4%) were non responders. Of the 20 unvaccinated 1/20 (5%) was reactive and 19 (95%) were non reactive.

4.1 Distribution of reactive and non reactive in vaccinated medical laboratory students according to gender

Table 4.1 shows the sex distribution of the study group. The percentage of immune response to vaccine in vaccinated students was 29/70 (41.4) % in males and 33/70 (47.1) % in females.

Table 4.1: Immune response of vaccinated students to Hepatitis B vaccine

Gender	Vaccinated		Total
	Reactive	Non reactive	
Male	29 (41.4)%	6 (8.5)%	35 (49.9) %
Female	33 (47.1)%	2 (3) %	35 (50.1) %
Total	62 (88.5)%	8 (11.5)%	70 (100) %

P. value: 0.000

4.2 Distribution of reactive and non reactive in non vaccinated medical laboratory students according to gender

Table 4.2 Only 1 male was found to be raective and other students were non reactive.

Table 4.2: Immune response of non vaccinated students to Hepatitis B vaccine

Gender	Non vaccinated		Total
	Reactive	Non reactive	
Male	1 (5) %	9(45) %	10 (50) %
Female	0 (0) %	10 (50) %	10 (50) %
Total	1(5) %	19 (95) %	20 (100) %

P value : 0.5

4.3 Relation of age and vaccination to HBV

Out of the 70 medical laboratory students included in this study 56 (80) % were among (21 – 23) years and 14 (20)% were among (26- 30) years (Table 4.3).

Table 4.3: Age groups reactive and non reactive in vaccinated students

Age group	Reactive	Non reactive	Total
21-25	48 (68.5) %	8 (11.4) %	56 (80) %
26-30	14 (20) %	0 (0) %	14 (20) %
Total	62 (88.5) %	8 (11.4) %	70 (100) %

P value: 0.27

4.4 Relation of diabetes on HBV vaccination

Five students out of the 70 tested students were diabetic. Two diabetic students out of them were found to be reactive (Table 4.4).

Table 4.4: Distribution of vaccinated medical laboratory students in relation to diabetes

Diabetic students	Reactive	Non reactive	Total
Yes	2(40) %	3(60)%	5 (100) %
No	60(92.3)%	5(7.7) %	65 (100) %

P value : 0.00

4.5 Relation between smoking and HBV vaccination

Only one student was smoker and was found to be reactive (Table 4.5).

Table 4.5: Distribution of vaccinated medical laboratory students according to smoking

Smoking	Reactive	Non reactive	Total
Yes	1 (20)%	4(80) %	5 (100) %
No	61(93.9) %	4(6.1) %	65 (100) %

P value : 0.00

CHAPTER FIVE

Chapter Five

5.1 Discussion

Occupational exposure to blood and/or other body fluids within healthcare sector facilities is a major risk of HBV transmission to medical students, this risk is highest during medical training.

In the present study, results revealed immune response among Sudanese laboratory medical students, of which 62/70 (88.5 %) were responders to HBV vaccine. Study of Tripathy *et al* (2007) was found 95% of Indian medical students had anti-HBs levels more than 100 IU/L. These results agree with seroconversion rate of Hepatitis B vaccine globally which ranges from 85-90 %. However, Jain (2005) found seroconversion rate of 98.45 %. Hussein and Hussein (2012) were found the protective level among medical students was high in Egypt (96%). This indicate that Hepatitis B vaccine is a protective vaccine throughout the world.

Chisary and Ferrari (1995) reported that unresponsiveness to HB vaccine has been attributed to a number of environmental and genetic factors, the most important ones being the haplotype of HLA antigen and immunological tolerance . Of the 20 non vaccinated controls, 1(5%) had protective antibody response. This is similar to the study by Singh *et al* (2010), due to subclinical infection and exposure person might had developed anti HBs antibody.

In the present study, the males showed less response to HBV vaccine (41.4%) than females (47.1%), however statically there was significant relation (*P*:0.000). This study did not agreed with study of MacMohan (2007) who reported males had higher antibody level than females. Whereas Ane and Fang (1994) found that female children responded with a significantly higher antibody level than male children. In the study conducted by Abdul Ahad (2009) in Bangladesh the level of anti HBs antibody was 85.88% males and 92.31 % of females. Glaser (1992) found in his study that antibody level becomes less in persons undergoing more stress than less stress one.

Despite the small number of smokers in this study, the results suggest that non-smokers are more likely to demonstrate vaccine response statistically (*p* value: 0.00). This finding confirms the results made by Bock *et al* (1996) . It has been supposed, the diminished response in smokers may be due to the increasing of T suppressor lymphocytes (*P*: 0.06).

It has been reported that antibody response decreases with age Looney and Hasan (2001) they found that antibody response was dramatically different between young and elderly group, their results were dissimilar to our study in that there was no relation between age and immune response because all participants were adults.

Also the present study displayed significant relation between response to vaccine and systemic disease (*P* value 0.00); none of participants who had systemic disease responded to vaccine; which point out that some other non immunological factors (diabetes) influence the outcome of vaccination.

5.2 Conclusion

The study concluded that Hepatitis B vaccine was an effective vaccine. Age, smoking and diabetes are factors that hinder Hepatitis B vaccine immune response.

5.3 Recommendation

1. More studies are required, using large sample size to acquire more accurate result.
2. It is essential to check the post vaccination status of all Health care workers as it does not only ensure safety of employee but also reduces rate of transmission.
3. There is a need to strictly implement policy for hepatitis B immunization among the health care setting in developing countries.

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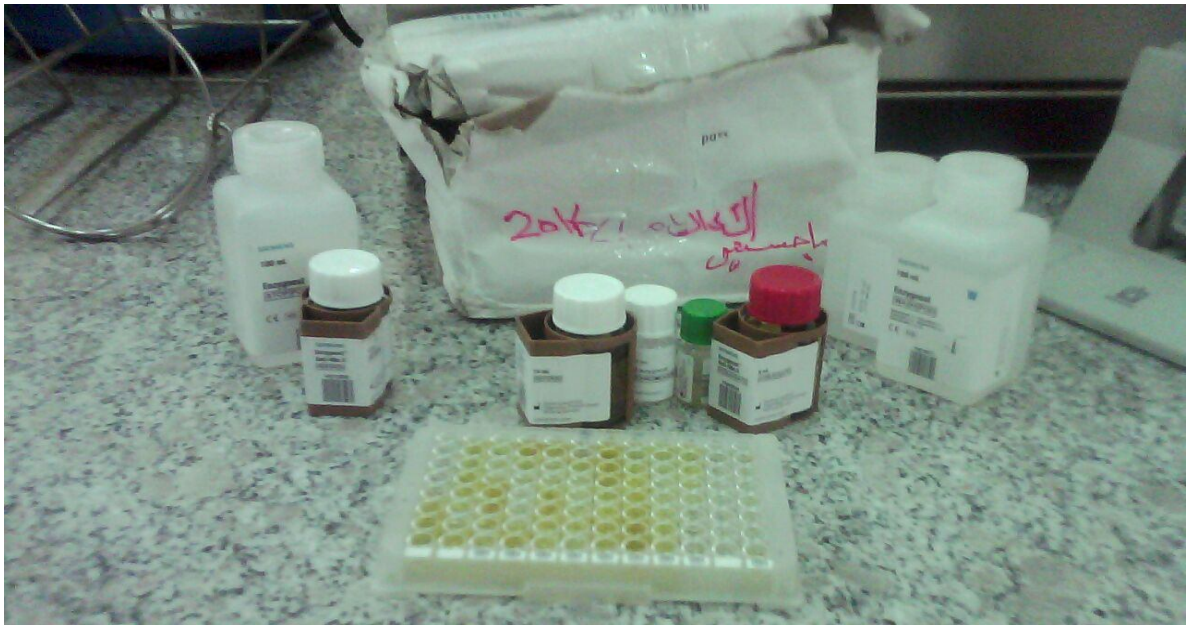
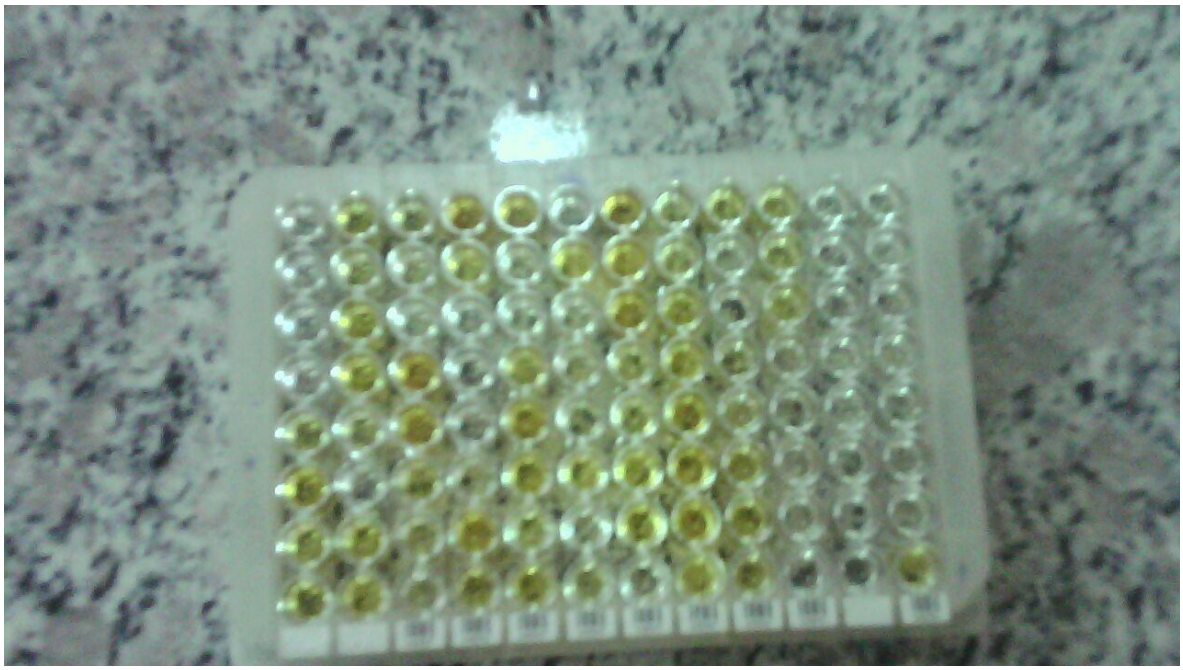
Appendix



Appendix(1**) ELISA KIT image**

Appendix(2**) ELISA Reader**



Appendix(3**)ELISA Result**

SUDAN University of Science and Technology

Collage of graduate studies

Microbiology Department

Questionnaire

Study of immune respone among medical students

Date…/…/2015 **questionnaire NO……**

Name of student……..

Sex:

Male ☐ **Female** ☐

Age :

……

Yes or No :

Smoking

YES ☐ **NO** ☐

Chronic diseases

YES ☐ **NO** ☐

Test required : ELISA for Anti HBVs Ag

Result:

ELISA……