بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Serodetection of Hepatitis B Virus among Nurses at El-obied cityHospitals North KorfofanState

الكشف المصلي لفيروس التهاب الكبد الوبائي (ب) بين الممرضين في مستشفيات مدينة الابيض ولاية شمال كردفان

A Dissertation Submitted in Partial Fulfillment of the Requirements for the award of the degree of Master ofMedical Laboratory Science (Microbiology)

by:

Wadah Adam Omer Ahmed

B.Sc (Honor degree) in Medical Laboratory Sciences, Kordofan University, (2018)

Supervisor:

Prof.YousifFadlallaHamedelnil

December2022

Approval Page

(To be completed after the college council approval)

Name of Candidate: | Wadah | Adam | Omer | Ahmed |

Thesis title: Sero detection of Hepatitis B virus among nurses at Al-Obeid City Hospitals North Kordofan State

Degree Examined for: Master in Medical Laboratory Science (Microbiology)

1. External Examiner

Name: Dr. Mohammed Nahi Hammad

Signature: ..... **Date:** 29.01.2023

2. Internal Examiner

Name: Dr. WAFAA MOHAMMED ABDALLA

Signature: ..... **Date:** 29/1/2023

3. Supervisor

Name: Prof. Yousif Fadlalla Hamedabid

Signature: ..... **Date:** 29.1.2023

الاية

بسم الله الرحمن الرحيم

قال الله تعالى :

﴿**اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ** (1) **خَلَقَ الْإِنسَانَ مِنْ عَلَقٍ** (2) **اقْرَأْ وَرَبُّكَ الْأَكْرَمُ** (3) **الَّذِي عَلَّمَ بِالْقَلَمِ** (4) **عَلَّمَ الْإِنسَانَ مَا لَمْ يَعْلَمْ** (5).

صدق الله العظيم

سورة العلق الاية (1- 5)

Dedication

This is for you, Mom. I am extremely grateful to you for your love, prayers, caring and Sacrifices for educating and preparing me for my future. Thanks for always being there for me.

Acknowledgements

First and foremost, I must acknowledge my limitless thanks to **Allah**, the ever-magnificent; the ever-thankful, for his help and bless for giving me the power and patience to complete this study. I am totally sure that this work would have never become truth, without Allah guidance.I would like to express my deep and sincere gratitude to my research Supervisor, **Prof.YousifFadlalla**, for providing insight, expertise and invaluable guidance throughout this research. His dynamism, vision, sincerity and motivation have deeply inspired me. It was a great privilege and honor to work and study under his guidance. I am extremely grateful for what he has offered me. I would like to say thanks to El Obeid Educational Hospital and El Obeid Specialized Pediatric Hospital staff for their help during specimen collection and processing. Finally, my thanks go to all the people who have supported me to complete the research work directly or indirectly.

Abstract

Hepatitis B infection is one of the world's major infectious diseases and nurses have a high risk of occupational exposure to Hepatitis B Virus. This descriptive cross sectional study aimed to determine sero-prevalence of Hepatitis B virus and to determine the possible association between Hepatitis B virus and some risk factor among Nurses workersat El-obied city Hospitals North Korfofan Stateduring a period from May to October 2022.

Structured questionnaire was used to collect both demographic and clinical data,Venus blood (3 ml) was taken from each participantby standard procedure,were put in plain containers to clot then centrifuged at 5000 rpm for5 minutes and serum was obtained and kept at -20°C until used .

The serum sample were examined by Enzyme Linked ImmunosorbentAssay (ELISA) to detect HBsAg. A total of (n=78) nurses in EL-obeid city were included in this study, in which 7 (9%) were males and 71 (91%) were females, mostly were at age between 20-30 years (63%). Among Nurses there were 4 (5.1%) positive for HBs Ag and other were negative(95%). Among these There were3/4 (75%) females were positive. for HBs Ag and 1/4 (25%) males waspositive.

According to age groups, there was 2(50%) of the positive result between 20-30 years ,1 (25%) in age range from 30 to 39 years and 1(25%) in age more than50 years were positive for HBs Ag.

There was 2 (2/4 (50%))of positive HBsAg nurses had accidental needle stick and the othersdidn't expose to any of the possible risk factors were HBs Ag positive .

The study indicated insignificant association between Hepatitis B infection and effective factor (P> 0.05),gender ,age , accidental injury ,blood transfusion , surgicaloperation ,renal dialysis , (P.value: 0.25 , 0.75 , 0.71,0.50 , 0.98 , 0.74 , ,respectively).

This study concluded the frequencyof hepatitis B virus among the nurses are normal.

المستخلص

عدوى التهاب الكبد (ب) هي من الامراض المعدية الرئيسية في العالم , ويواجه الممرضون مخاطر عالية من التعرض المهني لمرض التهاب الكبد (ب) هدفت هذه الدراسة الوصفية المقطعية الى تحديد نسبة انتشار مرض التهاب الكبد الوبائي في مدينة الابيض ولاية شمال كردفان خلال الفترة من مايو حتى اكتوبر 2022.

عن طريق الاستبيان المحدد جمعت المعلومات الجغرافية والسريرية, اخذت 3 مل من الدم من الوريد بالطريقة المثالية وُضعت في وعاء خالي من مضادات التجلط وبعد عملية تجلط العينات,فصلت بواسطة جهاز الطرد المركزي 5000 لمدة 5دقائق وحفظت في درجة حرارة −20درجة الي حين استخدامها.

فحصت العينات بواسطة تقنية اليزا للكشف عن البروتين السطحي للفيروس ,تم تضمين مجموعة ثمانية وسبعون عينة من الممرضين بمدينة الابيض , والتي كان 7(9%) من الذكور و 71(91%) من الإناث , تتراوح اعمار معظمهم بين (20−30)(63%).

بين الممرضين كان هنالك 4(5.1%) إيجابية لفيروس إلتهاب الكبد الوبائي (ب) والاخرون كانوا سلبيون (95%). كان هنالك 4/3(75%) من الإناث إيجابيات لفيروس إلتهاب الكبد الوبائي (ب) وكان 4/1(25%) من الذكور إيجابيون لفيروس إلتهاب الكبد الوبائي(ب).

وفقا للفئات العمرية كان هنالك 2بين 20−30 عاما , واحد بين 30−39 عاما 1 في الفئة العمرية اكثر من50 عاماكان إيجابيا لفيروس إلتهاب الكبد الوبائي (ب).

كان هناك 4/2(50%) من الممرضين لديهم إصابة عصا إبرة عرضية, و الاخرين لم يتعرضو لاي عوامل الخطر المحتملة للإصابة بفيروس إلتهاب الكبد الوبائي (ب),

هذه الدراسة اثبتت عدم وجود اهمية احصائية بين الإصابة والعوامل المؤثرة اي انه ليس هنالك ي إرتباط بين النوع والعمر والجروح اثناء اداءة العمل ونقل الدم والعمليات الجراحية وغسيل الكلى (القيمة الإفتراضي.*0.25,0.75,0.71,0.50,0.98,0.74* على التوالي.

خلصت هذه الدراسة الى ان معدل انتشار التهاب الكبد الفيروسي ب عادي وسط الممرضين.

Contents

Content	Page No
الاية	I
Dedication	II
Acknowledgment	III
Abstract	IV
الخلاصة	V
List of contents	VI
List of tables	VIII
List of figures	VIII
Abbreviation	IX
Chapter One: Introduction	
1.1.Introduction	1
1.2. Rationale	3
1.3. Objectives	4
1.3.1. General Objective	4
1.3.2. Specific Objectives	4
Chapter Two: Literature Review	
2.1 Hepatitis B virus	5
2.1 Background	5
2.2 Virus biology	5
2.3 Classification	6
2.4 Genome	6
2.5 Genotypes	7
2.6 Replication	8
2.7 Transmission	9
2.8 Risk group	9
2.11. 1 Acute infection	12
2.11. 2 Chronic infection	12

2.11. 3 Hepatocellular carcinoma.	13
2.11.5 Immunomechanism	13
2.12 Laboratory diagnosis	15
2.12.1 Serologic and virologic markers	15
2.12.1.1Viral capsid (HBSAg) and antibody(anti-HBs)	15
2.12.1.2. Antibody directed against the core antigen (anti-HBc)	16
2.12.1.3. Secreted core antigen (HBeAg) and its antibody (anti-HBe)	17
2.12.1.4Polymerase chain reaction (PCR)	17
2.12.1.4.1. Real time PCR	17
2.12.1.5. Hepatitis B viral DNA (HBV-DNA)	18
2.13 Treatment	18
2.14 Prevention	18
2.14.1 Active immunization	19
2.14.2. Passive immunoprophylaxis	20
2.15. Previous studies	21
Chapter Three: Materials And Methods	
3.1. Study design	22
3.2. Study area	22
3.3Study duration	22
3.4. Study population	22
3.5. Sampling technique	22
3.6. Inclusion criteria	22
3.7. Exclusion criteria	22
3.8. Sample size	22
3.9. Ethical consideration	22
3.10. Data collection	22
3.11. Collection of blood specimens	22
3.12. Laboratory investigation	23
3.13.1 ELISA technique	24
3.13.1.1Assay procedure	24

313.1.2Interpretation of results	24
3.14.Method of data analysis	24
Chapter Four: Results	
Result	25
Chapter Five: Discussion, Conclusion and Recommendation	
5.1 Discussion	30
5.2 Conclusion	32
5.3 Recommendations	33
References	34
Appendixes	42
Appendix (A): Fortress diagnostics for HBsAg	42
Appendix (B): Colourplate (2)ELISA Kit	43
Appendix (C): HBsAg coated microplate	44
Appendix (D): Questionnaire	45

List of tables

Table No	Name of table	Page No
Table(4.1)	Frequency result of gender	25
Table(4.2)	Frequency result of age	25
Table(4.3)	Frequency result of education.	25
Table(4.4)	Frequency result of marital status	26
Table(4.5)	Frequency result ofHBV	26
Table(4.6)	Correlation between result of HBV and gender	26
Table (4.7)	Correlation between result of HBV and age	27
Table (4.8)	Correlation between result of HBV and Sharp instrument	27
Table (4.9)	The correlation between HBs Ag results and possible risk factors among nurses	28

List of Abbreviations

3TC Lamivudine

Ab Antibody

CCC DNA Covalently Closed Circular DNA

CDC Center for Disease Control and Prevention

CHB Chronic Hepatitis B

CTL Cytotoxic T-lymphocyte

DNA Deoxyribonucleic acid

ETV Entecavir

HBc Ag Hepatitis B core antigen

HBeAg Hepatitis B viral protein

HBIG Hepatitis B immunoglobulin

HBsAg Hepatitis B surface antigen

HBV Hepatitis B virus

HCC Hepatocellular carcinoma

HCWs Health care workers

HDV Hepatitis delta virus

HIV Human immunodeficiency virus

IDUs Intravenous Drug Users

IFN Interferon

mRNA Messenger RNA

MSM Men Who Have SexWithMen

MTCT Mother-To-Child Transmission

NAs Nucleotide analogues

SPSS Statistical Package For Social Science

STI Sexual Transmitted Infection

WHO World Health Organization

CHAPTERI

INTRODUCTION

CHAPTER I

1. Introduction

1-1 Introduction

Hepatitis means inflammation of the liver. It is most common caused by one of the several viruses, such as hepatitis A virus, hepatitis B virus, hepatitis C virus and other viruses, toxins, bacterial infections, certain drugs, and heavy alcohol use can also cause hepatitis (Jawetz*etal*., 2007)).Half a century ago, hepatitis B virus (HBV) is one of the world's most severe infectious illnesses. (Yang *et al*., 2017).HBV is an enveloped virus of the Hepadnavirus family that infects the liver, causing hepatocellular necrosis and inflammation. HBV, spread by percuta-neous or mucosal exposure to infected blood and various body fluids, can cause eitheran acute or chronic disease(WHO, 2022).

An estimated 296 million people, or 3.8% of the global population, had chronic hepatitis B infections as of 2019. Another 150 million developed acute infections that year, and 820,000 deaths occurred as a result of HBV. (WHO, 2022).Cirrhosis and liver cancer are responsible for most HBV-related deaths. (Nelson *et al*.,2016).

The disease is most prevalent in Africa (affecting 7.5% of the continent’s population) and in the Western Pacific region (5.9%).(WHO, 2021, 2022).

Infection rates are 1.5% in Europe and 0.5% in the Americas. (WHO, 2021, 2022).According to some estimates, about a third of the world's population has been infected with hepatitis B at one point in their lives. (Nelson*et al*., 2016).Hepatitis B was originally known as "serum hepatitis(Barker, 1970).

HBV seroprevalence rates ranged from 5.1 to 26.8% with an overall pooled prevalence of12.1%. According to study findings, Khartoum State hadthe highest prevalence of HBV infection in Sudan with aproportion of 12.7% (Badawi, *et al*, 2018).Likewise, this rate is comparableto other African countries such as Burundi (15.6%), Central African Republic (14%), while it is higher than Nigeria(5%) and Ethiopia (7%)(Mudawi, 2008).

World Health Organization (2022), estimated that about 3 million Health care workers(HCWs) are exposed to blood borne pathogens each year—occupational

exposure causes approximately 2 million to HBV infections.Occupational exposure to HBV is a well-recognized risk for HCWs and it is dependent on the frequency of percutaneous and permucosal exposure to blood or body fluids containing blood, which commonly occurs due to needle sticks or other sharp device injuries (NSIs)(Hisham*et al*., 2013).

If preventive measures are not employed properly, nurses and other health professionals are more likely susceptible and caught the virus. Nurses are frontline caretakers that play a critical role in the healthcare settings of patients suffering from multiple illnesses, including HBV infection. They offer emotional support as well as education about the disease's nature, diagnosis, and prevention. Therefore, nurses must be knowledgeable with HBV transmission and preventive measures to reduce the risk of spreading infection from patient to patient, nurse to patient, or patient to nurse.it is a dangerous disease that can impact nurses if they do not understand how the virus spreads and prevent it from spreading.

Studies have reported a lack of awareness of HBV among HCWs; consequently, proper precautions (e.g., use of disposable gloves) against blood-borne infections are lacking in these workers and this observation is consistent with other studies demonstrating that untrained individuals are more likely to be exposed to HBV infection and preventive vaccination against hepatitis B for hospital staff is standard in many countries, but is still not implemented in many resources-poor settings. Therefore WHO recommends monitoring immune responses to the vaccine in addition to compulsory vaccination of HCWs (Mueller *et al*., 2015).

HBV-infected HCWs also pose a potential risk for patients as there is documented risk of HBV transmission to patients from treating doctors or medicals vaccination is effective in protecting 90-95% adults (Vishal *et al*.,2015).

1.2. Rationale

Hepatitis B is a well-documented occupational risk for health professionals, including nursing staff (Elmukashfi*et al*., 2012).

The prevalence of disease is associated with a proper understanding of the mode of transmission of the disease.The observation that needle-stick injuries can transmit the virus indicates that only very small amounts of blood are necessary to transmit the disease(Jha*et al*.,2012).

Moreover, little is known about the situation and prevalence of the disease in El-Obeid city especially among nurses whom may represent a source of infection. Furthermore, the proper understanding of the prevalence in study area may help in setting further control programs.

The aim of this study was to determine the prevalence of HBV among nurses in El-Obeid city.

1.3. Objectives:

1.3.1. General objective:

To detect Hepatitis B Virus Infection among Nurses in El-Obeid city.

1.3.2. Specific objective:

1. To detect hepatitis B surface antigen (HBsAg), among Nurses in El-Obeid city using Enzyme Linked Immune Sorbent Assay technique.
2. To determine the frequency of HBV among nurses.
3. To detect possible association between hepatitis B virus and selected risk factors(vaccine , accidental injury , blood transfusion , Renal dialysis and surgical operation).

CHAPTER II

LITERATURE REVIEW

CHAPTER II

2. Literature Review

2.1. Hepatitis B virus (HBV)

Hepatitis refer to an inflammation of the liver cell and damage to the liver ,the liver function include detoxifying the blood ,storing vitamin and producing hormone ,hepatitis disrupt these process (WHO, 2022).

HBV infection was first identified in 1965 when Blumberg and co-workers found the hepatitis B surface antigen (HBsAg), originally termed as Australia antigen. Enhanced viral replication leading to a vigorous and extensive immune response may lead to massive liver injury resulting spontaneously into fulminant hepatic failure. The seriousness of disease incidence is mainly related to various host factors (age, gender, duration of infection, immune response and viral factors as viral load, genotype, quasispecies). (Jayalakshmi*etal*.,(2013)). viral hepatitis is caused by infection with five distinctly different human hepatitis viruses A, B, C, D and E, which cannot be distinguished from one another without serologic testing, other type of hepatitis can caused by over consumption of alcohol or an autoimmune condition (WHO, 2022).

2.2. Hepatitis B Virus biology

Human hepatitis B virus (HBV) is the prototype of a family of small DNA viruses that productively infect hepatocytes and, for the most part, non-cytopathic. Although hepadnaviruses have a strong preference for infecting liver cells, but small amounts of hepadna viral DNA can be found in the bile duct epithelium of the liver, rare exocrine cells and alpha and beta islets of the pancreas, proximal tubular epithelium of the kidney, and possibly a subset of splenic cells. Infection of the liver may be either transient (<6 months) or chronic and lifelong, depending on the ability of the host immune response to clear the infection. Chronic infections can cause immune mediated liver damage progressing to cirrhosis and hepatocellular carcinoma (HCC). The mechanisms of carcinogenesis are unclear(Seeger and Mason ,2016).

The incubation period for acute hepatitis B ranges from 45 to 180 days (average 120 days). The clinical manifestations of acute HBV infection are age dependent. Infants,

young children (younger than 10 years of age), and immunosuppressed adults with newly acquired HBV infection are usually asymptomatic. older children and adults are symptomatic in 30%–50% of infections. When present, clinical symptoms and signs might include anorexia, malaise, nausea, vomiting, abdominal pain, jaundice, dark urine, and clay-colored or light stools. Occasionally, extrahepatic manifestations occur and include skin rashes, arthralgia, and arthritis. Among adults with normal immune status, most (94%–98%) recover completely from newly acquired HBV infections, eliminating the virus from the blood and producing neutralizing antibodies that confer immunity from future infection. In infants, young children, and immunosuppressed persons, most newly acquired HBV infections result in chronic infection. Infants are at greatest risk, with a 90% chance of developing chronic infection if infected at birth. Although the consequences of acute hepatitis B can be severe, most of the serious sequelae occur in persons in whom chronic infection develops. Chronic liver disease develops in two-thirds of these persons, and approximately 15%–25% die prematurely from cirrhosis or liver cancer. Persons with chronic HBV infection are often detected in screening programs, such as those for blood donors, pregnant women, and refugees.Persons with chronic HBV infection are a major reservoir for transmission of HBV infections. Any person testing positive for hepatitis B surface antigen (HBsAg) is potentially infectious to both household and sexual contacts.(Winston..*et al*,2014).

2.3. Classification

HBV belongs to the genus Orthohepadnavirus of the family Hepadnaviridae and the virion is spherical with a diameter of 42 nm(Faseeha, 2015).

It is the best known hepadnavirusis that infects humans which is commonly referred to HBV and it is a major importance as an agent of disease and death(Levinson, 2014).Related viruses have been found in woodchucks, ground squirrels, and ducks, suggesting a long evolutionary history of this virus family(Shuping*et al*., 2013).

2.4. Genome

The partially double-stranded HBV genome is encased within the core particle, which is wrapped by an envelope consisting of host-derived lipids containing dispersed viral

envelope proteins (Shuping*et al*., 2013). The nucleocapsid encloses the viral genome consisting of two linear strands of DNA held in a circular configuration. One of the strands (the plus strand) is incomplete, so that the DNA appears partially double stranded and partially single stranded. Associated with the plus strand is a viral DNA polymerase, which has both DNA-dependent DNA polymerase and RNA-dependent reverse transcriptase functions. Although it is a DNA virus, it encodes a reverse transcriptase and replicates through an RNA intermediate and this polymerase can repair the gap in the plus strand and render the genome fully double stranded (Kumar, 2016).

2.5. Genotypes

HBV is differentiated into many genotypes, according to genome sequence. To date, eight well-known genotypes (A-H) of the HBV genome have been defined. Moreover, two new genotypes, I and J, have also been identified. Some HBV genotypes are further classified as sub-genotypes. HBV sequence is characterized by > 8% nucleotide differences for genotype, and 4%-8% nucleotide differences for sub-genotype. Over 30 related sub-genotypes belonging to HBV genotypes have been determined to date, but the mechanisms of different pathogenic characteristics of HBV genotypes are not known for certain. Many studies have reported that different genotypes and sub-genotypes show different geographical distribution, and are related to disease progression, clinical progression, response to antiviral treatment, and prognosis. A-D and F genotypes are divided into various sub-genotypes; no sub-genotypes have been defined for E, G and H genotypes.(Cooksley, 2010**;**Huang *et al*., 2013**;** Shi *et al*., 2013**;**Moura*et al*., 2013).

Genotype A is prevalent in Brazil, USA, Canada, Northwest Europe, South Asia, Central African countries, Tunisia and Benin and Genotype B is common in Japan, Taiwan, Philippines, Hong Kong, China, Vietnam, Thailand, Indonesia and United States of America (Mahmood, 2016). Genotype C occurs in Australia, Polynesia, Melanesia, Micronesia, Indonesia, China, Hong Kong, Vietnam, Thailand, Japan, Korea, Taiwan, India, Solomon Islands, Brazil and USA. Genotype D is predominant in Mediterranean region, Spain, Albania, Czech Republic, Russia, Turkey, Middle East, Iran, Afghanistan, South Asia, Solomon Islands, Tunisia, Polynesia, Melanesia, Micronesia, Brazil and USA (Mahmood, 2016).

Genotype E almost exclusively occurs in African people and its presence is more

commonly associated with the development of chronic HBV (CHB) infection. Moreover, an epidemiological link has been found between the distribution of HBV genotype Einfection and African countries with high incidences of hepatocellular carcinoma (Malagnino, 2018).

Genotype G (HBV-G) is an aberrant genotype with little sequence divergence, suggesting a recent origin. HBV-G is strongly associated with certain risk groups such as intravenous drug users (IDUs) and men who have sex with men (MSM), but hardly with geography. The origin and epidemiology of HBV-G remain unresolved, is also present in certain risk groups in Europe (Cornelissen*et al*., 2016).

Genotypes B, C and I are associated with a more frequent vertical transmission from mother to child, a higher transmission rate during sexual contact or injecting drug use has been reported for genotypes A, D and G (Velkov*et al*., 2018). Acute genotype A and D infection results in higher chronicity rates than B and C (Lin and Kao, 2015).

2.6. Replication

The replication of HBV is unique for several reasons, first, HBV has a distinctly defined tropism for the liver, Its small genome also necessitates economy, In addition, HBV replicates through an RNA intermediate produces and release antigenic decay particles (Murray *et al* .,2002).

The infectiousvirion attaches to cells and becomes uncoated . In the nucleus, the partially double-stranded viral genome is converted to covalently closed circular double-stranded DNA (cccDNA). The cccDNA serves as template for all viral transcripts, including a 3.5-kb pregenome RNA. The pregenome RNA becomes encapsidated with newly synthesized HBc (Geo. *et al* .,2010).

Within the cores, the viral polymerase synthesizes by reverse transcription a negative-strand DNA copy. The polymerase starts to synthesize the positive DNA strand, but the process is not completed. Cores bud from the pre-Golgi membranes, acquiring HBsAg-containing envelopes, and may exit the cell. Alternatively, cores may be reimported into the nucleus and initiate another round of replication in the same cell.(Geo. *et al* .,2010).

2.7. HBV transmission

The HBV has ability to survive outside the body for at least 7 days, during this time, the virus can still cause infection if it enters the body of a person who is not protected by the vaccine and the incubation period of the hepatitis B virus is 75 days on average, but can vary from 30 to 180 days(WHO, 2022b).

There are three important modes of transmission; parenteral transmission, perinatal transmission and sexual transmission(Kumar*et al*., 2013).Despite being transmitted vertically from infected mother to a child,sexually, contacting the infected needle sticks or sharp object injuries, HBV is not transmitted through breast-feeding, hugging, kissing, coughing, and sneezing, or sharing food and drink (Guvenir and Arikan, 2020).

2.8. Risk groups for HBV

2.8.1. Health care workers including nurses

Healthcare workers (HCWs) are considered a high-risk group for HBV infection due to occupational exposure to blood-borne pathogens. Previous studies in Africa found high HBV infection and exposure rates (roughly 10%) in HCWs in South Africa and Nigeria. Worldwide, approximately 2 million HCWs are infected with HBV through sharp injury (Sondlane*et al*., 2016). The risk of acquisition of this infection in an unvaccinated individual after a single exposure is estimated 32–67% when blood is positive for both hepatitis B surface antigen (HBsAg) and envelope antigen (HBeAg) and 6% when HBeAg is negative. The World Health Organizationrevealed that in 2000, 66,000 HBV infections among HCWs could have happened owing to their occupational exposure (Ganczak*et al*., 2019).

Health care worker are most exposed to HBV due to number of blood exposures sustained during medical procedures, the risk of transmission at each exposure and the prevalence of HBV in general population, particularly in hospitalized patients, lack of training in infection control, and not using protective equipment contribute to contracting HBV at hospital setting (Ganczak*et al*., 2019).

2.8.2. Sexual (heterosexual and homosexual) exposure

Among persons with case reports of HBV infection with information about sexual exposure, 26.4% reported having two or more sexual partners, 3.3% reported sexual contact with an HBV infected person, and 11.8% of males reported having had sex with another male. As many as 10%–40% of adults seeking treatment in clinics have evidence of current or past HBV infection. Among adults with acute HBV infection, 39% were screened or sought care for sexual transmitted infection (STI) prior to becoming infected with HBV(Mahmood*et al*., 2016).

2.8.3. Hemodialysis patients

Since the initiation of HBV vaccination and additional infection control precautions for HBV in dialysis centers, the incidence of HBV infection among hemodialysis patients has declined approximately 95%. Since 1995, the annual incidence has been stable and HBsAgsero-prevalence has remained at 1%.Receipt of dialysis was reported in <1% of acute HBV surveillance cases with information reported to CDC (Schillie*et al*., 2018).

2.8.4. Travelers to countries where HBV is endemic

Short term travelers to countries in which HBV infection is of high or intermediate endemicitytypically are at risk for infection only through exposure to blood in medical or disaster relief activities, receipt of medical care that involves parenteral exposures, sexual activity, or drug use. Monthly incidence of 25–420 per 100,000 travelers has been reported among long term travelers to countries where the disease is endemic(Schillie*et al*., 2018).

2.9. Epidemiology

Hepatitis B is considered as one of the most common infectious diseases worldwide. Thereare 350 million chronic HBV carriers globally have been estimated. The prevalence of chronic HBV infection differs geographically, from high (>8%), intermediate (2-7%) to low (<2%) prevalence (JinlinHou, *et al*, 2005).

High prevalence rate of chronic HBV infection effect more than 8% of population is common in Asia pacific and Sub-Saharan African regions populations, 45% of the world's population live in an area of high prevalence. Inter mediate prevalence

rate which effect (2%-7%) of populations include North Africa and Middle East, parts of eastern and south Asia. Low prevalence populations include Australia, Asia, Northern and Western Europe, Japan, North America and some countries of South America make up minority of global population (~12%) (Jennifer, *etal,*2015).

Sudan is classified among the African countries with high HBV endemicity. Exposure tothe virus varied from 47%–78%, with a hepatitis B surface antigen prevalence ranging from 6.8%in central Sudan to 26% in southern Sudan (Mudawi, 2008).

The reported prevalence of HBV chronic infection, characterized by the detectable level of HBV surface antigen (HBsAg), varied from region to region and ranged between 5 and 7% in the general population and 26% in hospital outpatients. The prevalence of adults having been in contact with HBV and identified by the presence of anti-core antibodies (anti-HBc) was high, ranging between 47.5 and 67%. The introduction of vaccination and the screening of blood and blood products during the past 8 years is expected to reduce the rate of HBV infection and the carrier pool (Mahgoub*et al*., 2011).

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

2.10. Pathogenesis

After entering the blood, the virus infects hepatocytes, and viral antigens are displayed on the surface of the cells. The pathogenesis of hepatitis B is probably is 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) (Livenson, 2014).

2.11. Clinical presentations

2.11.1. Acute infection

After exposure to the virus, there is a long, asymptomatic incubation period, which may be followed by acute disease lasting many weeks tomonths. The natural course of acute disease can be tracked using serummarker(Spearman *et al*., 2013).

During the acute infection, hepatitis B does not appear to induce an intra-hepatic innate immune response and instead, it acts as astealth‘ virus early in the infection (Spearman *et al*., 2013).

Hepatitis B surface antigen (HBsAg) appears before the onset of symptoms, peaks during overt disease, and then declines to undetectable levels in 3 to 6 months (Kumar *et al.*, 2013). Anti-HBs antibody does not rise until the acute diseases over and usually is not detectable for a few weeks to several months after the disappearance of HBsAg. It may persist for life, conferring immunity; this is the basis for current vaccination strategies using non-infectious. HBsAg, HBeAg, HBV-DNA, and DNA polymerase appear in serum soon after HBsAg, and all signify active viral replication. Persistence of HBeAg is an important indicator of continued viral replication, infectivity, and probable progression to chronic hepatitis. The appearance of anti-HBe antibodies implies that an acute infection has peaked and is on the wane (Kumar*et al*.,2013).

2.11.2. Chronic infection

Chronic HBV infection is defined as persistence of hepatitis B surface antigen (HBsAg) for more than six months, high levels of HBV DNA, and presence of (HBeAg) in the serum. Chronic HBV infection occurs in approximately five to ten percent of individuals with acute HBV infection.Theexactmechanisms by which chronic liver injury occurs inHBV infection are not known (KozielandSiddiqui,2010). Long-term effects of chronic HBV infection include cirrhosis, liver failure, and HCC. HBV infections acquired by infants or children are significantly more likely to progress to chronic HBV infections as compared to adults(Keeffe*etal*, 2008). Chronic HBV can cause serious destruction to the liver and it contributes greatly to the world wide burden of the disease states. Surprisingly, some of the patients infected persistently may have no clinical or

biochemical evidence of liver disease, while other may show signs of easy fatigability, anxiety, anorexia, and malaise (Mahony and kane,1999).

2.11.3. HBV and hepatocellular carcinoma

A number of HBV patients with chronic hepatitis will develop HCC. Persons at increased risk of developing HCC include adult male and chronic HBV with cirrhosis that contracted hepatitis B in early childhood. Only about 5% of patients with cirrhosis develop HCC. On the other hand between 60 and 90% of HCC patients have underlying cirrhosis (Hollinger and Liang, 2001).

Risks of HCC among HBV-infected patients vary by several factors, the major one being serum HBV-DNA levels. Although there is no discrete cutoff level, having greater than Log10 5/mL viral copies confers a 2.5- to threefold greater risk over an 8- to 10-year follow-up period than does having a lower viral load. The cumulative incidence of HCC increases with serum HBV-DNA levels. A recent hospital-based cohort study further validated the HCC risk, showing it started to increase when the HBV-DNA level was higher than 2000 IU/ml. In addition to HBV-DNA levels, the clinical significance of quantitative hepatitis B surface antigen (HBsAg) has become increasingly recognized (Omata*et al*., 2017).

There are three reported mechanisms by which HBV promotes carcinogenesis: HBV proteins are involved in many signaling pathways in hepatocytes, thereby affecting the expression and functions of specific genes and contributing to live disorders and most of these changes are associated with HCC, integration of HBV DNA into the host genome alters the function of endogenous genes or induces chromosomal instability, and Inflammation-mediated alteration of specific signaling pathways contributes to tumorigenesis. Chronic inflammation plays a vital role in the development of HCC and repeated cycles of inflammation-induced apoptosis and hepatocyte regeneration increase the risk of hepatocarcinogenesis(Xu*et al*., 2014).

2.11.5 Immunomechanism against HBV

The host innate and adaptive immune systems play critical roles in eliminating HBV upon infection. However, HBV has evolved and developed efficient strategies for escaping host immune surveillance, which results in persistent infections. The majority of HBV infections occur in newborn infants with the presence of immunological defects,

characterized by a lower quality and quantity of HBV-specific T cells and B cells. In addition, maternal hepatitis B e antigen (HBeAg) can induce the Kupffer cells (KCs) of the offspring by upregulating programmed death-ligand 1 (PD-L1) to suppress the HBV-specific CD8+ T cells response to support HBV persistence after birth(Zhao, *et al*., 2022).

In addition, HBV circumvents endogenous type I interferon (IFN-I) responsesand inhibits the function of innate and adaptive immune cells]. Prolonged exposure of T cells to large quantities of viral antigens, such as hepatitis B surface antigen (HBsAg) and HBeAg, induces a defective T-cell response with the loss of effector functions and increased inhibitory receptor expression, facilitating viral persistence. Moreover, HBV infection affects the expression of human leukocyte antigen (HLA)-II alleles, including HLA-DP, HLA-DQ, and HLA-DR, on antigen-presenting cells (APCs, which in turn impairs antigen presentation capacity with induction of an inefficient T-cell response, leading to persistent HBV infection (Zhao, *et al*., 2022)

The virus trigger adaptive immune response, which usually prompts the death of infected hepatocytes leading to hepatic injury and damage. The intention of which is to remove virus infected cells. In this immune response, both CD4 T cells (T helper cells) and CD8 T (cytotoxic T-lymphocyte (CTL) cells are activated CD4 T cells are robust producers of cytokines and are required for the efficient development of CTLs and B cells, which produce anti-HBV antibody to reduce the levels of circulating virus studies of HBV infected chimpanzees, suggest that CD4 T cells have no direct effect on viral clearance and liver disease (Lu, 2011).

CD8 T cells clear HBV-infected hepatocytes through cytolytic and non-cytolytic mechanisms, reducing the levels of circulating virus, whereas B-cell antibody production neutralizes free viral particles and can prevent reinfection. This antiviral immune response is induced in adults after acute HBV infection and leads to HBV control. In contrast, chronic HBV patients fail to mount such an efficient antiviral response (Tan *etal*., 2015).

Broadly reactive CD4 T cells are predominantly detectable during acute infection, whereas their numbers decline during chronic infection. Both, CD4 and CD8 T cell responses are deterministicof whether an acute infection is resolved, or whether it

progresses to chronic infection (Prieto and Dorner, 2017).Depletion of CD4 T cells at the peak of HBV infection in chimpanzees does not affect the rate of viral clearance or the extent of liver damage, thereby supporting this hypothesis. However, CD4
T cells may be necessary to instruct and maintain anti-HBV CTLs and the specific CTL response plays a significant role in viral clearance and the pathogenesis of liver damage (Lu, 2011).

In acute HBV infection, initial damage to the liver corresponds kinetically with the entry of HBV-specific CTLs into the liver. Furthermore, depletion of these cells at the peak of viremia delays the onset of liver damage and viral clearance in chimpanzees (Lu,2011).

The association of CTLs with liver injury is also observed in patients with acute viral hepatitis who successfully clear HBV and in patients with chronic HBV infection, CTLs seem to be suppressed, although low levels of CTLs exist in the infected liver. Reactivation of the killing mediated by CTLs usually leads to the clearance of HBV in patients with chronic infection (Lu, 2011).

2.12. Laboratory diagnosis

2.12.1. Serologic and virologic markers

Serological markers for HBV infection consist of HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBcIgM and IgG. The identification of serological markers allows to identify patients with HBV infection; to elucidate the natural course of chronic hepatitis B (CHB); to assess the clinical phases of infection; and to monitor antiviral therapy (Song and Kim, 2016).

Detection of antibodies to HBeAg and HBsAg is obscured during infection because the antibody is complexed with antigen in the serum(Murray *et al*., 2013).

2.12.1.1. Viral capsid surface antigen and the antibody directed against the surface antigen (anti-HBs)

Hepatitis B surface antigen (HBsAg) is the expressed protein on the surface of the virus and one of the early viral markers for HBV active or acute infection. HBsAg level in the serum is associated with cccDNA levels inside host hepatic cells, defining a clinical relevance of this marker (Al-Sadeq*et al.*, 2019).

Several studies have reported the association between transcription activity of cccDNA in the liver and serum HBsAg levels and differences in the serum HBsAglevels during the different phases of infection indicate the distribution of cccDNA during the respective phases of the disease. The serum HBsAg titers are higher in patients with HBeAg-positive CHB than in HBeAg-negative CHB and monitoring of quantitative HBsAg levels predicts treatment response to interferon and disease progression in HBeAg-negative CHB patients with normal serum alanine aminotransferase levels (Song and Kim, 2016).

Anti-HBs is known as a neutralizing antibody, and confer long-term immunity and in patients with acquired immunity through vaccination, anti-HBs is the only serological marker detected in serum. In the past HBV infection, it is present in concurrence with anti-HBcIgG (Song and Kim, 2016).

Occasionally, the simultaneous appearance of HBsAg and anti-HBs has been reported in patients with HBsAg positive and in most cases, anti-HBs antibodies are unable to neutralize the circulating viruses, thus these patients are regarded as carriers of HBV(Song and Kim, 2016).

2.12.1.2. Antibody directed against the core antigen (anti-HBc)

Hepatitis B core antigen is detected in infected hepatocytes, butis not released into serum; however, Immunoglobulin M antibody directed against HBcAg (anti-HBc) is usually the earliest anti-hepatitis B antibody detected in the infected patient (Frederick and Southwick, 2007).

The IgM anti-HBc is usually interpreted as a marker for early acute disease; however, in some patients, anti-HBcIgM levels can persist for up to 2 years after acute infection, and in patients with chronic active hepatitis, IgM antibody levels can rise during periods of exacerbation. An anti-HBcIgM titer is particularly helpful for screening blood donors, because this antibody is usually present during the window between HBsAg disappearance and anti-HBs appearance.

The Immunoglobulin G antibodies directed against the core antigen develop in the later phases of acute disease and usually persist for life (Frederick and Southwick, 2007).

2.12.1.3. Secreted core antigen (HBeAg) and its antibody (anti-HBe)

Secreted core antigen (HBeAg) marker indicates viral replication and risk of transmission of infection, and seroconversion of HBeAg to anti-HBe is associated with remission of liver disease. However, some anti-HBe reactive subjects continue to have active viral replication and hepatic disease caused by mutations in the pre-core and core region in the HBV genome, which reduces the production of HBeAg(Villar*et al*., 2015).

2.12.1.4. Polymerase chain reaction (PCR) test

PCR is a simple, yet elegant, enzymatic assay, which allows for the amplification of a specific DNA fragment from a complex pool of DNA (Garibyan and Avashia, 2013).

PCR can be performed using source DNA from a variety of tissues and organisms, including peripheral blood, skin, hair, saliva, and microbes. Only trace amounts of DNA are needed for PCR to generate enough copies to be analyzed using conventional laboratory methods. For this reason, PCR is a sensitive assay (Garibyan and Avashia, 2013).

It is based on the use of DNA fragment called the gene probe (39). Gene probe isrelatively small, single stranded DNA segment that can hunt for complementary fragment of DNA. To use a gene probe effectively, it is valuable to increase the DNA to be searched (Pommerville, 2004).Most PCR based methods of HBV DNA detection for clinical purposes have a sensitivity of 50-200 IU/mL with dynamic range of 4-5 log10 IU/m (Allain, 2004). In comparison, real-time PCR based assays have higher sensitivity (5-10 IU/mL) with a wider dynamic range 8-9 log 10IU/Ml(Datta*et al*., 2014).

2.12.1.4.1. Real time PCR

Real-time PCR is widely used for the quantitative detection of HBV DNA. Currently, most HBV DNA quantification reagents use one pair of primers and a single probe for a given HBV genotype test. If HBV genetic variations exist in these primer or probe regions, the actual viral load of HBV will be underestimated by the assay. Mutations in the probe region of the COBAS Amplicor test caused by lamivudine led to the underestimation of the HBV DNA level of a chronic hepatitis patient(Liu *etal*., 2017).

2.12.1.5. Hepatitis B viral DNA (HBV-DNA)

Quantitation of viral DNA in serum is most commonly used in the assessment of patients with chronic active hepatitis. In the patient with acute hepatitis, this test provides no significant advantages over that for HBeAg. Both tests indicate active viral replication and in patients with fulminant hepatitis, assays for HBV-DNA have been positive in the absence of other positive markers for HBV (Frederick and Southwick, 2007).

2.13. Treatment

Hepatitis B usually clears up on its own without treatment ,No antiviral therapy is typically used in acute hepatitis B. For chronic hepatitis B, entecavir (Baraclude) or tenofovir (Viread) are the drugs of choice. They are nucleoside analogues that inhibit the reverse transcriptase of HBV. Interferon in the form of peginterferon alfa-2a (Pegasys) is also used. Other nucleoside analogues such as lamivudine (Epivir-HBV), adefovir (Hepsera), and telbivudine (Tyzeka) are used less frequently. A combination of tenofovir and emtricitabine (Emtriva) is also used. These drugs reduce hepatic inflammation and lower the viral load of HBV in patients with chronic active hepatitis. Neither interferon nor the nucleoside analogues cure the HBV infection. In most patients when the drug is stopped, HBV replication resumes (Levinson, 2014).

2.14. Prevention

Prevention is far simpler than treatment, particularly in the case of HBV, which requires lifelong treatment in most cases. Besides avoiding transmission from infected people via blood supply screening and universal precautions, vaccination is the most important means of reducing the global burden of disease (Rajbhandari and Chung, 2016). Wherever possible, immunization before exposure to HBV is preferred (Joshiand Kumar, 2001). Efficient HBV vaccines have been available since the early 1980s (Jourdain*et al*., 2016).

Vaccination in adults is recommended in high-risk groups at risk for infection by sexual exposure (e.g., men who have sex with men, people with multiple sexual partners, those seeking evaluation and treatment for sexually transmitted disease), or in persons at risk for infection by percutaneous or mucosal exposure to blood (e.g., injection drug users, household contacts of HBsAg+ patients, patients on hemodialysis,

institutionalized patients, health-care workers, and public safety workers) (Rajbhandari and Chung, 2016).

Vaccination is also recommended in international travelers to regions with high or intermediate endemicity for HBV infection, persons with chronic liver disease, and with HIV infection. Vaccination in children is recommended as part of the regular schedule of childhood immunizations. Thirty-five years after the availability of a safe and effective vaccine, universal vaccination of all children is finally available now in 184 of 196 countries in the world .Global vaccine coverage with all three doses of vaccine is estimated at 82% (Rajbhandari and Chung, 2016).

2.14.1. Active Immunization

Prevention of primary infection by vaccination is an important strategy to decrease the risk of chronic HBV infection and itssubsequentcomplications.These includes either, Purified, noninfectious 22-nm spherical forms of HBsAg derived from the plasma of healthy HBsAg carriers or Plasma-derived vaccine, supplanted by a genetically engineered vaccine derived from recombinant yeast, consisting of HBsAg particles that are nonglycosylated but are otherwise indistinguishable from natural HbsAg(Weinbaum*et a*l., 2009) .Active immunization by the vaccine yields long-term immunity. Because in endemic areas the major infection route comes from maternal transmission and the outcome of perinatal transmission results in a very high rate (90%) of chronic infection and the best timing of initial HBV immunization, therefore, should be within 24 h after birth, followed by subsequent doses of HBV vaccine during infancy (Chang and Chen, 2015).

The development of an HBV vaccine using HBsAg protein from HBV carriers as the immunogen to induce anti-HBs, the protective antibody against HBV infection, is a successful pioneer in the history of vaccine development. During the past three decades, it is proved to be safe and successful in protecting people from HBV infection and the related diseases worldwide (Chang and Chen, 2015).Vaccination strategies against HBV include administration of traditional HBsAg vaccine, human anti-HBV surface antibody (anti-HBs), T cell vaccine, DNA vaccines, apoptotic cells expressing HBV antigens, and viral vectors expressing HBV proteins . Parenteral HBV immunoglobulin is occasionally used to provide instant protection until an effective

response in the host immune system occurs and also among individuals who do not form an effective immune response to conventional HBV vaccination (Das *et al*., 2019).

With regard to HBV protection, both monovalent and combined vaccines were found to provide similar sero-protection or vaccine response rates and HBV vaccines are available as a single-antigen formulation and in combination with other vaccines (Das, S., 2019). The single antigen vaccines are recommended for use at birth and the combined vaccines are usually not recommended at birth (Pediatrix‘ for individuals aged 6 weeks–6 years and Twinrix‘ for individuals aged ≥18 years)(Das *et al*., 2019).In the Islamic Republic of Iran (I.R. Iran), mass vaccinationof neonates against HBV infection began in 1993 as a national program in routine neonatal care. The program was supposed to affect the prevalence rate of HBV infection through the country and decrease the rate of infection. Therefore, a recent study in Iran showed that the rate of hepatitis B carriers varied between zero and 3.9% with an average of 1.7%.(Zali*et al*., 2003).

Also, 3.6% of the population was HBsAg positive, putting Khorassan among the highly affected areas in Iran.4,5 HBV prevalence has decreased dramatically in the Iranian population during the last decade.(Alavian 2007).

Generally, it is estimated that approximately 1.5 to 2.5 million people are suffering from HBV infection in I.R. Iran, and some of them are carriers who may unintentionally transmit the infection to others. (Zali, *et al*.,2003.,Alavian.,2007).

2.14.2. Passive immune prophylaxis

The best way for protecting nurses , other health care workersand other high risk groups is to combine passive and active immunization.

Hepatitis B immunoglobulin (HBIG) is a purified solution of human immunoglobulin that could be administered to the mother, newborn, or both and it offers protection against HBV infection when administered to pregnant women who test positive for HBeAgorHBsAg, or both. When HBIG is administered to pregnant women, the antibodies passively diffuse across the placenta to the child to protect against HBV infection. This works best during the last third of pregnancy(Eke *et al*., 2017).

Approximately 20years ago, China implemented an HBV immune prophylaxis strategy, which led to a 90% reduction (to 0.96%) in HBsAg carriers <5 years old by 2006.

Approximately 90% of infants born to both HBsAg-positive and HBeAg-positivemothers will become HBsAgcarriers if no immunoprophylaxis is given. The most effective way to prevent mother-to-child transmission (MTCT) of HBV infection is by immunizing all susceptible individuals with adequate hepatitis B immune globulin (HBIG) and hepatitis B vaccines at birth, especially newborn infants born to HBV-positive mothers(Wang *et al.*, 2016).

Standard HBV vaccination with or without HBIG for newborns born to HBeAg-positive mothers should affect the rate of chronic HBV progression. HBVvaccination combined with HBIG for newborns born to HBeAg-positive mothers can reduce the chronic progression of HBV infection acquired prenatally(Zixiong*et al*., 2015).

2.15. Previous studies

There were no specific studies related to the subject so the studies here wereexcluded from studies talks about health care worker in general.

Astudy among the health care workerinCameroon,who participated in this study, Nurses were (n= 224/398 (56.3%)) of the study, The prevalence of HBsAg positivity in nurses was 28/224 nurse (12.5%),Among the health care worker , 272/398 (68.3%) were women. The (16–25) years old age group was the most represented (167, 42.0%). (Akazong*et al*., 2020).

A study among the health care worker in Mozambique ,most of the 315 HCWs in the study 39.7% (125/315) in the professional category of nurses The prevalence of HBsAg positivity in nurses was 8/125 nurse (6.4%), Among the health care worker , 187/315 (59.4%) were women. The (20–39) years old age group was the most represented (167, 42.0%).Most HCWs weremarried77.8% (245/315)..(Mabunda*et al.*, 2022).

While in Sudan at different Khartoum hospitals, A total of 245 health workers partici-pated in this study, 168 (68.6%) females and 77 (31.4 %) males. which found that; 12 (4.9%) were positive for HBsAg. The nurses in this study were 73/245(29.8%),The prevalence of HBsAg positivity in nurses was 6/73 nurse (8.2%). (Elduma and Saeed ,2011).

Also in khartoum study in health care worker, a total of 90 HCWs enrolled in the study, HBsAg was detected in 4 (4.4%). The positivity of HBsAg, in this study, was more among males and staff more than 50 years old. Abdalwhab and Nafi, (2014).

CHAPTER III

MATERIALSAND METHODS

CHAPTER III

3. Materials and methods

3.1.Study design

This is cross sectional prospective descriptive hospital based study.

3.2.Study area

El Obeid Educational hospital and El Obeidspecialized pediatric Hospital

3.3.Study duration

The study was carried out during May2022 to October 2022.

3.4.Study population

Nurses works in specialized pediatric hospital or El Obeid Educational hospital in El-Obeid city.

3.5.Inclusion criteria

Nurses in specialized pediatric hospital and El Obeid Educational hospital during the study period were the candidates of the study.

3.6.Exclusion criteria

Nurses who were known HBV positive and who refused to participate in study.

3.7.Sample size

Seventy eight(n=78)

3.8.Sampling technique

non probability sample-convienence

3.9.Ethical consideration

Ethical approval was obtained from the Scentific Research Committee of College Medical Laboratory Science Sudan University of Science and Technology and Also from the Health Services Director in the North Kordofan State (El Obied city) and verbal consent was obtained from participants before collection of thedemographic and clinical

Data .

3.10.Data collection

A structured questionnaire was used to collect demographic and clinical

Data (Appendix4).

3.11. Lab processing

Under sterile condition 3 ml of venous blood sample was withdrawn from each participant, the sample were let to clot on bench and serum was separated by centrifugation at 5000rpm for five minutes, serum wascollected into plain containers then stored at -20C˚ until analysed.

3.12. Laboratory investigation

The surface antigen (HBsAg) was screened by HBsAg - ELISA Kit.

3.12.1. ELISA technique: Detection of HBsAg using ELISA

Fortress HBsAg is an *in vitro* diagnostic kit for the detection of hepatitis B surface antigen (HBsAg) in human serum or plasmait wasSandwish

3.12.2. Assay procedures

The reagent and samples were allowed to reach room temperature. The wells including two negative control (B1, C1) and one blank(A1), (A1, neither samples nor HPR conjugate should be added into the blank wells) and D1, E1 positive control. Twenty ul of sample diluents was added to each well except the blank and mixed by toping the plate gently,then 100ul of positive control and negative control and specimen were added to theirrespective wells by using separate disposable tip for each specimen negative control and positive control to avoid contamination. Then incubated at 37 ˚C for 45 minute. Then 50 ulHRP conjugate was added to each well except the blank and mixed by tapping the plate gently. The plate was then covered and incubated for 30minutes at 37˚C.After incubation, the cover was removed and the plate content was discard. Each well was washed 5 times with diluted wash buffer.After washing dispense 50ul of chromogen A and 50ul of chromogen B solutions was added into each well including the blank and mixed by tapping the plate gently.was incubated at 37˚C for 15 minutesStopped the reaction by using a multichannel pipette, added 50ul stop solution into the each well and mixed gently the absorbance measured at 450 nm. and calculated the cut-off value and evaluated the result and read the absorbance within 5 minutes after the stopping the reaction .

3.12.1.2 Interpretation of results

Cut off value was calculated accourding to following formula:

Cutoff value (C.O.) =*NC*2.1

*NC=the mean absorbance value of two negative controls.

Negative result: sample gave an absorbance less than the 1.0 value are considered negative, which indicates no HBsAg has been detected with this HBsAg ELISA kits.

Positive result: sample gave an absorbance greater than the 1.0 are considered initially reactive, which indicates HBsAg has been detected with this HBsAg ELISA kit.

3.13. Method of data analysis:

The data that collected from questionnaire and laboratory results was analysed byStatistical Package For Social Scienceversion 26 computerized program – using Cross tabulation chi square test.

P.value of < 0.05 was considered as significant for all study results**.**

CHAPTER IV

RESULTS

CHAPTER IV

4. RESULTS

A total of (n=78) blood specimen were collected from nurses in Hospitals of El-Obeid city were included in this study, in which 7 (9%) were males and 71 (91%) were females (table (4.1)

Table (4.1): Distribution of nurses according to gender group.

Gender	Frequency	Percent	
Female	71	91%	
Male	7	9%	
Total	78	100%	

Mostly were at age between 20-30 years (62.8%) (table (4.2).

Table (4.2):Distribution of nurses according to age group

Age group	Frequency	Percent
20-30	49	62.8%
31-40	14	18%
41-50	6	7.7%
>50	9	11.5%
Total	78	100%

Majority of nurses education level were diploma(74.4%) followed by bachelor (24.4%) and master (1.2%) as showed in (table (4.3).

Table (4.3): Distribution of nurses according to education level.

Educational level	Frequency	Percent
Bachelor	19	24.4%
Diploma	58	74.4%
Master	1	1.2%
Total	78	100%

Majority of nurses were single (64%) , (35%) were married and 1% was divorced as showed in table (4.4).

Table (4.4): Distribution of study group according to marital status

Marital status	Frequency	Percent
Divorced	1	1%
Married	27	35%
Single	50	64%
Total	78	100%

From the total of 4 positive results,There was 3 (75%) females positive for HBs Ag and 1(25%) male was positive as showed in table (4.5).

Table (4.5): Frequency of anti HBsAg antibodies among study group.

Result of HBV	Frequency	Percent
Negative	74	95%
Positive	4	5%
Total	78	100%

The percentage of femalewas more than male. There were insignificant correlation between gender and positivity of HBs Ag as showed in (table (4.6).

Table (4.6): association HBV infection and gender

Gender	Result of HBV		Total	*p.value*
	Negative	Positive		
Female	68(91.9%)	3(75%)	71(91%)	0.25
Male	6(8.1%)	1(25%)	7(9%)	
Total	74(100%)	4(100%)	78(100%)	

According to age groups, there weretwo (50%) of positive result were between 20-30 years , one (25%) in age range 30-40 years , and one (25%) in more than 50 years were positive for HBs Ag and there was insignificant correlation between age groups and HBs Ag positivity*p.value* = 0. 75 as explained in (table (4.7).

Table (4.7): association HBV infection and age group

Age group	Result of HBV		Total	*p.value*
	Negative	Positive		
20-30	47(63.5%)	2(50%)	49(62.8%)	0.75
31-40	13(17.6%)	1(25%)	14(17.4%)	
41-50	6(8.1%)	0(0%)	6(7.7%)	
>50	8(10.8%)	1(25%)	9(11.5%)	
Total	74(100%)	4(100%)	78(100%)	

There weretwo (50%) nurses had accidental needle stick and two (50%) didn't expose to needle stick as showed in (table (4.8).

Table (4.8): Association between of HBV infection and history of needle stick.

historyof needle stick.	result of HBV		Total	*p.value*
	Negative	Positive		
No	44(59.5%)	2(50%)	46(59%)	.71
Yes	30(40.5%)	2(50%)	32(41%)	
Total	74(100%)	4(100%)	78(100%)	

There was one (25%) nurses had blood transfusion and surgical operation and three (75%) didn't expose to any of the possible risk factors were HBs Ag positive and there was no association between sero-positivity of HBsAg and accidental needle stick injury, hemodialysis, previous surgical operation ,vaccination and blood transfusion as explained in table (4.9).

Table (4.9):The association between HBs Ag results and possible risk factors among nurses.

Risks factors		result of HBV		Total	*p.value*
		Negative	Positive		
Blood transfusion	No	43 (58.1%)	3 (75%)	46 (59%)	.50
	yes	31 (41.9%)	1 (25%)	32 (41%)	
Surgical operation	No	56 (75.7%)	3 (75%)	59 (75.6%)	.98
	yes	18 (24.3%)	1 (25%)	19 (24.4)	
Hemodialysis	No	72 (97.3%)	4 (100%)	76 (97.4%)	.74
	yes	2 (2.7%)	0 (0%)	2 (2.6%)	
Vaccination	No	60 (81.1%)	4 (100%)	64 (82.1%)	.34
	yes	14 (18.9%)	0 (0%)	14 (17.9%)	

CHAPTER V

Discussion, Conclusion and Recommendation

CHAPTER V

5. DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1. Discussion

HBV is a serious growing problem in African and Arab countries, and they have great affect on community and future generation health. So we need great effort to conduct studies that may give guidelines for proper planning to deal with the health problem that related to HBV infection, chronic infection with HBV is also noted to be associated with development of hepatocellular carcinoma (Nabil, 2005).

In this study there were 78 nurses was investigated for HBs Ag at El-Obeid city and only 4/78 (5.1%) were positive for HBs Ag, which wasnearly similar to those reported from khartoum by Abdalwhab and Nafi, (2014) which was (4.4%) and Mozambique by Mabunda, N.,2022), in Which (6.4%) was positive.but slightly lower than study conducted by Akazong*et al*(2020*).* In Cameroon which was(12.5%).

This differences may be due to the different levels of exposure to potential risks environment and could be due to different in sample size.In the present study the frequency was more common in females (3/4(75%))of posivity, which was similar with the result conducted in Khartoum which was 168 (68.6%) females and 77 (31.4 %) males.According to age groups, there was 2/4 (50%) from the positive result between 20-30 years and 1/4 (25%) between 30-39 and the other 1/4 (25%) from more than 50 years, which was different with the result conducted in Khartoum..(Elmukashfi*et al*., 2012).

The age group representation was 58.4% for the age group 30-49 while in cameroon The (16–25) years old age group was the most represented (167, 42.0%).(Akazong*et al*., 2020)..There was no significant association between sero-positivity of HBsAg and accidental needle stick injury, hemodialysis, previous surgical operation and blood transfusion but in study conducted in a Tertiary Hospital in Tanzania which was done for health care workers showed that some risk factors (needle stick injuries, blood transfusion, operation and intramuscular/intravenous drug administration) were

found to be significantly associated with chronic hepatitis B infection (HBsAg+) (Mueller *etal*., 2015).

5.2. Conclusions

The frequency of hepatitis B virus infection among nurses was normal, and the frequency of infection was higher in females than males, And the higher percentage of HBV infection observed in the age group 20-30 years.

5.3. Recommendations

Nurses should be screened regularly for Hepatitis B virus and other blood-borne infections, To eradicate the infection among nurses a vaccination program must be set place by the Ministry of Health for all of HCWs, and ensure they are assessed for immunity (post-vaccination management), Health care facilities must be improved, seminars and workshops on laboratory safety and laboratory management should be established by the Ministry of Health,Increase the Educational level about the virus, it is transmission and prevention for the nurses, and further studies with large number of samples are required to validate the results of the present study.

References

References

Abdallah T M, Mohamed MH, and Ali AA (2011).Seroprevalence and epidemiological factors of hepatitis B virus (HBV) infection in Eastern Sudan. Inte. J. Med. Med. Sci; 3(7): 239-241.

Abdalwhab, M and Nafi, M. (2014).Sero- frequency of Hepatitis B infection among health care workers in Khartoum. American Journal of Research Communication, 2 (12):148-154.

Akazong W, E.,Tume, C., Njouom, R., Ayong, L., Fondoh, V. and Kuiate, J., (2020). Knowledge, attitude and prevalence of hepatitis B virus among healthcare workers: a cross-sectional, hospital-based study in Bamenda Health District, NWR, Cameroon. BMJ Open, 10(3), p.e031075.

Alavian, Seyed. (2007). Ministry of Health in Iran Is Serious about Controlling Hepatitis B. Hepatitis Monthly. 7.

Allain, P.J. (2004). Occult hepatitis B virus infection Transfusion. Clinique etBiologique, 11(1):18-25.

Al-Sadeq, W.D., Sara, A.T., Roan, E., Aied., S.M., Maria, K.S., Balsam, R.R., Asmaa, A., HadiM.Y and Gheyath, K. (2019). Hepatitis B Virus Molecular Epidemiology, Host-Virus Interaction, Co-infection and Laboratory Diagnosis in the MENA Region. Pathogens, 8 (2): 63.

Annals of Internal Medicine, (2017).Hepatitis B Vaccination, Screening, and Linkage to Care.167(11), p.I-22.

Badawi, M.,Atif, M. and Mustafa, Y., (2018). Systematic review and meta-analysis of HIV, HBV and HCV infection prevalence in Sudan.Virology Journal, 15(1).

Barker, L., 1970.Transmission of Serum Hepatitis. JAMA: The Journal of the American Medical Association, 211(9), p.1509.

Chang, H.M and Chen, S.D. (2015). Prevention of Hepatitis B. Cold Spring Harbor Perspectives in Medicine, 5(3).

Cooksley, W.,(2010). Do we need to determine viral genotype in treating chronic hepatitis B?.Journal of Viral Hepatitis, p.no-no.

Cornelissen, M., Fokla, Z., Sylvia, M., Margreet, R., Ben, B and Antoinette, C.K. (2016). Widespread hepatitis B virus genotype G (HBV-G) infection during the early years of the HIV epidemic in the Netherlands among men who have sex with men. Bio Med Central Infectious Diseases, 16: 268.

CornellissenCN , Fisher BD and Harvey RA(2012) .Hepatitis B and Hepatitis D (Delta Viruses . in :Lippinocotts illustrated reviews . Microbiology .3rd ed .lippincotts Williams and Wilkins , Philadelphia . Pp.273-282.

Das, S., Kirubakaran, K., Sapan, P.B., Mahalakshmi, G., Alphienes. S.X., andSandhiya S(2019). Hepatitis B Vaccine and Immunoglobulin: Key Concepts. Journal of Clinical and Translational Hepatology, 7(2): 165–171.

Datta, S., Chatterjee, S. and Veer, V. (2014). Recent advancesin molecular diagnostics of hepatitis B virus. World Journal of Gastroenterology, 20 (40): 14615–14625.

Elduma, A.H. and Saeed, N.S. (2011) "Hepatitis B virus infection among staff in three hospitals in Khartoum, Sudan, 2006-07," Eastern Mediterranean Health Journal, 17(06), pp. 474–478. Available at: https://doi.org/10.26719/2011.17.6.474.

Eke, A.C., George, U.E., Uzoamaka, A.E., Yun, X , and Jiao, L (2017). Hepatitis B immunoglobulin during pregnancy for prevention of mother-to-child transmission of hepatitis B virus. Cochrane Database System Review, 17 (2).

Elmukashfi, T.A., Omer, A.I., Isam, M.E., Abdelgadir, A.B and Mohammed, A.A. (2012). Hazards Analysis, within Departments and Occupations, for Hepatitis B Virus among Health Care Workers in Public Teaching Hospitals in Khartoum State Sudan. Global Journal of Health Science, 4 (6): 51–59.

Faseeha, N. (2015). Hepatitis B virus infection: An insight into infection outcomes and recent treatment options Virus disease, 26 (1-2): 1–8.

Frederick, S. and Southwick, M.D. (2007).Infections disease A clinical short course. 2ndEd. USA: McGraw - Hill Companies.

Ganczak, M., Topczewska, K. Maria, B., and Marcin K., (2019). Seroprevalence of anti-HBc, risk factors of occupationally acquired HBV infection and HBV vaccination

among hospital staff in Poland: a multicenter study. Bio Med Central Public Health, 19: 298.

Garibyan, L and Avashia, N. (2013). Research Techniques Made Simple: Polymerase Chain Reaction (PCR). Journal of Investigative Dermatology, 133 (3):6.

Geo. F . Brooks, Karen C. Carroll , Janet S. ButelandStephen A. Morse March (2010). Basic pathology.Pp (533-684).

Guvenir, M. and Arikan, A., 2020. Hepatitis B Virus: From Diagnosis to Treatment. Polish Journal of Microbiology, 69(4), pp.391-399.

Hisham, Z., El-Hattab, M., Shingheer, N., Zorgani, A.andElahmer, O. (2013). Hepatitis B vaccination status among healthcare workers in a tertiary care hospital in Tripoli, Libya.Journal of Infection and Public Health, 6: 246—251.

Hollinger FB and Liang TJ. (2001). Hepatitis B virus. In: knipe DM et al. ,eds .Feilds Virology,4th ed. Philadelphia, LipponcottWilliamsandwilkins, 2971-3036.

Huang, C.,Kuo, T., Yeh, C., Hu, C., Chen, Y., Tsai, Y., Chen, M., Chou, Y. and Chang, C., (2013). One single nucleotide difference alters the differential expression of spliced RNAs between HBV genotypes A and D. Virus Research, 174(1-2), pp.18-26.

Jawetz,Melnick, andAdelberg(2007).Hepatitis B virus anddisease.Medical Microbiology, 24thEdition.The McGraw-Hill companies.UAS.Pp 470-498.

Jayalakshmi, M.K., Kalyanaraman, N. and Pitchapp, R. (2013) "Hepatitis B virus genetic diversity: Disease pathogenesis," Viral Replication [Preprint]. Available at: https://doi.org/10.5772/53818.

Jennifer H, MacLachlan, Benjamin C. Cowie. (2015). Hepatitis B virus epidemiology. PMC. 5(5):a021410.

Jha, A.,Chadha, S., Bhalla, P. and Saini, S., 2012. Hepatitis B Infection in Microbiology Laboratory Workers: Prevalence, Vaccination, and Immunity Status. Hepatitis Research and Treatment, 2012, pp.1-5.

JinlinHou, Zhihua Liu, Fan GU.(2005). Epidemiology and Prevention of Hepatitis B Virus Infection.PMC. 2(1):50–57.

Joshi, N and Kumar, A. (2001). Immunoprophylaxis of hepatitis B virus infection. Indian journal of microbiology, 19 (4): 172-183.

Jourdain, G.HuongN,CresseyT,Harrison L(2016). Prevention of mother-to-child transmission of hepatitis B virus: a phase III, placebo-controlled, double-blind, randomized clinical trial to assess the efficacy and safety of a short course of tenofovirdisoproxilfumarate in women with hepatitis B virus e-antigen. Bio Med Central Infectious Diseases, 16: 393.

Keeffe EB, Dieterich DT, Han SH. (2008). A treatment algorithm for the management of chronic hepatitis B virus infection in the United States. CGH. 6(12):1315-1341.

Koziel, MJ. and Siddiqui , A. (2010).Hepatitis B Virusand Hepatitis Delta Virus in Mandell GL, BennttJE and Dolin R. Principles and Practice of Infectious Diseases , 7th Ed. Elsevier: Philadelphia.

Kumar, S. (2016). Chapter 67: Essentials of Microbiology. 1stEd.New Delhi: Jaypee Brothers Medical Publishers, p.p. 467-471

Kumar, V., Abbas, A.K. and Aster, J.C. (2013).Robbins Basic Pathology.Ninth Edition.Elsevier Canada: Saunders, p.p. 615.

Levinson, W. (2014). Review of Medical Microbiology and Immunology. Thirteenth Edition. United States: McGraw-Hill Companies, p.p. 331, 334.

Lin, C.-L.and Kao, J.-H.(2015) "Hepatitis B virus genotypes and variants," Cold Spring Harbor Perspectives in Medicine, 5(5). Available at: https://doi.org/10.1101/cshperspect.a021436.

Liu, C., Chang, L., Jia, T., Guo, F., Zhang, L., Ji, H., Zhao, J. and Wang, L. (2017). Real-time PCR assays for hepatitis B virus DNA quantification may require two different targets. Journal of Virology, 14: 94.

Lu, X. (2011). Pathogenesis of Hepatitis B Virus (HBV)-Mediated Liver Injury North American. Journal of Medicine and Science, 4 (1): 1.

Mabunda, N., Vieira, L., Chelene, I., Maueia, C., Zicai, A., Duajá, A., Chale, F., Chambal, L., Vubil, A. and Augusto, O., 2022.Prevalence of hepatitis B virus and immunity status among healthcare workers in Beira City, Mozambique.PLOS ONE, 17(10), p.e0276283.

Mahgoub, S., Daniel, C., Magdy, E. and Jean, P.A. (2011). Hepatitis B Virus (HBV) Infection and Recombination between HBV Genotypes D and E in Asymptomatic

Blood Donors from Khartoum, Sudan. Journal of Clinical Microbiology, 49 (1): 298–306.

Mahmood, M., Muhammad, A.A., Azra, K.,Nasib, Z and Abida, R. (2016). Distribution and clinical significance of hepatitis B virus genotypes in Pakistan. Bio Med Central Gastroenterology, 16 (1): 104.

Mahoney D and Kane R. (1999).Liver biopsy assessment in chronic viral hepatitis.J Viral Hepat. 14(1): 16-21.

Malagnino, V. (2018). High rates of chronic HBV genotype E infection in a group of migrants in Italy from West Africa: Virological characteristics associated with poor immune clearance. PLoS One, 13 (3): 0195045.

Medicalnewstoday.com. 2022. Hepatitis B: Causes, symptoms, and treatment. [online] Available at: <https://www.medicalnewstoday.com/articles/306288> [Accessed 15 October 2022].

Medicalnewstoday.com. 2022. Viral hepatitis: Types, symptoms, and prevention. [online] Available at: <https://www.medicalnewstoday.com/articles/145869> [Accessed 15 October 2022].

Morse March 2010 P(533-684).

Moura, I., Lopes, E., Alvarado-Mora, M., Pinho, J. and Carrilho, F., 2013.Phylogenetic analysis and subgenotypic distribution of the hepatitis B virus in Recife, Brazil.Infection, Genetics and Evolution, 14, pp.195-199.

Mudawi, H. M.(2008). Epidemiology of viral hepatitis in Sudan.Clinicaland Experimental Gastroenterology,1:9–13.

Mudawi, H., 2008. Epidemiology of viral hepatitis in Sudan.Clinical and Experimental Gastroenterology, p.9.

Mueller, A.,Stoetter, L., Kalluvya, S.,Stich, A., Majinge, C.Weissbrich, B. and Kasang, C.(2015). Prevalence of hepatitis B virus among health care workers in tertiary hospital in Tanzania. Bio Med Central Infectious Disease, 23 (15):386.

Murray RP, Rosenthal SK, Kobayashi SG and PfallerAM.(2002). Hepatitis viruses. In: Medical microbiology 4th ed. Mosby, united state of America. Pp 591-605.

Murray, P.R., Rosenthal, K.S and Pfaller, M.A. (2013).Medical Microbiology.Seventh Edition. China: Elsevier Saunders, p.p. 592.

N. and Burnett, R. J., et al. (2016). High prevalence of active and occult hepatitis B virus infections in healthcare workers from two provinces of South Africa. Vaccine, 34(33): 3835-3839.

Nabil , HB . (2005). Health and environmental effects of pesticides.Sudanese J Dermatol.3(1):6-8. 31

NCBI (2017).

Nelson, N., Easterbrook, P. and McMahon, B., 2016. Epidemiology of Hepatitis B Virus Infection and Impact of Vaccination on Disease.Clinics in Liver Disease, 20(4), pp.607-628.

Omata, M.Cheng,A., Kokudo,N.(2017). Asia–Pacific clinical practice guidelineson the management of hepatocellular carcinoma. Hepatology International, 11 (4): 317–370.

Pommerville, j.(2004).AlcamosFundamentals of Microbiology. 7th Ed. London: Oxford University press.

Prieto, A.O and Dorner, M. (2017).Immune Evasion Strategies during Chronic Hepatitis B and C Virus Infection. Vaccines (Basel), 5 (3): 24.

Rajbhandari, R and Chung, T.R. (2016). Treatment of Hepatitis B: A Concise Review. Clinical and Translational Gastroenterology, 7(9):190.

Schillie, M.D.,Vellozzi, C., Reingold, A., Harris, A., Haber, P., Ward, J.W. and Nelson, N.P.(2018). Prevention of Hepatitis B Virus Infection in the United States: Recommendations of the Advisory Committee on Immunization Practices. MMWR Recommendations and Reports, 67(1): 1–31.

Seeger.C, Mason .W.S.(2016), Molecular Biology of Hepatitis B Virus Infection, Virology, 672-686.

Shi, W., Zhang, Z., Ling, C., Zheng, W., Zhu, C., Carr, M. and Higgins, D., 2013. Hepatitis B virus subgenotyping: History, effects of recombination, misclassifications, and corrections. Infection, Genetics and Evolution, 16, pp.355-361.

Shuping, T.,Jisu, L.i., Jack, R. W. and Yu-mei Wen2 (2013). Hepatitis B virus geneticvariants:biological properties and clinical implications. Emerging Microbes and Infections, 2 (3): 10.

Sondlane, T.,Mawela, L., Razwiedani, L., Selabe, S., Lebelo, R., Rakgole, J., Mphahlele, M., Dochez, C., De Schryver, A. and Burnett, R., (2016). High prevalence of active and

occult hepatitis B virus infections in healthcare workers from two provinces of South Africa.Vaccine, 34(33), pp.3835-3839.

Song, E.Jand Kim, Y.D. (2016). Diagnosis of hepatitis B. Annals of Translational Medicine's, 4(18): 338.

Spearman CW, Sonderup MW, Botha JF, van der Merwe SW, Song E, Kassianides C, Newton KA, Hairwadzi HN; (2013). South African guideline for the management of chronic hepatitis B: 2013. S Afr Med J. 2013 May;103(5 Pt 2):337-49. PMID: 23967497.

Tan, A., Koh, S. andBertoletti, A. (2015). Immune Response in Hepatitis B Virus Infection. Cold Spring Harbor Perspectives in Medicine, 5 (8).

Velkov, S., Jördis, J. and Thomas, M.(2018). The Global Hepatitis B Virus Genotype Distribution Approximated from Available Genotyping Data. Genes (Basel), 9 (10): 495.

Villar , M.L., Cruz, H.M., Barbosa. J.R., Bezerra, C.S.,Portilho, M.M. and Scalioni, L. (2015).Update on hepatitis B and C virus diagnosis. World Journal Virology, 4 (4): 323–342.

Vishal, B.,Amitava, G., Sunil, D., Dinesh, K and Bhargava, N. (2015). Hepatitis B immunization in healthcare workers. Annals of Gastroenterology, 28 (2): 276–280

Wang, C., Wang, C., Jia, Z.F., Wu, X., Wen, S.M., Kong, F., Hu, K.Q., Li, J. and Jiang, J, N,J. (2016). Protective effect of an improved immunization practice of mother-to-infant transmission of hepatitis B virus and risk factors associated with immune prophylaxis failure. Medicine (Baltimore), 95 (34).

Weinbaum CM, Mast EE and Ward JW. (2009). Recommendations for identification and public health management of persons with chronic hepatitis B virus infection. Hepatology. 49:35-44.

Winston. E. Abara .MD, Sarah . F,.Schillie .MD and MPH,(2014), Hepatitis B, VPD

Surveillance Manual Hepatitis B: Chapter 4.1 :1-15.

WHO.int.(2022) . Hepatitis B. [online] Available at: <https://www.who.int/news-room/fact-sheets/detail/hepatitis-b> [Accessed 15 October 2022].

WHO.int.(2022b). Global progress report on HIV, viral hepatitis and sexually transmitted infections, 2021. [online] Available at: <https://www.who.int/publications-detail-redirect/9789240027077> [Accessed 15 October 2022].

Xu, Z.H., Yun-Peng, L., Bayasi, G. andJian-Lin, R.(2014). Hepatitis B Virus-Related Hepatocellular Carcinoma: Pathogenic Mechanisms and Novel Therapeutic Interventions. Gastrointest Tumors, 1 (3): 135–145.

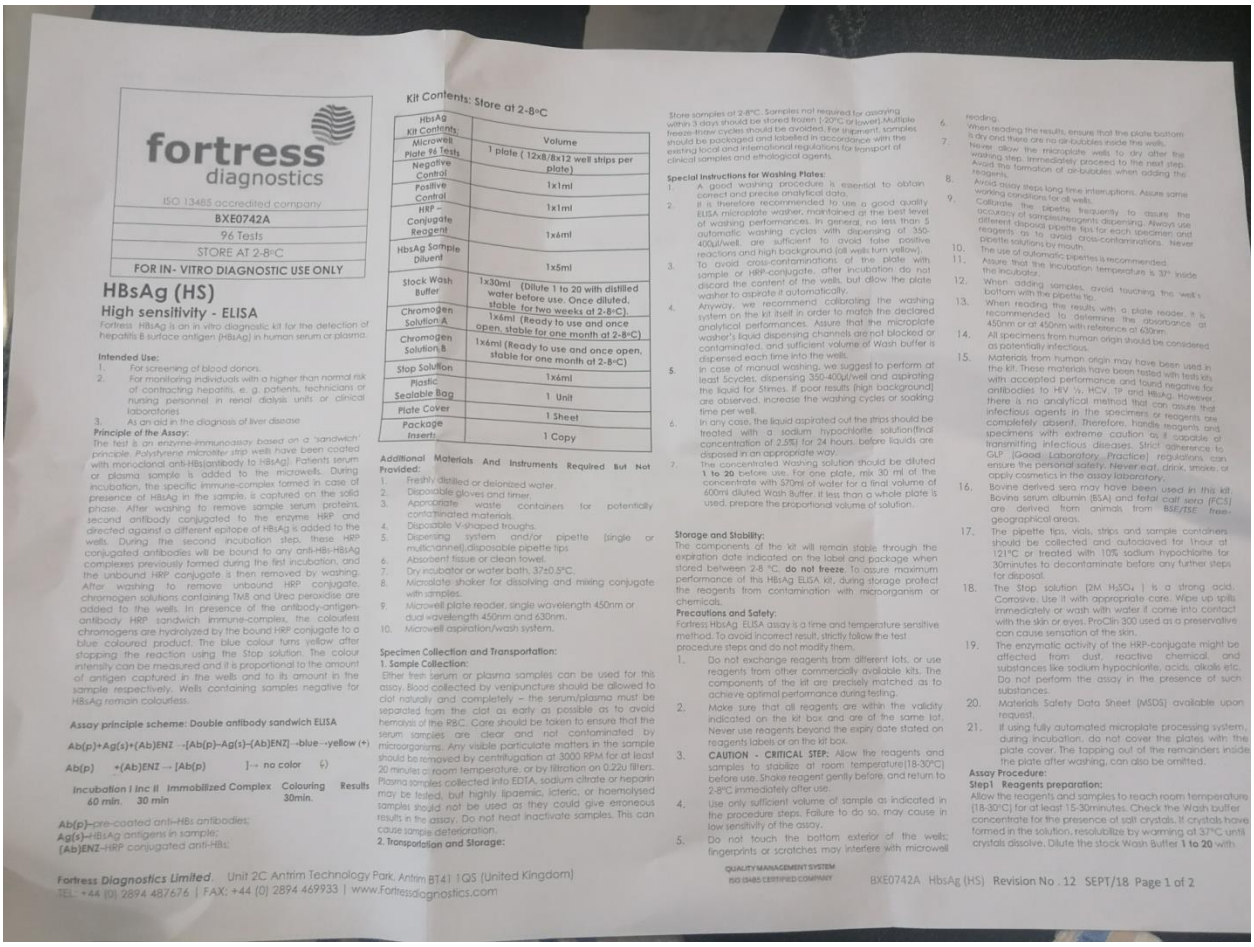
Yang, A.,Guo, Z., Ren, Q., Wu, L., Ma, Y., Hu, A., Wang, D., Ye, H., Zhu, X., Ju, W. and He, X. (2017). Active immunization in patients transplanted for hepatitis B virus related liver diseases: A prospective study. PLoS One, 12(11).

Zali, M., NooriNayer, B., Shahraz, S., Sherafat, R., Mohammad, K., Noorbala, M. and Mohraz, M., (2003).Rate of HBS AG positivity in Iran following hepatitis B mass vaccination.Journal of Hepatology, 38, p.184.

Zhao, H., Hu, Y., Han, Q. and Zhang, J., (2022). Innate and adaptive immune escape mechanisms of hepatitis B virus.World Journal of Gastroenterology, 28(9), pp.881-896.

Zixiong, L.,Xiaomei, H. and Guangwen, C. (2015). Is mother-to-infant transmission the most important factor for persistent HBV infection. Emerging Microbes & Infections, 4 (5).

Appendix I Fortress diagnostics for HBsAg (HS) High sensitivity – ELISA



fortress diagnostics

ISO 13485 accredited company

BXE0742A

96 Tests

STORE AT 2-8°C

FOR IN- VITRO DIAGNOSTIC USE ONLY

HBsAg (HS)

High sensitivity - ELISA

Fortress HBsAg is an in vitro diagnostic kit for the detection of hepatitis B surface antigen (HBsAg) in human serum or plasma.

Intended Use:

1. For screening of blood donors.
2. For monitoring individuals with a higher than normal risk of contracting hepatitis, e. g. patients, technicians or nursing personnel in renal dialysis units or clinical laboratories
3. As an aid in the diagnosis of liver disease

Principle of the Assay:

The test is an enzyme-immunoassay based on a 'sandwich' principle. Polystyrene microtitre strip wells have been coated with monoclonal anti-HBs(antibody to HBsAg). Patients serum or plasma sample is added to the microwells. During incubation, the specific immune-complex formed in case of presence of HBsAg in the sample, is captured on the solid phase. After washing to remove sample serum proteins, second antibody conjugated to the enzyme HRP and directed against a different epitope of HBsAg is added to the wells. During the second incubation step, these HRP conjugated antibodies will be bound to any anti-HBs-HBsAg complexes previously formed during the first incubation, and the unbound HRP conjugate is then removed by washing. After washing to remove unbound HRP conjugate, chromogen solutions containing TMB and Urea peroxidase are added to the wells. In presence of the antibody-antigen-antibody HRP sandwich immune-complex, the colourless chromogens are hydrolyzed by the bound HRP conjugate to a blue coloured product. The blue colour turns yellow after stopping the reaction using the Stop solution. The colour intensity can be measured and it is proportional to the amount of antigen captured in the wells and to its amount in the sample respectively. Wells containing samples negative for HBsAg remain colourless.

Assay principle scheme: Double antibody sandwich ELISA

Ab(p)+Ag(s)+(Ab)ENZ →[Ab(p)–Ag(s)–(Ab)ENZ] –blue→yellow (+)

Ab(p) +(Ab)ENZ → [Ab(p)]→ no color (-)

Incubation I Inc II Immobilized Complex Colouring Results
60 min. 30 min 30min.

Ab(p)–pre-coated anti-HBs antibodies;
Ag(s)–HBsAg antigens in sample;
(Ab)ENZ–HRP conjugated anti-HBs;

Kit Contents: Store at 2-8°C

HbsAg Kit Contents	Volume
Microwell Plate 96 Tests	1 plate (12x8/8x12 well strips per plate)
Negative Control	1x1ml
Positive Control	1x1ml
HRP – Conjugate Reagent	1x6ml
HbsAg Sample Diluent	1x5ml
Stock Wash Buffer	1x30ml (Dilute 1 to 20 with distilled water before use. Once diluted, stable for two weeks at 2-8°C).
Chromogen Solution A	1x6ml (Ready to use and once open, stable for one month at 2-8°C)
Chromogen Solution B	1x6ml (Ready to use and once open, stable for one month at 2-8°C)
Stop Solution	1x6ml
Plastic Sealable Bag	1 Unit
Plate Cover	1 Sheet
Package Inserts	1 Copy

Additional Materials And Instruments Required But Not Provided:

1. Freshly distilled or deionized water.
2. Disposable gloves and timer.
3. Appropriate waste containers for potentially contaminated materials.
4. Disposable V-shaped troughs.
5. Dispensing system and/or pipette (single or multichannel),disposable pipette tips
6. Absorbent tissue or clean towel.
7. Dry incubator or water bath, 37±0.5°C.
8. Microplate shaker for dissolving and mixing conjugate with samples.
9. Microwell plate reader, single wavelength 450nm or dual wavelength 450nm and 630nm.
10. Microwell aspiration/wash system.

Specimen Collection and Transportation:

1. Sample Collection:

Either fresh serum or plasma samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid hemolysis of the RBC. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matters in the sample should be removed by centrifugation at 3000 RPM for at least 20 minutes at room temperature, or by filtration on 0.22u filters. Plasma samples collected into EDTA, sodium citrate or heparin may be tested, but highly lipaemic, icteric, or haemolysed samples should not be used as they could give erroneous results in the assay. Do not heat inactivate samples. This can cause sample deterioration.

2. Transportation and Storage:

Store samples at 2-8°C. Samples not required for assaying within 3 days should be stored frozen (-20°C or lower).Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labelled in accordance with the exerting local and international regulations for transport of clinical samples and ethological agents.

Special Instructions for Washing Plates:

1. A good washing procedure is essential to obtain correct and precise analytical data.
2. It is therefore recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than 5 automatic washing cycles with dispensing of 350-400µl/well are sufficient to avoid false positive reactions and high background.(all wells turn yellow).
3. To avoid cross-contaminations of the plate with sample or HRP-conjugate, after incubation, do not discard the content of the wells, but allow the plate washer to aspirate it automatically.
4. Anyway, we recommend calibrating the washing system on the kit itself in order to match the declared analytical performances. Assure that the microplate washer's liquid dispensing channels are not blocked or contaminated, and sufficient volume of Wash buffer is dispensed each time into the wells.
5. In case of manual washing, we suggest to perform at least 5cycles, dispensing 350-400µl/well and aspirating the liquid for 5times. If poor results (high background) are observed, increase the washing cycles or soaking time per well.
6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution(final concentration of 2.5%) for 24 hours, before liquids are disposed in an appropriate way.
7. The concentrated Washing solution should be diluted **1 to 20** before use. For one plate, mix 30 ml of the concentrate with 570ml of water for a final volume of 600ml diluted Wash Buffer. If less than a whole plate is used, prepare the proportional volume of solution.

Storage and Stability:

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8 °C, **do not freeze**. To assure maximum performance of this HBsAg ELISA kit, during storage protect the reagents from contamination with microorganism or chemicals.

Precautions and Safety:

Fortress HbsAg ELISA assay is a time and temperature sensitive method. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.

1. Do not exchange reagents from different lots, or use reagents from other commercially available kits. The components of the kit are precisely matched as to achieve optimal performance during testing.
2. Make sure that all reagents are within the validity indicated on the kit box and are of the same lot. Never use reagents beyond the expiry date stated on reagents labels or on the kit box.
3. **CAUTION - CRITICAL STEP:** Allow the reagents and samples to stabilize at room temperature(18-30°C) before use. Shake reagent gently before, and return to 2-8°C immediately after use.
4. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.
5. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
6. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.
7. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air-bubbles when adding the reagents.
8. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
9. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Always use different disposal pipette tips for each specimen and pipette solutions by mouth.
10. The use of automatic pipettes is recommended.
11. Assure that the incubation temperature is 37° inside the incubator.
12. When adding samples, avoid touching the well's bottom with the pipette tip.
13. When reading the results with a plate reader, it is recommended to determine the absorbance at 450nm or at 450nm with reference at 630nm.
14. All specimens from human origin should be considered as potentially infectious.
15. Materials from human origin may have been used in the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV ½, HCV, TP and HBsAg. However, there is no analytical method that can asure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Never eat, drink, smoke, or apply cosmetics in the assay laboratory.
16. Bovine derived sera may have been used in this kit. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
17. The pipette tips, vials, strips and sample containers should be collected and autoclaved for 1hour at 121°C or treated with 10% sodium hypochlorite for 30minutes to decontaminate before any further steps for disposal.
18. The Stop solution (2M H_2SO_4) is a strong acid. Corrosive. Use it with appropriate care. Wipe up spills immediately or wash with water if come into contact with the skin or eyes. ProClin 300 used as a preservative can cause sensation of the skin.
19. The enzymatic activity of the HRP-conjugate might be affected from dust, reactive chemical, and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of such substances.
20. Materials Safety Data Sheet (MSDS) available upon request.
21. If using fully automated microplate processing system, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.

Assay Procedure:

Step1 Reagents preparation:

Allow the reagents and samples to reach room temperature (18-30°C) for at least 15-30minutes. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37°C until crystals dissolve. Dilute the stock Wash Buffer **1 to 20** with

Fortress Diagnostics Limited. Unit 2C Antrim Technology Park, Antrim BT41 1QS (United Kingdom)
TEL: +44 (0) 2894 487676 | FAX: +44 (0) 2894 469933 | www.Fortressdiagnostics.com

AppendixIIColourplate (2)ELISA Kit

Appendix **III** HBsAG coated MICROPLATE

Appendix's (IV)

Study Questionnaire

Sudan University of Science and Technology

Serodetection of Hepatitis B among Nurses in El-obeidCity Hospital at North Kordofan State

ID. Number: ……..…

Age: ……

Gender: Male ( ) female ( )

Marital status: single ( ) married ( ) divorced ( )

Educational level Diploma ( ) Bachelor ( ) Master ( )

Medical history:

Have you taken a sharp instrument? Yes ( ) No ( )

If the answer is yes, what is the procedure used in the hospital to encroach the accident? …….

Previous bloodtransfusion Yes ( ) No ( )

PrevioussurgeryYes() No ( )

Hemodialysis Yes ( ) No ( )

HBV Vaccination Yes ( ) No ( )

Investigation results:

HBsAg: +ve ( ) -ve ( )