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Evaluation of Immune Globulin level among Vaccinated Health Care Workers *Against Hepatitis B Virus* in Khartoum State Hospitals

A thesis submitted for partial fulfillment for requirements of M.Sc. degree in

(Microbiology)

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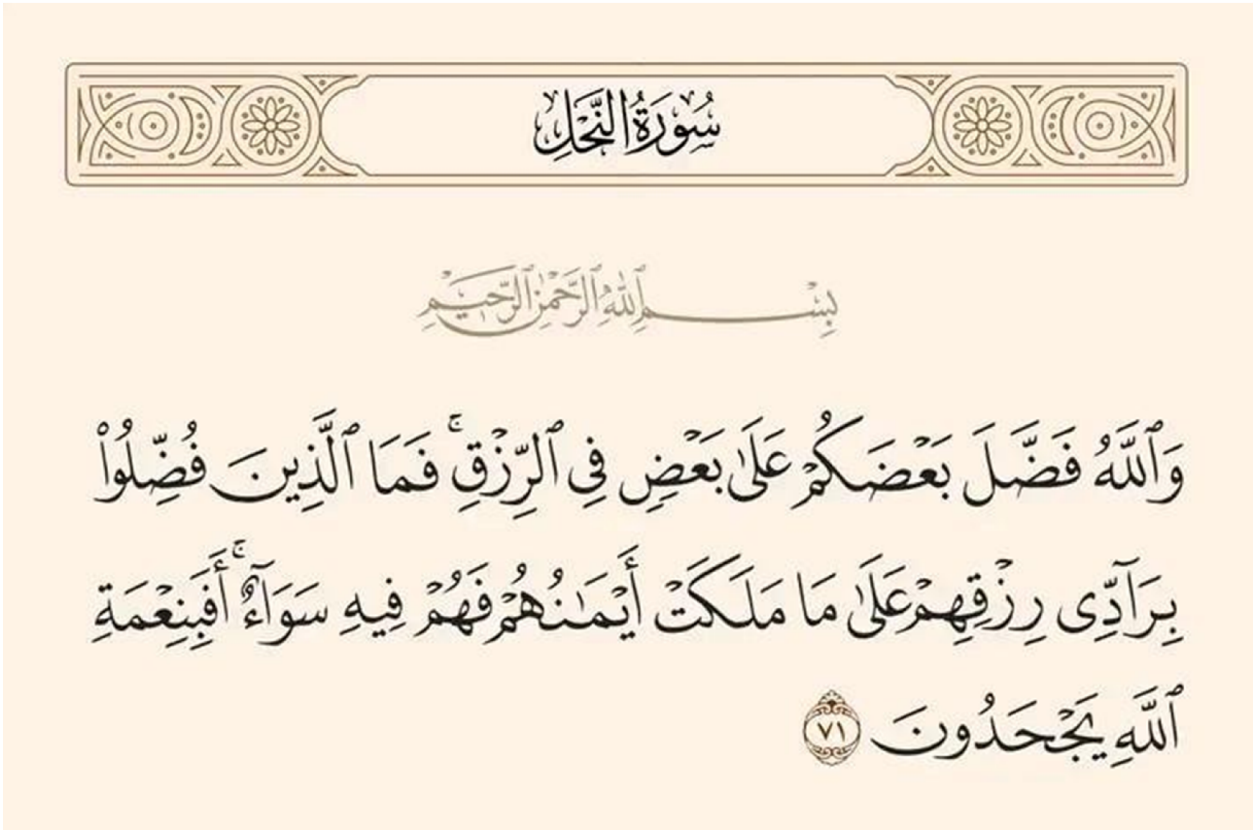

صدق الله العظيـــــم

سورة النحل الآية "٧١"

DEDICATION

To...

My father,,, My mother and

Aunt soul and MySisters,,,

For their hard efforts, support and encouragement.

To my friends…

With love …and respect

I dedicated this work…

ACKNOWLEDGEMENTS

Firstly, thanks to ALLAHA to reconcile and help me for complete .this study

Then my thankfulness to my supervisor Dr. Leila Mohammed Abd Elgadeer for his supervision, great efforts, beneficial advices,and encouragement.

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Also to my colleagues at Shendi university, for their help, effortand beauty emotions.

Table of content

List of Tables

List of Figures

List of abbreviations

ACIP	Advisory Committee on Immunization Practices
CpGODN	Immun ostimulatory cytidine-phosphate Guanosineoligo deoxynucleotide
CccDNA	Covalently closed circular DNA
DNA	Deoxyribonucleic Acid
EDTA	Ethylene di-amine tetra acidic acid
ELISA	Enzyme Linked Immuno-sorbent Assay
HB	Hepatitis B
HBV	Hepatitis B Virus
HbcAb	Hepatitis B core antibody
HbsAb	Hepatitis B surface antibody
HbsAg	Hepatitis B surface antigen
HBIG	Hepatitis B Immunoglobulin
HCWs	Health Care Workers
HIV	Human Immunodeficiency Virus
ICT	Immuno-chromatography test
IM	Intramuscular
IV	Intravenous
OD	Optical Density
ORF	Open Reading Frame
RcDNA	Relaxed circular DNA
RNA	Ribonucleic Acid
STDs	Sexual Transmitted Diseases
SPSS	Statistical package for Social sciences
WHO	World health organization

ABSTRACT

Background: vaccination of health care workers (HCWs) for the hepatitis B virus (HBV) is a crucial part of the hospital infection control programs, they are considered to be a population at high-risk to develop HBV infection due to the high transmissibility of the and the risk infection related to occupational injuries. The risk is particularly high in HCW with greater exposure to accidental inoculation at work and is related to the duration of professional activity, the characteristics of the healthcare center and the type of population served.

Aim: The current study conducted to evaluate immunity status of the vaccinated HCWs against Hepatitis B Virus and associate of Hepatitis B virus with demographic data (age, Gender, Type of employment, Employment duration and duration of vaccination) in Khartoum Hospitals .

Methods: This cross sectional descriptive study conducted in the period of December2021 to 2024, data were collected through a structured questionnaire, regarding demographic data (age, gender, type of employment, length of employment and duration of vaccination) were recorded for each participant after complete three doses of fully Vaccinated with recombinant vaccine ,serum samples were collected and examined, Elisa technique was used to detect the Anti HBs level, collected data were analyzed using SPSS.

Results: this study consent 90 of HCWs, found that 70% of study populations have protective level of HBs-Ab while 30% of them have not protective level of HBs-Abs.

Conclusion: The study show that the results of Health Care Workers Antibodies level were protective.

المستخلص

خلفية الدراسة:تحصين العاملين في الرعاية الصحية ضد التهاب الكبد البائي ب هو جزء من برنامج مكافحة العدوى بالمستشفى، فهم يعتبرون من السكان المعرضون لخطر كبير للإصابة بعدوى فيروس التهاب الكبد ب بسبب القابلية العالية لانتقال العدوى والمخاطر المتعلقة بإصابات العمل يزداد الخطر في العاملين بالرعاية الصحية مع فترة وطبيعة النشاط المهني وخصائص مركز الرعاية الصحية ونوع السكان الذين تم تقديم الخدمة لهم.

الأهداف: أجريت الدراسة الحالية لتقييم حالة مناعة العاملين في مجال الرعاية الصحية الذين تم تلقحيهم ضد فيروس التهاب الكبد ب وربطها بالبيانات الديموغرافية (بالعمر والجنس ونوع الوظيفة وطول فترة العمل ومدة التطعيم) في مستشفيات ولاية الخرطوم.

طرق البحث :دراسة وصفية مقطعية أجريت خلال الفترة من ديسمبر ٢٠٢١ إلي ٢٠٢٤ تم جمع البيانات من خلال استبيان منظم بشأن البيانات الديموغرافية (العمر والجنس ونوع العمل ومدة التوظيف ومدة التطعيم لكل مشارك) . تم جمع العينات من كل مشارك اكمل الثلاث جرعات تحصينا كاملا بrecombinant vaccine. جمعت عينة الامصال وتم فحصها بواسطة Elisa للتحقق من مستوى bodyAnti-HBs anti. البيانات التي جمعت تم تحليلها بواسطةspss.

النتائج:شملت هذه الدراسة ٩٠ من العاملين في الرعاية الصحية، يوجد منهم ٧٠٪ داخل المدي الوقائي للتحصين بلقاح فيروس الكبد البائي، وال٣٠٪ خارج المدي الوقائي.

الخاتمة :أوضحت الدراسة ان نتائج مستوي الأجسام المضادة للعاملين في الرعاية الصحية انها كانت وقائية.

Chapter One

1. Introduction

1.1 Background

Five viruses are responsible for most cases of viral hepatitis, which is an inflammation of the liver due to viral infection. These are the hepatitis A virus(HAV) and hepatitis B virus (HBV) hepatitis C virus (HCV) hepatitis D virus(HDV) and hepatitis E virus(HEV). All the hepatitis viruses can cause acute hepatitis, however, only HBV, HCV and HDV frequently cause chronic hepatitis, which can lead to liver cirrhosis and hepatocellular carcinoma(WHO2017)

Hepatitis B Virus (HBV) is a member of the Hepadnaviridae family with a small DNA virus and unusual features of replication similar to retroviruses, in which HBV can replicate through an RNA intermediate and forming a stable minichromosome (cccDNA)

in the nucleus,These features of the HBV replication cycle give it the ability to persist in infected cells.

HBV can survive outside the body for up to seven days; the virus incubation period is around two and a half months. The virus can be transmitted perinatally from a mother to her baby, horizontally by exposure to infected blood or blood products, or through direct spread percutaneously or tomucosal membranes.(WHO2017)

HBV infects more than 300 million people worldwide; Sudan is classified among the countries with high HBV seroprevalence. Exposure to the virus varied from 47 to 78%, with a hepatitis B surface antigen prevalence ranging from 6.8% in central Sudan to 26% in southern Sudan HBV vaccine is a recombinant DNA vaccine that contains HBsAg genetically engineered from the yeast Saccharomyces cerevisiae. It provides a seroprotection rate of 85–100% that was seen one month after

the last dose of vaccine and it confers immunity for at least 10 years(Hamadanil Y, 2017).

Hepatitis B vaccine is recommended for unvaccinated adults who are at risk for HBV infection like people whose sex partners have hepatitis B, sexually active persons who are not in a long-term monogamous relationship, persons seeking evaluation or treatment for a sexually transmitted disease, men who have sex with men, people who share needles, syringes, or other drug-injection equipment, household contacts of HBV-infected persons, health personnel and public safety workers at risk for exposure to blood or body fluids, residents and state of facilities for developmentally disabled persons, persons in correctional facilities, victims of sexual assault or abuse, travelers to regions with increased rates of hepatitis B, people with chronic liver disease, patients on hemodialysis, HIV infection, or diabetes, and anyone who wants to be protected from HBV. The vaccine is usually given as three shots over a period of a six-month period (Abdullahi M, 2020).

Serum antibody to the hepatitis B surface antigen (anti-HBs) has long been established as a marker of vaccine-induced protection against hepatitis B. An anti-HBs level of ≥10 IU/ml has been suggested to indicate protection (Abdullahi M, 2020). against hepatitis B disease . Old age in adults, male gender, increased body mass index, smoking, and concomitant disease are some of the factors that may decrease the immunologic response to HBV vaccine (Abdullahi M, 2020).

1.2. Study problem

HCWs are at high risk for HBV infection because of particular exposure of mucus membranes and breached skin to blood. HBV-infected HCWs also pose a potential risk for patients as there is documented risk of HBV transmission to patients from treating doctors or medical staff. According to WHO5,9% of HCWs are each year exposed to blood borne HBV infections corresponding to about 66000HBV infections in HCWs worldwide. Approximately70%of HCWs in hyper or intermediate endemic countries have been reported to have needle stick injuries, with an average of two needle pricks a year (Batraa,et al,2015).

1.3. Rationale

Hepatitis B vaccine can prevent hepatitis B. Hepatitis B is a liver disease that can cause mild illness lasting a few weeks, or it can lead to a serious, lifelong illness.

Persistence of anti-HBs and ability of the immune system to mount a response to exposure of HBV later in life is necessary for long term protection against HBV infection, some studies have confirmed persistence of antibodies and immune memory that following HB vaccination.

A further study with better design and laboratory confirmation of vaccination status should be done in a large group of HCWs.

This study was conducted to Evaluate of hepatitis B immune status among vaccinated health care workers in Khartoum state, selection of this state due to some reasons:

There is large number of Health Care Workers in Khartoum , and also to Availability to chose differ hospital s And due to eazy to obtaining devices and other facilitators help to conducting study.

1.4.Objectives

1.4.1.General objective

To Evaluate Immune globulin level among vaccinated HCWs against hepatitis B Virus in Khartoum state hospitals.

1.4.2. Specific objectives

1.To measure the Anti-HBs Abs titre using ELISA technique.

2.To Associaate between vaccination level and age, gender and time of last dose of given vaccine.

Chapter Two

2 .Literature Review

2.1. Hepatitis B Virus

While the discovery of human HBV, hereafter denoted as HBV, occurred in the 1960 s, recent research has shown that HBV have actually been present since the time of the dinosaurs. In fact, the earliest known HBV is approximately 82 million years old and was identified from the DNA of infected birds from the Mesozoic period. Although multiple theories of the origins of HBV exist, it appears that the infection of mammals is a much more recent event. The jump into humans, in particular, may have been only about40,000 years ago. Despite the evolutionary timeline, modern HBV is remarkably similar to these ancient hepatitis B viruses (Lamontagne, et al, 2016).

Hepatitis is an inflammation of the liver due to viral infections and there are groups of viruses that affect the liver. HBV infection threatens the health of populations across the globe. It is a DNA virus that cause viral hepatitis and can lead to liver cirrhosis and hepatocellular carcinoma (Bedaso,et al,2018). Infection with HBV remains an important global public health problem with significant morbidity and mortality (Thomas,et al,2017), can result in acute hepatitis, fulminant hepatitis, a chronic asymptomatic carrier, chronic hepatitis cirrhosis or hepatocellular carcinoma (Juergen,and Michael, 2017).

HBV is a member of the hepadnaviridae family, the only DNA virus among the agents which commonly cause viral hepatitis, the viral particle (called the Dane particle) is42 nm in diameter. The lipoprotein (HBs-Ag) which encoats the virus is seen not only as a viral envelope but also by electron microscopy as free non-infectious tubular and spherical structures. These forms of HBs-Ag circulate in considerable excess

compared with the virion and may play a permissive role in viral persistence. However, HBs-Ag may be present in blood

When replication cannot be documented. Therefore, the presence of HBs-Ag does not necessarily imply contagiousness (Juergen, and Michael, 2017).

Hepadnaviruses (hepatitis B viruses) cause transient and chronic infections of the liver.

Transient infections run a course of several months, and chronic infections are often lifelong. Chronic infections can lead to liver failure with cirrhosis and hepatocellular carcinoma.

The replication strategy of these viruses has been described in great detail, but virus-host interactions leading to acute and chronic disease are still poorly understood Studies on how the virus evades the immune response to cause prolonged transient infections with high-titer viremia and lifelong infections with an ongoing inflammation of the liver are still at an early stage, and the role of the virus in liver cancer is still elusive. The state of knowledge in this very active field is therefore reviewed with an emphasis on past accomplishments as well as goals for the future. (Seeger, et al, 2020)

HBV infection is a major health concern and worldwide over 2 billion subjects (1/3 of the world population) have evidence of HBV infection (Coppeta, et al, 2019).

HBV infection and its sequelae, including chronic liver disease,cirrhosis and hepatocellular carcinoma are major global health problems (Zeeshan, etal, 2007).

Chronic hepatitis B virus (HBV) infection has a complicated course Three phases are identified: an immune tolerant phase with high HBV DNA and normal alanine aminotransferase (ALT) levels associated with minimal liver disease; an immune active phase with high HBV DNA and

elevated ALT levels with active liver inflammation; and an inactive phase with HBV DNA levels < 2000 IU/ ML and normal ALT levels with minimal inflammation and fibrosis on liver biopsy. Affected persons can move progressively from one phase to the next and may revert backward. The primary adverse outcomes of chronic HBV infection are hepatocellular carcinoma (HCC) and cirrhosis. Published natural history studies were reviewed and ranked by the strength of evidence regarding the study development of HCC or cirrhosis from population-based design. Factors with the highest evidence of risk for

Prospective cohort studies include male sex, family history of HCC, HBV DNA level above 2000 IU/ML in persons above age 40, HBV genotypes C and F, and basal core promoter mutation. Those with the next highest level of evidence include aflatoxin exposure, and heavy alcohol and tobacco use.

Improved methods to identify persons at highest risk of developing HCCor cirrhosis are needed to allow intervention earlier with antiviral therapy in appropriate patients(Coppeta, et al, 2019).

Future studies should include prospective follow-up of established population-based cohorts as well as new cohorts recruited from multiple centers stratified by HBV genotypes/sub genotypes and clinical phase to determine the incidence of the various HBV phases, HCC, and cirrhosis. Also, nested case-control studies assessing immunological and host genetic factors among persons with active and inactive disease phases, HCC, and cirrhosis could be condu chronic hepatitis B virus infection is a global public health threat that causes considerable liver-related morbidity and mortality. It is acquired at birth or later via person-to-person transmission Vaccination effectively prevents infection and chronic hepatitis B virus carriage. In chronically infected patients, an

elevated serum hepatitis B virus DNA concentration is the main risk factor for disease progression, although there are other clinical and viral parameters that influence disease outcomes. In addition to liver biochemistry, virological markers, and abdominal ultrasonography, non-invasive assessment of liver fibrosis is emerging as an important assessment modality(Coppeta, et al, 2019).

2.2. Prevalence

With almost 350 million people chronically infected with HBV worldwide, HBV infection is a major public health challenge. The majority of the infected cases are living in developing countries of sub-Saharan Africa. WHO estimates that about two million HCWs face occupational exposure to HBV each year and that 90% of the infections that result from these exposures are in low income countries, especially those in sub Saharan Africa (Ogoina,et al,2014).

The prevalence of HBV-related hepatitis varies across countries: in industrialized West European countries and North America, the prevalence of HBs-Ag positivity in the general population is less than 2% (low endemicity);in most countries of the Mediterranean, East Europe, and Asia it ranges between 2 –8%(intermediate endemicity);whereas it is over 8% in some developing countries in Far-East Asia and Sub-Saharan Africa (high endemicity), as shown in figure (2,1) (Coppola,et al, 2015).

The prevalence of chronic HBV infection varies greatly in different part of the world; it could be categorized as high, intermediate and low endemicity. The age at the time of infection is associated with the endemicity of HBV infection, the prevalence of HBV infection varies markedly throughout regions of the world, Hepatitis B is highly endemic in developing regions with large population such as South East Asia, China, sub-Saharan Africa and the Amazon Basin, where at least 8% of the population are HBV chronic carrier. In these areas, 70-95% of the

population shows past or present serological evidence of HBV infection. Most infections occur during infancy or childhood. Since most infections in children are asymptomatic, there is little evidence of acute disease related to HBV, but the chronic liver disease and liver cancer in adults are high (Jinlin,et al,2013).

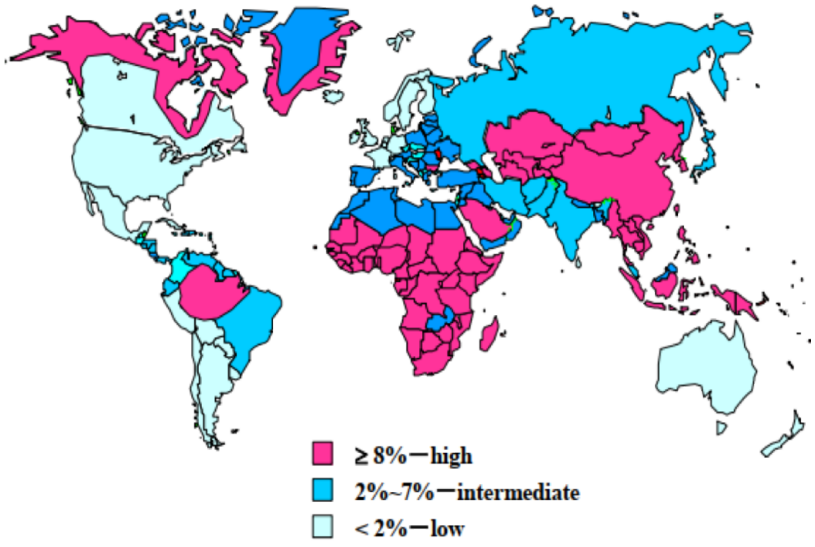


Fig 2.1: geographic distribution of chronic HB infection (jinlin,et al ,2013).

2.3. Viral genome structure

Studies have identified a minimum of eight HBV genotypes, designated A-H, with genetic differences greater than8%, but less than 17% between each genotype. Two additional potential genotypes have been described, genotype has genetic divergence around 21 with a strong homology to genotype C, making its classification as a distinct genotype more controversial than that of the more well-accepted genotypes. A potential 10th genotype, genotype J, has also been described recently and is likely

the result of recombination of genotype C and gibbon HBV (Lamontagne, et al, 2016).

Dane particle (42 nm), spherical (20 nm) and filamentous (22 nm) particles are three different viral structures that were observed in serum of HBV-infected patients by electron

Microscopy, all the three particles have a common HBs-Ag on their surface, the core region of Dane particle contains a small, circular, partially double-stranded DNA molecule and viral DNA polymerase that surrounded by nucleocapsid. Assembled HB c AG build nucleocapsid that is covered with a lipid envelope containing HBs-Ag, the nucleocapsid structure of HBV contains HBV genome with is 3, 2 kilo base (kb) in length and partially ds-relaxed circular DNA (rc DNA) molecule. Nucleocapsidis formed by composition of 240 viral capsid proteins with 27 nm diameter in an icosahedral structure, Andit contains single copy of viral genome DNA and viral polymerase enzyme covalently attached to 5' end of the negative chain, some cellular proteins including protein kinesis are also packed in nucleocapsid structure, one of unique characteristics of HBV genome is asymmetric structure of the two chains. Viral genome contains overlapping and open reading frames (ORF)for S, C, P, and X coding for four different proteins, overlapping structure of coding regions facilitates the use of HBV genome with 150% efficiency (Inan and Tabak, 2014).

Partially double-stranded genome is depicted with attached RNA primer and polymerase protein. Open reading frames (ORFs) are indicated by the thicker, colored lines. The outermost black circles represent the viral transcripts with the shared polyadenylation site; (B) schematic representation of the overlapping nature of the HBV ORFs; (C) the mature HBV virion consists of two main parts: a nucleocapsid (core) consisting of a partially DS-DNA genome bound to polymerase (P) and

encapsulated by dimmers of core protein, and a viral envelope consisting primarily of S-HBs-Ag (S), with an intermediate amount of M-HBs-Ag (M) and lower levels of L-HBs-Ag (L), as shown in figure (2,2) (Lamontagne, et al, 2016).

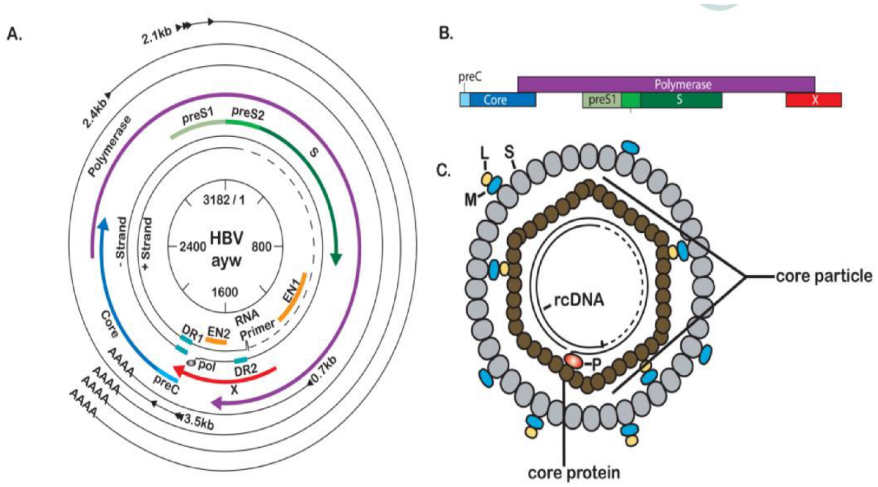


Fig. 2 .2: (B) HBV genome. (A) Internal circle shows genomic position relative (lamontagne, et al, 21016)

2.4. Replication

Cycle Hepatitis B virus (HBV), the causative agent of type B hepatitis in humans, is the prototypic member of the hepadnaviridae, a family of small enveloped DNA-containing viruses with pronounced host and tissue specificity. This property has greatly hampered progress in understanding the initial events of mechanisms of the late steps of the infectious cycle in some detail. During the last few years, such studies have emphasized the differences between hepadnaviral and retroviral replication. Very recent research, however, indicates that the border separating the two viral families may not be as strict as previously thought. In this article, we will briefly summarize the pertinent differences, and will then focus on the new data, with particular emphasis on the initiation of reverse transcription. (M Nassal, et al, 2010).

Hepadnaviruses, including human hepatitis B virus (HBV), replicate through reverse transcription of an RNA intermediate, the pregenomic RNA (pg RNA). Despite this kinship to retroviruses, there are fundamental differences beyond the fact that hepadnavirions contain DNA instead of RNA. Most peculiar is the initiation of reverse **transcription:**

It occurs by protein-priming, is strictly committed to using an RNA hairpin on the pgRNA, ε, as template, and depends on cellular chaperones; moreover, proper replication can apparently occur only in the specialized environment of intact nucleocapsids. This complexity has hampered an in-depth mechanistic understanding. The recent successful reconstitution in the test tube of active replication initiation complexes from purified components, for duck HBV (DHBV), now allows for the analysis of the biochemistry of hepadnaviral replication at the molecular level.Here we review the current state of knowledge at all steps of the hepadnaviral genome replication cycle, with emphasis on new insights that turned up by the use of such cell-free systems. At this time, they can, unfortunately, not be complemented by three-dimensional structural information on the involved components. However, at least for the ε RNA element such information is emerging, raising expectations that combining biophysics with biochemistry and genetics will soon provide a powerful integrated approach for solving the many outstanding questions. The ultimate, though most challenging goal, will be to visualize the hepadnaviral reverse transcriptase in the act of synthesizing DNA, which will also have strong implications for drug development (Beck, et al, 2007).

After liver cell membrane attachment to most likely cell associated heparin sulfate proteoglycans, the viral particle binds specifically to a Hepatocyte-specific pres% -receptor. Both, endocytosis and direct fusion of the viral envelope with the plasma membrane have been proposed as

potential pathways. After uncoating/release into the cytoplasm and transport of the nucleocapsid to the nucleus, the partially viral relaxed circular DNA (rc DNA) is repaired by both viral and cellular enzymes (Daniel and Robert, 2011).

Replication can broadly be divided into three phases

(1) Infectious virions, contain in their inner core the genome as a partially ds-circular but not covalently closed DNA (relaxed circular, or RC-DNA);

(2) upon infection, the RC-DNA is converted, inside the host cell nucleus, into a plasmid-like covalently closed circular DNA (cccDNA);

(3)from the cccDNA, several genomic and sub genomic RNAsare transcribed by cellular RNA polymerase II; of these, the pregenomic RNA (pgRNA) is selectively packaged into progeny capsids and is reverse transcribed by the co-packaged P protein into new RC-DNA genomes. Matured RC-DNA containing-but not immature RNA containing-nucleocapsids can be used for intracellular cccDNA amplification, or be enveloped and released from the cell as progeny virions,, as shown in figure (2,3) (Juergen,et al, 2010).

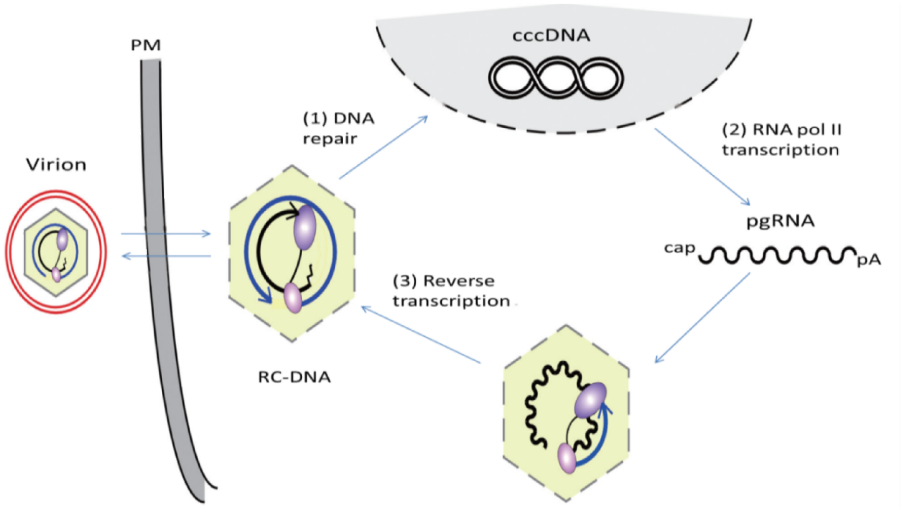


Fig. 2 .3: Replication cycle of HBV genome (Juergen, et al, 2010).

2.5. Pathogenesis

Viral hepatitis is a necro-inflammatory liver disease of variable severity. Persistent infection by HBV is often associated with chronic liver disease that can lead to the development of cirrhosis and hepatocellular carcinoma (HCC). Many studies suggest that HBV is not directly cytopathic for the infected hepatocyte. For example, during the early phase of HBV infection in chimpanzees (i.e., before virus-specific T cells enter the liver),

100% of the hepatocytes may be infected without histological or biochemical evidence of liver disease. Furthermore, when cellular immune responses are deficient or pharmacologically suppressed, HBV can replicate at high levels in the liver of patients [and in immunologically tolerant HBV transgenic mice] in the absence of cytological abnormalities or inflammation. (Chisari, et al, 2011).

Viral clearance and disease pathogenesis are largely mediated by the adaptive immune response in HBV infection .For HBV to persist it must either not induce a response or it Must overwhelm, evade or counteract it. Interestingly, HBV evades the innate immune response by simply not inducing it, acting as a stealth virus in this regard. On the other hand, viral persistence is characterized by a state of relative hypo-responsiveness of HBV specific T cells. Several viral proteins have been

shown to regulate the adaptive immune response to HBV (as described below) suggesting that HBV may employ active evasion strategies targeting the adaptive immune response. Indeed, it has been shown that antiviral treatment can overcome CD8+T cell hypo-responsiveness in chronic HBV infection, suggesting that the T cells are present in these subjects but suppressed.

Importantly, a recent study suggests induction of an effective HBV specificcd2+ T cell response is dependent on early CD4 + T cell priming which might be regulated by the size of the viral inoculums. (Chisari, et al, 2011).

The adaptive immune response is thought to be responsible for viral clearance and disease pathogenesis during hepatitis B virus infection. It is generally acknowledged that the humeral antibody response contributes to the clearance of circulating virus particles and the prevention of viral spread within the host while the cellular immune response eliminates infected cells. The T cell response to the hepatitis B virus (HBV) is vigorous, polyclonal and multi specific in acutely infected patients who successfully clear the virus and relatively weak. (francic, et al, 2010)The adaptive immune response is thought to be responsible for viral clearance and disease pathogenesis during hepatitis B virus infection. It is generally acknowledged that the humeral antibody response contributes to the clearance of circulating virus particles and the prevention of viral spread within the Host while the cellular immune response eliminates infected cells. The T cell response to the hepatitis B virus (HBV) is vigorous, polyclonal and multi specific in acutely infected patients who successfully clear the virus and relatively weak. (francis, et al, 2010).

2.6 .Clinical manifestation

Clinical manifestations approximately 70% of patients with acute HBV infection have subclinical or anicteric hepatitis, while 30% develop icteric hepatitis. The disease may be more severe in patients co-infected with other hepatitis viruses or with underlying liver disease. Fulminant hepatic failure is unusual, occurring in approximately 0, 1- 0, 5% of Patients. Fulminant HB is believed to be due to massive immune-mediated lysis of infected hepatocytes.

This explains why many patients with Fulminate HB have no evidence of HBV replication at presentation. The reasons why HBV has a fulminate course in some patients are not well understood (Wilkins, et al, 2010).

A case-control study evaluated risk factors for a fulminant course in an outbreak among injection drug users. Compared with control patients, case patients were more likely to have used acetaminophen during their illness (p = 0, 08), used more alcohol and methamphetamine, and lost more weight in the six months before illness. Furthermore, all nine isolates were genotype D. It is unclear whether viral or environmental factors led to the fulminant course in this outbreak, or if the risk factors identified in this outbreak can be generalized to acute HBV in other settings (Wilkins,etal, 2010).

Hepatitis B virus (HBV) infection is a serious public health problem worldwide. In the last few decades, major advances have been achieved that have contributed to greater understanding of the natural history and clinical manifestations of this infection. The fluctuation between viral replication and the host's immune response has implications in the pathogenesis and progression of the hepatic lesion. In immuno competent adults, most HBV infections resolve spontaneously in contrast with progression to chronic infection in most infants. Patients with chronic

hepatitis due to HBV or chronic hepatitis B can present at four phases: 1. the immune tolerance phas, 2.HBeAg-positive chronic hepatitis B, 3. inactive HBsAg carrier state, and 4. HBeAg-negative chronic hepatitis. HBeAg-positive or-negative chronic hepatitis can progress to cirrhosis, liver failure and hepatocellular carcinoma. Progression to these complications is more frequent in HBeAg-negative forms, associated with mutations that affect the pre-core region and maintain active viral replication. Risk factors are HBV-DNA positive serum levels, an increase in serum transaminase levels and some genotypes. These factors highlight the need to evaluate and monitor all HBV carriers to identify those who could benefit from early antiviral treatment, thus avoiding progression to more advanced forms of liver disease. These measures could improve prevention and treatment of hepatitis B (M Carneiro. et al. 2008).

Most people do not experience any symptoms when newly infected. However, some people have acute illness with symptoms that last several weeks, including yellowing of the skin and eyes (jaundice), dark urine, extreme fatigue, naus vomiting and abdominal pain. People with acute hepatitis can develop acute liver failure, which can lead to death. Among the long-term complications of HBV infections, a subset of persons develops advanced liver diseases such as cirrhosis and hepatocellular carcinoma, which cause high morbidity and mortality. (Who. https, 2022).

2.7. Transmission

HBV is spread through contact with infected body fluids and the only natural host is human. Blood is the most important vehicle for transmission, but other body fluids have also been implicated, including semen and saliva, currently, three modes of HBV transmission have been recognized: perinatal, sexual and parenteral/percutaneous transmission. There is no reliable evidence that airborne infections occur and feces are not a source of infection. HBV is not transmitted by contaminated food or

water, insects, or other vectors. The risk of chronicity is low (<5%) for transmission through sexual contact, intravenous drug use, acupuncture, and transfusion, individuals at risk for these transmission modes usually acquire HBV infection during adolescence or adulthood without immune tolerance. Instead, the disease progresses directly to the immune clearance phase and is of short duration, which probably accounts for high spontaneous recovery(Jinlin, et al, 2013).

The virus owes its remarkable diffusion to its environmental resistance (about 7 days at room temperature) (Cristina, et al, 2017).

HBV is carried in blood and other body fluids, including (saliva, tears, semen and vaginal secretions (Franco,et al,2012).

2.7.1.Horizontal transmission

Meta-analysis of seroprevalence surveys shows that horizontal transmission of hepatitis B virus (i.e., that occurring without apparent parenteral, sexual, or prenatal exposure) is common in areas endemic for the virus. It occurs especially in pre-adolescent children. In developed countries, where endemicity of hepatitis B virus is low, horizontal transmission (probably via saliva or open wounds) may occur in households with a persistent carrier, but it is less efficient a means of infection than is sexual or prenatal transmission. Horizontal transmission also seems possible in pre-school day-care centres in developed countries, despite the small numbers of carriers in such places (L Gray Davis. et al. 2011).

2.7.2Vertical transmission

More than 240 million people worldwide are chronically infected with hepatitis B virus (HBV). Mother-to-child transmission remains the most important mechanism of infection in countries with a high prevalence of HBV. Universal screening of all pregnant women, at-birth prophylaxis with specific anti-HBV immune globulin, as well as HBV vaccination for

newborns of infected mothers are effective in reducing the risk of vertical transmission. However, in cases of a high viral load and hepatitis B e antigen positivity, there is a residual risk of HBV transmission to the newborn despite prophylaxis. This review focuses on the above-indicated strategies and on the efficacy and safety of antiviral drugs administered during the third trimester of pregnancy (Ivan Geneital. et al. 2014).

Mother-to-child transmission (MTCT) of HBV is responsible for approximately half of the HBV transmission routes and continues to be a challenging problem worldwide. Even after the development of effective vaccines and clear World Health Organization guidelines toward HBV several decades ago, 1–9% newborns of HBV-carrying mothers still acquire HBV in early life as a result of in utero infection. The prevention of MTCT is of high importance, because chronically infected individuals function as a reserve for sustained HBV transmission, and 25% of them can develop asymptomatic liver cirrhosis and hepatocellular carcinoma. In this article, we review the canonical and novel HBV infection routes/mechanisms, influencing factors, diagnostic criteria, and interruption strategies for HBV MTCT. The preventative strategy of HBV MTCT has evolved from routine postpartum HB immune globulin (HBIG) plus HB vaccine schedules to Administration of HBIG or nucleoside analogs during pregnancy and minimizing the exposure of maternal body fluids to the newborn during delivery.(Lin Ma, et al, 2014) Chronic hepatitis B virus (HBV) infection caused by mother-to-child transmission (MTCT, also known as vertical transmission) during the prenatal period is a major public health problem worldwide. Despite the availability of the combined active-passive immunization with a hepatitis B vaccine and Hepatitis B immunoglobulin after birth, about 9% of newborns are still infected with HBV, especially those born to hepatitis B e antigen (HBe AG)-positive mothers. Currently, the management of HBV infection

during pregnancy remains controversial. This article briefly reviews the recent advances in the epidemiology of HBV, immunization against it, and management strategies in the third trimester (Yao Hu, et al, 2020).

2.7.3. Experimental transmission

The ability of human semen and saliva to transmit hepatitis B virus (HBV) by parenteral and nonparenteral routes was studied. Semen, donated by a hepatitis B surface antigen (HBsAg)- and hepatitis B e antigen (HBeAg)-positive carrier, was administered to one gibbon by subcutaneous inoculation and to another by intravaginal instillation. Both developed HBsAg, followed by the development of antibody to HBsAg (anti-HBs) and antibody to hepatitis B core antigen (anti-HBc). Saliva from two donors who were HBsAg- and HBeAg-positive was pooled and administered subcutaneously to two gibbons and orally to five others. The animals inoculated subcutaneously developed HBsAg followed by anti-HBs, but none of the gibbons exposed orally developed evidence of HBV infection. Thus, semen and saliva of HBsAg carriers can be infectious, and venereal transmission of HBV by semen can occur. Transmission of HBV in saliva can also occur through breaks in the skin, but experimental transmission of HBV by saliva administered orally has not been accomplished. (Scott, et al. 2013)

2.7.4. Endoscopic transmission

Although transmission of hepatitis B virus (HBV) infection has long been recognized as a potential hazard of gastrointestinal endoscopy, there has been little evidence of direct patient-to-patient cross-infection after such procedures. We wish to report a case of type B viral hepatitis almost certainly acquired at endoscopy from an instrument sterilized in the conventional manner, but which had been used on the previous day on a patient with bleeding esophageal varies who was incubating type B viral hepatitis. (Birnie, G. G, et al, 2012).

2.8.The Role of immune response in HBV

Hepatitis B virus (HBV) infection has a low rate of chronicity compared to HCV infection, but chronic liver inflammation can evolve to life threatening complications. Experimental data from HBV infected chimpanzees and HBV transgenic mice have indicated that cytotoxic T cells are the main cell type responsible for inhibition of viral replication, but also for hepatocyte lysis during chronic HBV infection. Their lower activation and impaired function in later stages of infection was suggested as a possible mechanism that allowed for low levels of viral replication. The lack of an interferon response in these models also indicated the importance of adaptive immunity in clearing the infection Increased knowledge of the signaling pathways and pathogen associated molecular patterns that govern activation of innate immunity in the early stages of viral infections in general has led to a re-evaluation of the innate immune system in HBV infection Numerous studies have shown that HBV employs active strategies to evade innate immune responses and induce immunosuppression. Some of the immune components targeted by HBV include dendritic cells, natural killer cells, T regulatory cells and signaling pathways of the interferon response. This review will present the current understanding of innate immunity in HBV infection and of the challenges associated with clearing of the HBV infection. The role of innate immunity in controlling HBV replication has been neglected for a number of years due to results from studies with HBV infected animals that failed to detect induction of type 1 interferon responses following HBV challenge The ability of antibody responses to HBV surface antigen to protect adults from HBV challenge also indicated the importance of adaptive immunity in controlling viral replication. However, adaptive immunity alone cannot explain the persistence of HBV during chronic

infection the immune pathogenesis that characterizes chronic hepatitis B .(Kumar A. et al. 2014) .

A better understanding of how the immune systems works has lead to a reconsideration of the role of innate immunity in HBV infection. Recent research studies mentioned in this review have shown a different image of HBV pathogenesis, with active mechanisms aimed at inactivating various components of the innate immune system. Thus, the lack of interferon responses during acute infection seems to be the result of inactivation of various signaling pathways that normally induce IFN production in other viral infection. HBx and HBV polymerase are the proteins associated most frequently with inactivation of the TLR and RIG-I pathways and ultimately with impaired IFN production. These mechanisms may constitute important factors of viral persistence during natural infection, since in vitro studies have shown that HBV is sensitive to antiviral properties of IFNs. During the course of infection HBV also contributes to a sustained immunosuppressive state that may favour its replication, since the number of Tregs increase as disease progresses, which also decreases the number and function of HBV specific T lymphocytes.

Other components of the innate immune system that are targeted by the virus are DCs, NK and NKT cells. Their activation during acute infection or immunization is linked to a favourable clinical outcome and subsequent robust adaptive immune responses. By disrupting innate immunity pathways early during infection, the virus also compromises the quality of adaptive immune response, in favour of its survival. The interplay between innate and adaptive immunity for successful control of HBV infection is complex and needs to be addressed in future therapeutic approaches aimed at controlling viral replication.(Kumar A. et al. 2014) .

Innate and Adaptive Immune Response against HBV Infection Innate immune response is important in the early management of HBV infection and limits the disease at initial stage; later, it helps in generating a proficient adaptive immune response that clears the infection. PRRs recognize different viral components including envelope proteins, nucleocapsid, nucleic acids, and specific viral structures that activate immune cells and signaling pathways to encourage the production of pro-inflammatory cytokines, chemokines, and interferons Both circulating as well as the intrahepatic innate immune system can sense and respond to HBV infection. However, robust immune response also leads to hepatic Necro-inflammation causing severe liver damage. Numerous innate immune cells including dendritic cells (DCs), macrophages, monocytes, natural killer (NK) cells, myeloid derived suppressor cells (MDSCs), and innate lymphoid cells (ILCs) play a protective as well as pathogenic role during chronic HBV infection. Immediately after a pathogen encounter, activation of the innate immune system occurs that is necessary for the recruitment and activation of adaptive immunity.

Adaptive immune systems act through the expansion and functional maturation of discrete T and B cell subsets that particularly recognize and kill HBV infected hepatocytes; a process that induces hepatic inflammation.

Persistent exposure of viral factors, including hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), and hepatitis B x antigen (HBx), leads to immune exhaustion and subsequent downregulation of host response by setting up chronic infection.

HBsAg is present on the surface of HBV and is responsible for binding and entry into hepatocytes. HBsAg can be detected in the blood after several weeks of infection and its presence indicates that the patient has contracted the infection. Production of anti-HBs antibodies is critical for

viral clearance, long term protection, and defines functional cure whereas, HBeAg is regarded as an accessory protein as it is not required for the viral genome replication. It can be found between icosahedral nucleocapsid core and the lipid envelope (the outermost layer of HBV) and its existence designates active viral replication (Khanam A. et al. 2021).

HBeAg exerts its immunoregulatory effect by eliciting tolerance in hepatitis B core antigen (HBcAg)/HBeAg-specific T cells HBcAg is located at the surface of the nucleocapsid core (the innermost layer of the HBV).

Presence of anti-HBc antibodies reflect past or current HBV infection and these antibodies appear within a few days of infection; however, do not provide any protection against HBV, unlike the surface antibody Moreover, HBxAg stimulates virus gene expression and replication and is crucial for the establishment and maintenance of chronic carrier state. Intrahepatic inflammatory reactions lead to the induction of several suppressive pathways and subsequent recruitment of regulatory cells that drive functional demolition of T cell.

These cells start overexpressing inhibitory receptors that further dampen their functional status causing immune exhaustion, resulting in viral persistence and further disease progression Concisely, both innate and adaptive immune systems work synergistically to cause immune related pathologies in CHB. (Khanam A. et al. 2021).

2. 9.Laboratory Diagnosis

When HBV infection is suspected, it is the appearance of serum markers of HBV infection that establishes the diagnosis of the disease. Of these, HBs-Ag is considered to be the sentinel marker for the confirmation of acute infection. Its presence can be detected as early as 6 weeks after exposure, and should therefore be assessed when prodromal (malaise,

anorexia, fever, rash, arthralgia) or more classical symptoms (dark urine, jaundice) are observed. Generally, some 8 to 16 weeks will usually have elapsed from exposure to the virus until the appearance of telltale symptoms.

Hbs-Ag is also the key marker in determining whether HB infection has become chronic: defined as the persistence of the surface antigen for at least 6 months. By contrast, if acute disease resolves, HBs-Ag declines followed by a subsequent rise of (anti-HBs) upon recovery, as shown in figure (2,4)(Sablon and Shapiro, 2014).

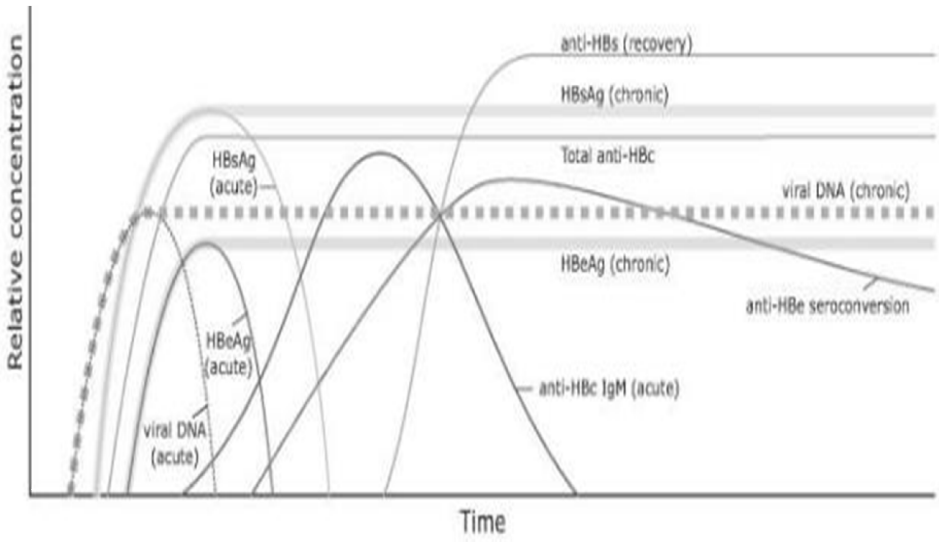


Fig. 2 .4: Evolution of HBV markers in acute and chronic infection (SablonandShapiro, 2014).

The diagnosis of HBV infection requires the evaluation of the patient's blood for hbs-Ag,HBs-Ab, and HBcab. Although the presence of hbs-Ag indicates that the person is infectious, the presence of HBs-Ab indicates recovery and immunity from HBV infection or successful immunization against HBV.

Hbcabappears at the onset of acute HBV infection, but may also indicate Chronic HBV infection. Interpretation of HBV immunologic markers

HBV DNA sometimes may be the only marker present in early infections (Wilkins, et al, 2010).

Hepatitis B virus (HBV) reactivation under systemic chemotherapy or immunosuppressive therapy is a serious complication among HBV-resolved patients. Some medications, such as more than 2 weeks of corticosteroid therapy, can influence HBV reactivation; therefore, screening tests that measure hepatitis B surface antigen (HBsAG), hepatitis B core antibody, and hepatitis B surface antibody before therapy are required. Additionally, because HBV reactivation has been reported in patients positive for HBsAGtreated with immune checkpoint inhibitors (icis), the prophylactic administration of nucleos(t)ide analogues prior to administering icisis recommended for HBsAG-positive patients. Under these circumstances, highly sensitive novel biomarkers are expected to be used for the early diagnosis of HBV reactivation. A fully automated high-sensitivity HBsAGassay (detection limit: 5 miu/ml) by LumipulseHbsag-HQ, with 10-fold higher sensitivity than that of conventional assays, is currently used. Furthermore, ultra-sensitive HB sagassays using a semi-automated immune complex transfer chemiluminescence enzyme immunoassay (ICT-CLEIA; detection limit: 0.5 miu/ml) have been developed. Recently, a fully automated, novel high-sensitivity hepatitis B core-related antigen assay (itact-hbcrag; cut-off value: 2.1 Log U/ml) has been developed and reported. The utility of ICT-CLEIA and itact-hbcragfor the diagnosis of HBV reactivation appears comparable to the use of HBV DNA. In this review, we provide the latest information related to medications that influence HBV reactivation and recently developed novel biomarkers that predict and monitor HBV reactivation (Takakoinoue, et al, 2021).

2.10. Treatment

Five oral nucleotide reverse transcriptase inhibitors were approved for the treatment of HBV infection. These medications require renal function monitoring. If HBV DNA levels do not become undetectable within six to 12 months, a second antiviral agent should be used. The incidence of sero conversion increases in a stepwise fashion with ongoing treatment and with the duration of undetectable HBV DNA levels. After three years of therapy with oral antiviral agents, the incidence of sero conversion approaches that of 12 months of therapy with pegylated interferon alfa-2 a. Oral therapy should be continue for at least an additional six months once sero conversion is achieved. If sero conversion does not occur, treatment should be continuing. Regardless of patient sero conversion status, HBV DNA and liver enzyme levels should be monitored, and therapy should be reinitiated if needed (Wilkins, et al, 2010).

For the 350 million persons chronically infected with HBV, the two therapeutic approaches presently available to control infection and its sequelae are immune modulatory agents and/or antiviral chemotherapy. Such treatment saiat interrupting the progression and clinical outcomes of the disease (cirrhosis, hepatocellular carcinoma) by stimulating the anti-HBV-specific host immune response or by markedly decreasing viral replication (Sablon and Shapiro, 2014).

2.10.1.Treatment to prevent hepatitis B infection after exposure

If you know you've been exposed to the hepatitis B virus, call your health care provider immediately. It is important to know whether you have been vaccinated for hepatitis B. Your health care provider will want to know when you were exposed and what kind of exposure you had.

An injection of immunoglobulin (an antibody) given within 24 hours of exposure to the virus may help protect you from getting sick with hepatitis B. Because this treatment only provides short-term protection,

you also should get the hepatitis B vaccine at the same time if you never received it. (Mayoclinic. https. 2022).

2.10.2Treatment for acute hepatitis B infection

If your provider determines your hepatitis B infection is acute meaning it is short lived and will go away on its own you may not need treatment. Instead, your provider might recommend rest, proper nutrition, plenty of fluids and close monitoring while your body fights the infection. In severe cases, antiviral drugs or a hospital stay is needed to prevent complications. (Mayo clinic. https. 2022).

2.10.3Treatment for chronic hepatitis B infection

Most people diagnosed with chronic hepatitis B infection need treatment for the rest of their lives The decision to start treatment depends on many factors, including if the virus is causing inflammation or scarring of the liver, also called cirrhosis; if you have other infections, such as hepatitis C or HIV; or if your immune system is suppressed by medicine or illness. Treatment helps reduce the risk of liver disease and prevents you from passing the infection to others.

2.10.4Treatment for chronic hepatitis B may include

Antiviral medications

Several antiviral medicines including entecavir (Baraclude), tenofovir (Viread), lamivudine (Epivir), adefovir (Hepsera) and telbivudine can help fight the virus and slow its ability to damage your liver. These drugs are taken by mouth. Your provider may recommend combining two of these medications or taking one of these medications with interferon to improve treatment response.

2.10.5.Interferon injections

Interferon alfa-2b (Intron A) is a man-made version of a substance produced by the body to fight infection. It's used mainly for young people with hepatitis B who wish to avoid long-term treatment or women who

might want to get pregnant within a few years, after completing a finite course of therapy. Women should use contraception during interferon treatment. Interferon should not be used during pregnancy. Side effects may include nausea, vomiting, difficulty breathing and depression.

2.10.6Liver transplant

If your liver has been severely damaged, a liver transplant may be an option. During a liver transplant, the surgeon removes your damaged liver and replaces it with a healthy liver. Most transplanted livers come from deceased donors, though a small number come from living donors who donate a portion of their liver. (Mayoclinic.https.2022).

2.11. Prevention

Prevention of primary infection by vaccination is an important strategy to decrease the risk of chronic HBV infection and its subsequent complications. The first-generation hepatitis B vaccine, an inactive plasma-derived vaccine, became available in1982.Consequently, the second generation of HB vaccine; a DNA recombinant HB vaccine was also available for general use in 1986. Both of the vaccines were proven to be safe and efficacious in preventing HBV infection. In 1991, WHO recommended that HB vaccination should be included in national immunization system in all countries with a HB carrier prevalence (HBs-Ag) of 8% or greater by 1995 and in all .countries

By May 2002, 154 countries had routine infant immunization with hepatitis B vaccine. Hepatitis B Immune Globulin (HBIG) is a sterile solution of ready-made antibodies against hepatitis B. HBIG is prepared from human blood from selected donors who already have a high level of antibodies to hepatitis B and used in passive immunoprophylaxis Changes in sexual practice and improved screening measures of blood products have reduced the risk of transfusion-associated hepatitis. Behavior modification is thought be more beneficial in developed

countries than in developing countries, where neonates and children in early childhood are at the greatest risk of acquiring infection. In these groups, immunoprophylaxis, both passive and active, will be more effective (Jinlin, et al, 2013)

2.12. Vaccination

Vaccination is the most effective means of preventing hepatitis B, cirrhosis and hepatocellular carcinoma worldwide, the first vaccines, available between1981 and 1982, were produced by harvesting the hepatitis B surface antigen from plasma of chronic HBs-Ag carriers and contained highly purified 22 nm HBs-Ag particles inactivated through a combination of urea, pepsin, formaldehyde and heat These immunogenic plasma derived vaccines have been used with success in several hundred million individuals and are still produced in Asia and used in a number of countries(Franco,et al, 2012).

HBs-Abs decrease over time following hepatitis B immunisation; hence, it is unclear whether people vaccinated in 3-dose or 4-dose schedules of the hepatitis B vaccine during their primary vaccination are still immune when the HBs-Abs level in their body is undetectable, or lower than the level usually considered protective (Poorolajal and Hooshmand, 2016).

2.12.1. Hepatitis B Vaccine

HBV vaccine contains recombinant HBs-Ag, derived from yeast cells, adsorbed onto aluminum hydroxide adjuvant. The vaccine is effective at preventing infection in individuals who produce specific antibodies to HBs-Ag (anti?HBs).EMI Guidelines, the first HBV vaccine (a heat-treated form of HBV) was developed by Blumberg and Millman in1996.(Das,et al, 2019).

In the late 1970s, two vaccines against HBV were developed in the United States and France, both containing purified HBs-Ag obtained from serum of HBs-Ag carriers. These plasma-derived vaccines contained

HBs-Ag that had been subjected to a combination of aggressive biophysical and biochemical treatments, which led to partial disruption of the surface antigen. The final purified HBs-Agwas subjected to formaldehyde treatment and adsorbed to alum. A United States product contained 22 nm HBs-Ag particles devoid of the pre-S proteins while a French HBV vaccine contained additional small and inconsistent amounts of pre-S2 and pre-S1 antigens. Later, similar vaccines were produced in Korea and China. Plasma derived vaccines have been shown to be highly immunogenic, efficacious, and safe,HB vaccines have now been available for over 20 years.

WHO has recommended that HB vaccination should be included in routine immunization for all children, worldwide As a result, various immunization strategies have been developed for routine infant vaccination, prevention of perinatal transmission and catch-up vaccination for older age groups. Knowledge of the structure and genomic organization of the HBV, has led to the development of immunogenic HBV vaccines with an excellent record of safety and immunogenicity. The purpose of this short overview is to summarize the Structure and properties of available licensed HBV vaccines relevant to the hepatitis B consensus conference and to discuss potential applications for some of the newly developed vaccines (Shouval, 2011).

Worldwide, universal HBV vaccination is administrated in infancy, but this practice produces lower immunogenicity than vaccination in adolescence or adulthood (Coppola, et al, 2015) Immune response to HBV vaccine is assessed by measuring antibody level after 6–8 weeks of completion of 3doses.HBs-Abs higher than10 mIU/ml is generally taken to be protective. Factors associated with decreased immune response include increasing age, smoking, obesity, gender and genetic factors (Zeeshan, et al, 2007).

It is a major public health challenge in the world infecting more than 66,000health professionals each year, vaccination against Hepatitis B saves the lives of these health professionals Around 45% of the global population live in high HBV infection prevalence (> 8%) areas Acute HBV has a case fatality rate of 0,5–1% Worldwide, 2 billion people have evidence of past or present infection with HBV, and 360 million are chronic carriers of HBs-Ag,and more than 686,000 people die each year from its complications. Overall, HBV infection reported more in lower and middle income countries causing a significant economic burden in terms of years of life lost (Abiye, et al, 2019) .

Hepatitis B virus (HBV) immunization is safe and has been accepted worldwide as a routine practice. The target of such vaccination is to induce the immune response in the host, resulting in the prevention of replication of HBV. There are several immunological and clinical factors which determine the clinical efficacy and safety of the HBV vaccine. In this article we have highlighted the response of the host immune system to HBV vaccination (immunogenicity), efficacy, and safety of the vaccine, issues with booster dosing, paths of development (preclinical and clinical) of the HBV vaccine, novel and upcoming strategies for improvement of HBV vaccination, and the concept of therapeutic HBV vaccination. The different aspects and regulatory recommendations pertaining to HBV vaccine development are also discussed. The new strategies for improvement of HBV vaccination include pre-S1 and pre-S2 portions of the HBV surface antigen, increasing the antigen dose,

The Accelerated vaccination schedules, alternative vaccination route, use of adjuvants like immunostimulatory DNA sequences, etc. Therapeutic vaccination is being explored for initiation of a multifunctional and multispecific T cell response against the major HBV antigens and also

effective activation of humoral immunity for viral control.(Saibal. D, et al, 2019).

United States Food and Drug Administration approved a plasma derived HBV vaccine produced by Merck Pharmaceuticals in 1981 that involved inactivation of viral particles in the blood which had been collected from HBs?Ag-positive donors. In 1986, the subsequent generation of genetically engineered (or DNA recombinant) a highly purified HBV vaccine was synthetically prepared without containing any of the blood products. In the present time, all recombinant vaccines which contain HBs-Ag are expressed in yeast Saccharo mycescerevisiae, Hansenula polymorpha, Pichiapastoris or mammalian (Chinese hamster ovary) cells (Das,et al, 2019).

Existing HB vaccines use an aluminum adjuvant, On November 9,2017HeplisavB (HepB-CpG), a single-antigen HB vaccine with a novel immune stimulatory sequence adjuvant, was approved by the Food and Drug Administration for the prevention of HBV in persons aged ≥18 years .

The vaccine is administered as 2 doses, 1 month apart, on February 21, 2018, the Advisory Committee on Immunization Practices (ACIP) recommended HepB?CpG for use in persons aged ≥18 years. HepB-CpG contains yeast-derived recombinant HepB surface antigen (HBs-Ag) and is prepared by combining purified HBs-Ag with small synthetic immune stimulatorycy tidine-phosphate guanosineoligodeoxy nucleotide (CpG-ODN) motifs (1018 adjuvant). The1018adjuvant binds to Toll-like receptor 9 to stimulate a directed immune response to HBs-Ag,HepB-CpG is available in single-dose 0,5 mL vials. Each dose contains 20 μg of HBs-Ag and 3,000 μg of 1018 adjuvant. HepB-CpGis formulated without preservatives and is administered as an intramuscular injection in the

deltoid region of the upper arm,HepB-CpG is the fifth inactivated HepB vaccine

Currently recommended for use in the United States(Schillie, et al, 2018)

Post-vaccination testing

Studies have shown that the vaccine induced antibody persists over periods of at least 10-15 years and that the duration of anti-HBs is related to the antibody peak level achieved after primary vaccination. Follow-up of those vaccinated has shown that the antibody concentrations usually decline over time but clinically significant breakthrough infections are rare. (Franco, E,et al, 2012).

Booster

The term 'booster' (or revaccination) refers to an additional dose of hepatitis B vaccine (HBV) given some time post-primary vaccination to induce immune memory and improve protection against HBV infection. (Poorolajal and Hooshmand, 2016).An administration of the booster dose is strongly recommended for HCWs with anti-HBs <10 mIU/mL if lacking certification of protective antibody titers, and anti-HBs dosage one month after booster administration should be performed (Coppeta,et al, 2019).

2.13. Immunization and health care Workers

In 1982, the Advisory Committee on Immunization Practices (ACIP) recommended hepatitis B vaccination for infants born to HBs-Ag-positive mothers and certain high-risk adult populations. Initial strategies for preventing HBV infection focused on 3-dosevaccination of high-risk groups: health care personnel, men who have sex with men (MSM), injection drug users (IDU) and recipients of certain blood products.

In 1991, the ACIP recommended that all infants be immunized with three doses of HB vaccine and the ACIP recommendation for high-risk adult populations was broadened to include international travelers to countries

with high or intermediate HBV endemicity or persons working in countries with high or intermediate HBV endemicity.

In 2006, ACIP further expanded the adult hepatitis B vaccination recommendations to include universal vaccination of unvaccinated adults attending certain healthcare and treatment settings that serve high-risk adults including sexually transmitted disease (STD) clinics, HIV counseling and treatment centers, correctional facilities, drug-abuse treatment centers, and healthcare settings with services targeting MSM(Lua,P,etal,2018).

Immunization of HCWs and medical students for the HBV is a crucial part of the hospital infection control programs, HCWs are considered to be a population at high-risk to develop HBV infection due to the high transmissibility of the virus and the risk related to occupational injuries (Coppeta, et al, 2019).

Studies in HCWs with percutaneous exposure to blood contaminated by HB virus suggest the risk of transmission is <30% if the source patient is HBs-Ag and HBeAg positive, and <6% if HBeAg is negative. HCW are one of the main groups at risk of HB infection. The risk is particularly high in HCW with greater exposure to accidental work and is related to the duration of professional activity (although it is higher during training), the characteristics of the healthcare center and the type of population served. Infected HCW are also a potential source of infection of the patients they care for. HB vaccination is the best tool for the primary prevention of HB infection. For these reasons, HCW were one of the first risk groups in whom vaccination was recommended (Dom-ınguez,et al, 2017).

2.14 previous studies

In Khartoum, Sudan by(Shamsoun K, 2013)

In this study a total of 90 individuals previously vaccinated with HBV found that 68.٦% of study populations have protective level of HBs-Ab while 31.4% of them have not protective level of HBs-Abs.

In Ethiopia by (Abebaw TA, 2017)

A total of 410 HCWs were enrolled in the study 96.9% of study populations have protective level of HBs-Ab while 3.1% of them have not protective level of HBs-Ab.

In pakistan, by (Mohammed Z, 2007)

Atotal of 666 HCWs were enrolled. in this study 86%of study populations have protective level of HBs-Ab while 14%of them have not protective level of HBs-Ab.

In Khartoum, Sudan, by (Abdullahi M, 2020)

A total of 106 HCWs enrolled in this study 73% of study population have protective level of HBs-Ab while 27% of them have not protective level of HBs-Ab.

Chapter Three

3. Materials and Methods

Materials:

3.1. Study design: The current is descriptive cross-sectional laboratory based study.

3.2. Study area : The study was conducted in different Khartoum State hospitals(Ebn seena, Ahmed gasem, Algooda, Alturki, Ebraheam malik, Elsaha, Royalcare, Bahri elaskari, Elzitoona, Fedail).

3.3 .Study duration : study conduct during the period from December 2021 to 2024.

3.4. Study population: Health care workers(Labtechnologists, Doctors, Nurses, Dentits, Midwifes) .

3.5. Inclusion criteria: Vaccinated HCWs, with Negative HBs-Ag, who completed the HBV vaccine doses.

3.5. Exclusion criteria: Vaccinated HCWs with positive HBs-Ag and non complete vaccine doses.

3.7. Ethical Considerations: The study was endorsed by the ethical review committee of shandi University (Faculty of Medical Laboratory Sciences).

Verbal consent was obtained from every participant before collecting data and clinical specimens.

Methods:

3.8. Data collection: Data were collected through a structured questionnaire; information regarding age, gender, type of employment, length of employment and duration of vaccination were recorded for each participant. Serum was collected from each participant and tested with ELISA titre for detection of immunization level.

3.9. Laboratory methods

3.10 .Specimen collection: About 3 ml of whole blood samples was collected from each participant under a hygienic conditions, serum was obtained and stored at -20 C° before testing.

Detection of : Ag-HBs

HBs-Ag was detected using ICT according to the manufacturer's protocol.

3.10.1. Measure Anti -HBs level using ELISA technique

Assay principle: For detection of antibodies, this kit uses antigen Sandwich ELISA (Precheck, United Kingdom), where polystyrene microwell strip precoated with recombinant HBs-Ag, patient serum is added to the micowell together with second HBsAg conjugated to horseradish peroxidase, in case ofpresent of HBs-Abs in the sample, the pre-coated and conjugated antigens will bound to the two variants domain of antibody and during incubation the immune complex formed is captured in the solid phase after washing to remove unbound

HRP conjugates, chromogen is added to wells, in the presence of antigenantibody complex, the colorless chromogen hydrolyzed to blue colored product then turn to yellow after adding stop solution sulfuric acid, wells contain samples negative to anti-HBs remain colorless.

Assay procedure: All regents and samples were allowed to reach room temperature, 50 ul of samples,calibrationcurvestandar dwells, were transferred in to their respective wells, and 50 ul of HRP conjugate to each well except the blank well then incubated for 60 min at 37 C, then the reagent wells were washed 5 times with diluted washing buffer, the wash buffer in each well was left for 30 to 60 seconds per washing. After washing, all liquid from the microplate were disposed by tapping it on absorbent paper with the openings facing downwards to remove all the residual washing buffer, 50ul of substrate A and B were pipetted to each well and incubated 10 min at 37C (protect from direct sun light).

50ul of stop solution was pipetted into each of the microplate Wells in the same order, and at the same speed as the Chromogen/substrate solution introduced. After that the photometric measurement of the color Intensity was made at a wavelength of 450 nm within 30min of adding the stop solution, prior to measuring micro plate was slightly shake to ensure homogeneous distribution of the solution.

3.11. Data analysis: Data was analyzed using SPSS. Descriptive statistics in terms of frequency tables with percentage and, graphs.

Tables presented with relevant graphical representation for quantitative data chi-square tests were used for statistical associations (P.value≤ 0.05 was considered significant).

Chapter Four

4. The Results

The current is descriptive cross sectional laboratory based study recruited Ninety HCW from different Khartoum state medical centers and hospitals during the period from December 2021 to 2024,to estimate HBs-Ab levels in their plasma using ELISA test, beside. (22%) of them were males while the rest (78%) were females as show in figure (4.1).

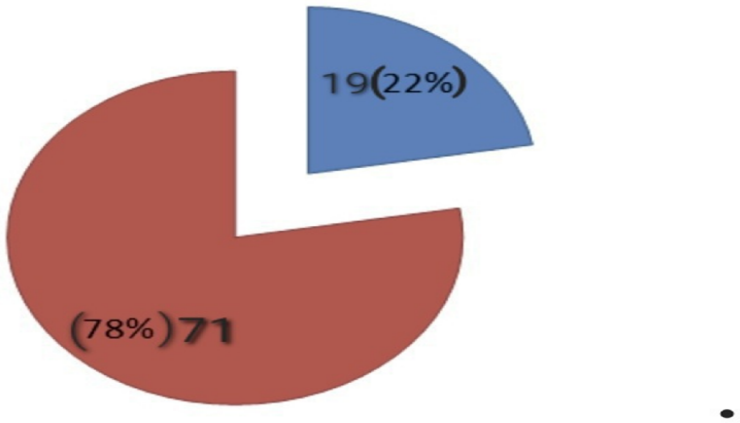


Figure (4.1): distribution of 90 HCWs according to their Gender

According to type of job, HCWs divided into five groups, 24.4%of them are doctors, 48.9%Are lab technologists, 18.9% are nurses, 2.2% are midwifes and 5.6% are dentists, as show in Table (4.1).

Table(4.1)Distribution of HCWs according to their jobs

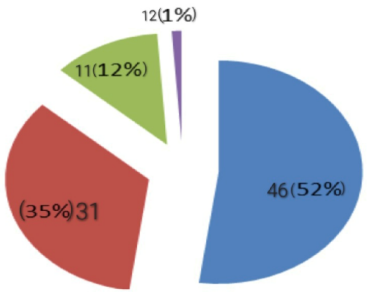
Jobs	Distribution
Lab technologists	**44(38.9%)**
Doctors	**22(24.4%)**
Nurses	**17(18.9%)**
Dentists	**5(5.6%)**
Midwifes	**2(2.2%)**
TOTAL	**90(100%)**

Most of HCWsemployment duration was less than 5 years 54.5%, 43.3% of them length working period were 5- 30 years and 2.2% of them length working period were more than 30 years, as show in Table (4.2)

Table (4.2) Employment duration distribution among 90 HCWs

Employment duration	distribution
<5 years	**49(54.5%)**
5 – 30 years	**39 (43.3%)**
>30 years	**2(2.2%)**
Total	**90(100%)**

We categorized the study population according to vaccination duration to four ranges, vaccination duration <5 years was 52.2%, 5-10 years was 34.5%, 11-15 years were 12.2%, and < 15 years were 1.1%as show in figure (4.2)



l

Figure (4.2) Vaccination duration among 90 HCWs

Generally, the study found that 70% of study populations have protective level of HBs-Ab while 30% of them have not protective level of HBs-Abs, as show in figure (4.3)

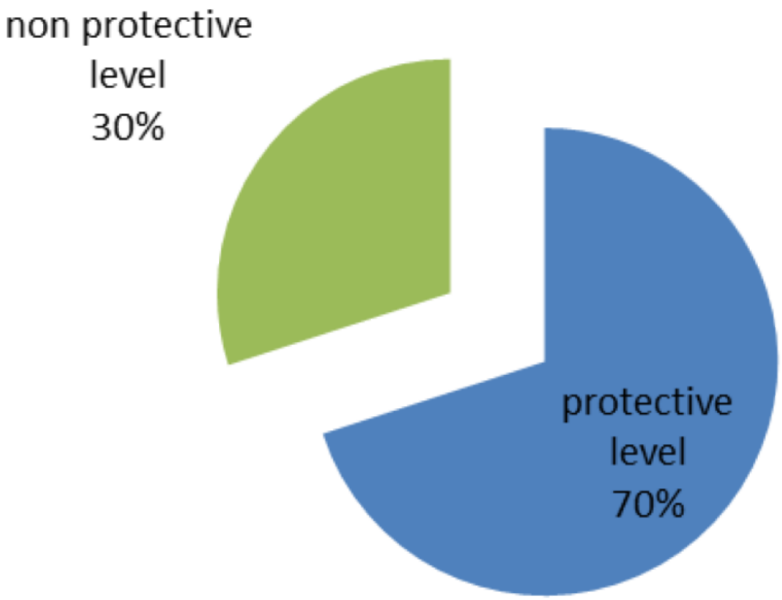


Figure (4.3) HBs-Abs level distribution among 90 HCWs

Table (4.3) and figure (4.4): Show the association between immunization level and gender among .90HCWs, in which females show higher level of protection level against HBV than males, with no statistical difference

Table (4.3) Association between immunization level and gender

		Vaccination Level		Total No. (%)
		Protective No. (%)	Non Protective No. (%)	
Gender	Male	**16**(17.8)	**04**(04.4)	**20**(22.2)
	Female	**47**(52.2)	**23**(25.6)	70(77.8)
Total		**63**(70.0)	**27**(30.0)	**90**(100)

*P.value:*0.268

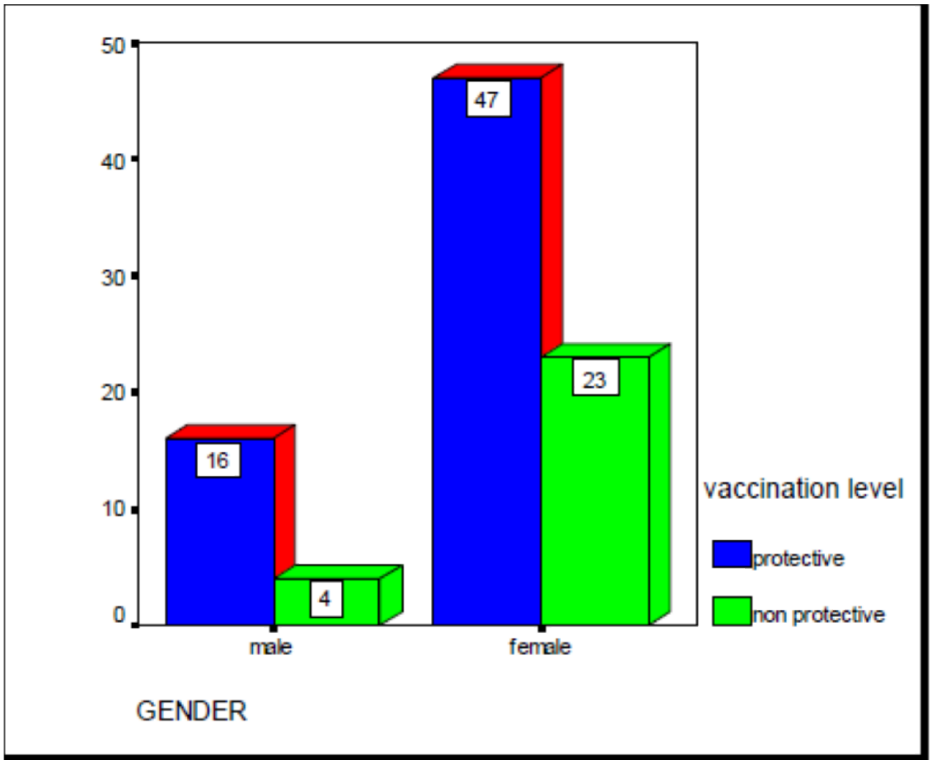


Figure (4.4) Association between immunization level and gender

Table (4.4) and figure (4.5): Show the association between immunization level and age groups among 90 HCWs, in which it categorized into three ranges, most of protective group were young and located in less than 35

years old, with no significant statistical difference between age and protective level.

Table (4.4) Association between immunization level and age groups

		Vaccination Level		Total No.(%)
		Protective No.(%)	Non-Protective No.(%)	
Age groups(Years)	< 35	50(55.6)	21(23.4)	71(78.9)
	35-50	10(11.1)	03(03.3)	13(14.4)
	> 50	03(03.3)	03(03.3)	06(06.7)
Total		63(70.0)	27(30.0)	90(100)

*P.value*0.485

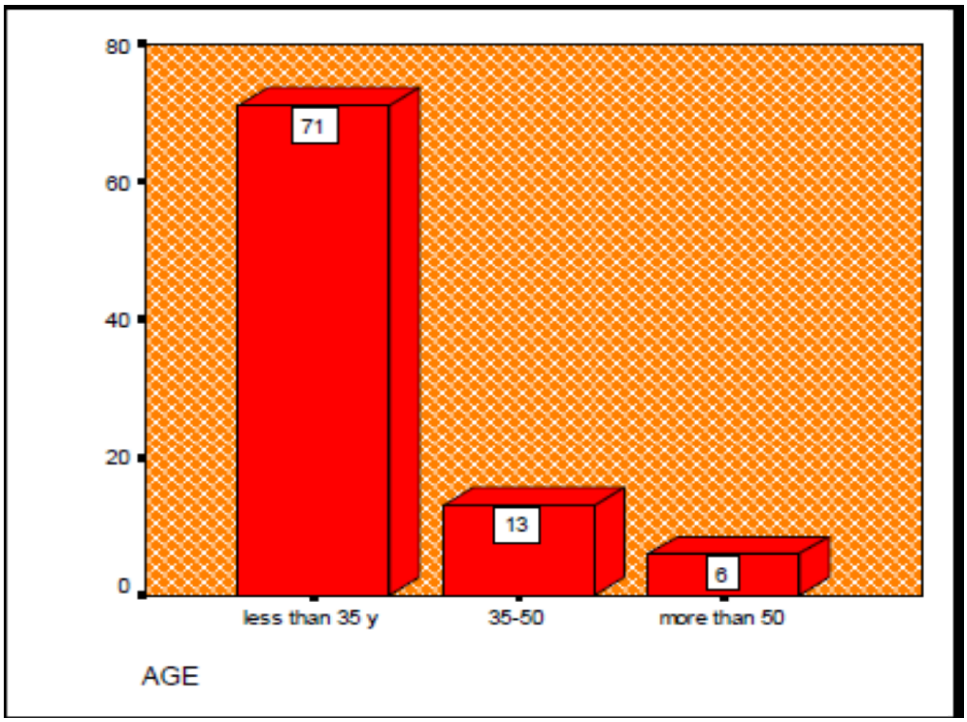


Figure (4.5) Association between immunization level and age groups

Table (4.5): Show the association between immunization level and type of employment among HCWs, in which it categorized into five categro,

higher percentage level against HBV was noticed in group of lab technologist with no significant statistical different

Table (5.4) Association between immunization level and type of employment

		Vaccination Level		Total No. (%)
		Protective No. (%)	**Non Protective No.** (%)	
Type of Employment	Lab technologists	32 (35.5%)	12 (13.4%)	**44(48.9%)**
	Doctors	13 (14.4%)	9 (10.0%)	**22(24.4%)**
	Nurses	12 (13.4%)	5 (5.6%)	**17(18.9%)**
	Dentists	4 (4.4%)	1 (1.1%)	**5(5.6%)**
	Midwifes	2 (2.2%)	0 (0.0%)	**2(2.2%)**
Total		63 (70.0%)	27 (30.0%)	**90(100%)**

p.value = 484

Table (4.6): Show the association between immunization level and employment duration length among . HCWs, in which it categorized into **three** ranges, higher percentage level against HBV was noticed in group of less than 5 years period with no significant statistical different

(Table 4.6) Association between immunization level and employment duration

		Vaccination Level		Total
		Protecti ve	**Non-Protective**	
Employment duration(Years)	**<5**	**36** (40.0%)	**13**(14.4%)	**49** (54. 4%)
	5 – 30	26 (28.9%)	**13** (14.4%)	39 (43.3%)
	> 30	**1** (1.1%)	**1** (1.1%)	**2** (2.2%)
Total		**63** (70.0%)	**27** (30.0%)	**90** (100.0%)

P. value0.127

Table (4.7): Show the association between immunization level and vaccination duration among 90 HCWs, in which it categorized into four ranges, most of protective level was located in group of less than 5 years, with no significant statistical difference.

Table (4.7) Association between immunization level and vaccination period

		Vaccination Level		Total
		Protective	**Non-Protective**	
Vaccination duration(Years)	**1**	**12**(13.3%)	**4**(4.4%)	**16**(17.7%)
	2 – 9	44(48.9%)	18(19.9%)	**62**(68.9%)
	10-16	**7**(7.8%)	**5**(5.6%)	**12**(13.4%)
Total		**63**(70.0%)	**27**(30.0%)	**90** (100.0%)

*P.value*0.123

Chapter Five

5. Discussion, Conclusions, and Recommendations

5.1. Discussion

The results of current study reveal that 70% of the studied vaccinated HCWs have protective level of HBs-Ab, Of these 23.3% had an anti-HBs titre between 10 and 100 mIU/ml and the rest 46.7% had an anti-HBs titre of >100 mIU/ml, while 30% of them are not protective, where 22.2%of them were males while the rest 77.8% were females with mean age of them is 30 years, Majority of participants were in the, labtechnoloegist /doctors/Nurse/Dentists/Midwife, and level of protection increased when employment duration less than 5 years 36(40%) and decrease after30 years 1(1.1%).

The Result show that the increase in protection of vaccination duration in up to 9years44(48.9%) and decrease in less than10years7(7.8%).

It is higher When compare with study conducted by (Shamsoun K, 2013) done in Khartoum ,68.6% of the studies Vaccinated HCWs have protective level of HBs-Ab while 31.4% is non protective. Among Of these protective 20.0% had an anti-HBs titre between 10 and 100 mIU/ml and the rest 48.6% had an anti- HBs titre of >100 mIU/ml.

The anti-Hbs response was similar in both male and female.

Age range of the study participants was 20–55 years.There was a decline in immune response as the age was increasing.

The mean months after the last dose of vaccination were 60.36..

they found that 96.5% of the vaccinated HCWs developed protective immensity to hepatitisB in this months.

And lower than other study done by (Abebaw TA, 2017)done in Ethiopia . 96.9% of study participants showed evidence of post-vaccination immunity measured as anti-HBs titre level >10IU/ml ,while 3.1% HCWs had anti-HBs titre levels <10IU/ml , The mediam age of participants was

29 years. Majority of participants were in the Nurse/Midwife/Health Assistants.

64% have worked for <5 years,the most protective occur in it.

most participants were females 82%. and18% male.

The mean range of duration of vaccine after last dose 4_8years 79 %protective in this years.

And our study lower when compare with study reported by (Mohammed Z, 2007 %) done in pakistan which 86%were found responders and14%were found non responders to vaccine.

The participants were mean age groups 25years most of protective HCWs in this level of Age.

22.2% were males and 77.8% were females,while responders 71.4% were females and 28.6% were males with significant difference between the two genders.

The vaccine duration between 1_10years 77%of responder in level less than 5 years.

Our study is closely similar compare with study conducted by(Abdullahi M, 2020)done in Khartoum, shown 73% of study populations have protective level of HBs-Ab while 27% non protective. about49. 2% of participants in this study did show excellant immune response, HBs_Abs titer >100miu/ml and23.8%had protective level of HBs-Ab titer between 10and100mIU/ml.

post immunization, Although the percentage of female protective responders was greater 72.6% compared to male protective responders 27.4 % the association of anti-HBs status and gender was not statistically significant.

increase in age leads to decline of anti-HBs protective responders. The highest number of protective responders is seen in age group less than 27

years. Whereas, highest number of non-protective is seen in age group of more than 60 year.

according to vaccine duration the study show high level of Immunity protective aginst HBs-Ab when last dose take in less than 5years46(50.3%)

We concluded that HBV post vaccination Immunity Status Of Health Care Workers in Khartoum state is lower than post vaccination HBV Immunity in other parts of the world.

5.2. Conclusions

This study show that the high immunization level of Antibodies titer Against HBV among health care workers after fully Vaccinated 70%of HCWs are protective level, While 30 %is non protective.

Also showed there is no significant statistical differ between Variables and immunization level.

5.3. Recommendations

- Regular check of post vaccination status for all HCWs, not only to ensure their safety but also to reduce rates of transmission.
- Administrate the booster dose to HCWs under protective level of Anti - HBsAg.
- Vaccination against HBV should be take place for all HCWs and medical students.
- A further study with better design and laboratory confirmation of vaccination status should be done in a large group of HCWs.

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Appendix 1

Questionnaire

Shendi University

College of Graduate studies

Faculty Of Medical Laboratory Sciences

Department of Medical Microbiology

Study Questionnaire

Evaluation of Hepatitis B Virus Immunization Level in Health Care Workers in Khartoum State

1/Serial No....

2/Age (years)

Up to 30... 31_50...

More than50...

3/Gender

Male.... Female....

4/Type of employment:..

Doctors... Lab technologist s.... Nurses...

Dentists... Midwifes...

5/Employment duration (years)

>10.... 10_20..... <20...

6 /Duration of vaccination (years)

>10.... 5_10....

Appendix2

إقرار بالموافقة

الاسم:.....

العمر:..... العنوان:.....

أوافق بمحض إرادتي بالمشاركة في البحث العلمي المتعلق بدراسة: **تحديد الحالة المناعية لعمال الرعاية الصحية المحصنيين ضد التهاب الكبد البائي في ولاية الخرطوم**

****الطالبة: عائشة محمد المأمون***

بعد ان شرحت لي طبيعة الدراسة وأعطيت فرصة كافية للاستفسار عنها وقد تمت الاجابة علي كل الأسئلة بصورة كافية واعلم ان المشاركة في هذا البحث لن تؤثر باي حال من الاحوال علي كما انه يحق لي بدون إبداء الإنسحاب من هذا البحث في اي مرحلة من المراحل.

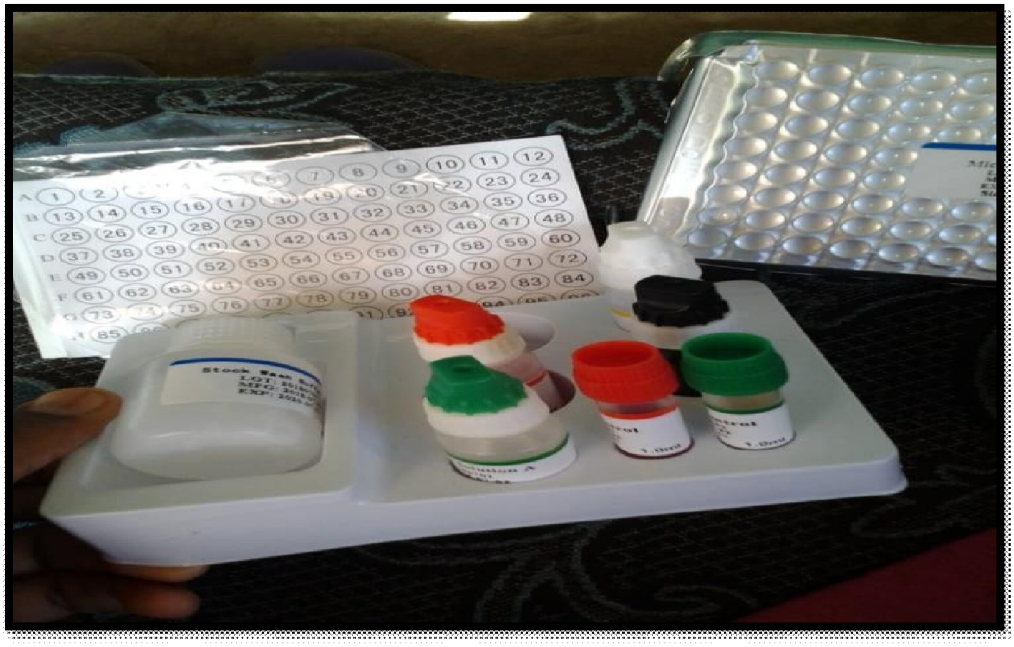
****البحث بإشراف:***

د. ليلى محمد احمد عبد القادر*

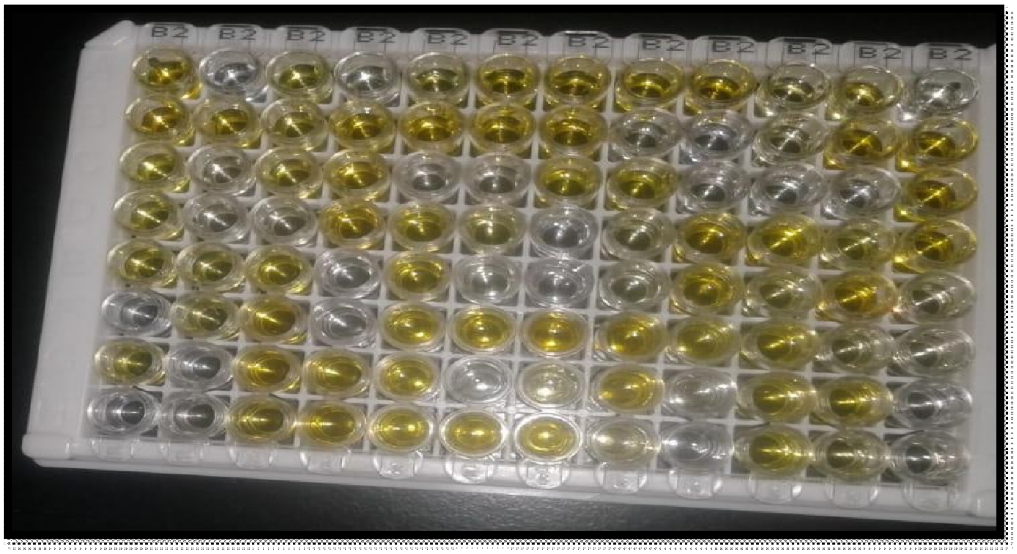
التوقيع:.....

التاريخ:.....

Appendix3

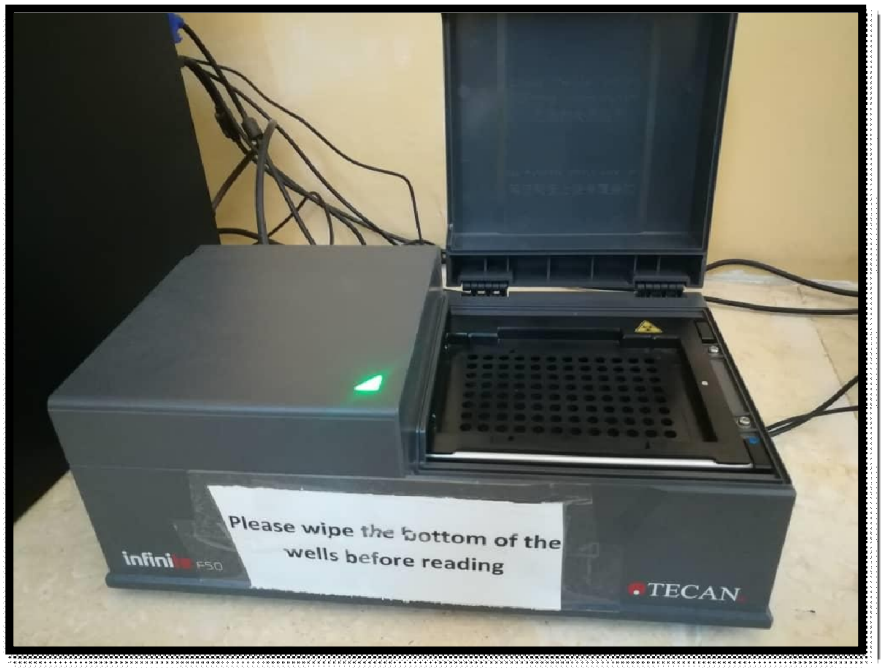


Diagnostic Elisa Kit for invitro qualitative detection of HBs-Abs in human serum



Micro-titerplate Negative control color less ($F^1$and H^1),while positive control(E^1andG^1)sampls were Show yellow color)

Elisawasher machine



Elisa reader

Machine(TECAN)