Sudan University of Science and Technology

College of Graduate Studies

Sero-detection of Hepatitis (B) Virus among Butchers in Khartoum State

الكشف المصلي لفيروس إلتهاب الكبد الوبائي (ب) وسط الجزارين في ولاية الخرطوم

A dissertation submitted in partial fulfillment for the requirements of M.Sc. degree in Medical Laboratory Science (Microbiology)

By:

ALrayan Abdalgafar Osman

B.Sc. in Medical laboratory Science, Sudan University of Science and Technology, Microbiology (2015)

Supervisor:

Dr. Wafaa Mohammed Abdalla

December, 2020

الآية

قال تعالى:

(وَإِذَا مَرِضْتُ فَهُوَ يَشْفِينِ)

صدق الله العظيم

سورة الشعراء، الآية (80)

DEDICATION

To my mother **Fatima Osman Mohamed** who did the impossible to make me what I am today, and to my brothers and sisters for their love and support.

AKNOWLEDGEMENTS

First of all thanks to **ALMIGHTY ALLAH** for giving me the strength and patience to do this study. Then I would like to thank my supervisor **Dr. Wafaa Mohammed Abdalla** who helped and supported me patiently to complete this work.

I would acknowledge my brother Mrs. **Mohamed Abdalgafar** and my sister miss. **Marwa Abdalgafar** for their great help in collection of the specimens.

Thanks extended to all my friends for their help and great assistance through the many stages of this thesis. A special thanks to my sister miss. **Asma Abdualgafar** and my brother Mrs. **Abubaker Abdalgafar** without whom I would never have completed this study. My appreciation must be made to the Central Laboratory staff and butchers for their help.

ABSTRACT

Hepatitis B is a viral infection that attacks the liver and can cause both acute and chronic disease. The virus is most commonly transmitted from mother to child during birth and delivery, as well as through contact with blood or other body fluids, including sex with an infected partner, injection-drug use that involves sharing needles, syringes, or drug-preparation equipment and needle sticks or exposures to sharp instruments. This descriptive, cross-sectional study was conducted during January 2020 to December 2020 to detect Hepatitis B Virus (HBV) serologically and related risk factors among butchers in Khartoum State. Ninety (n=90) blood specimens were collected from each eligible participant, and structured questionnaire was used to collect data from them. Enzyme Linked Immunosorbent Assay (ELISA) technique was used to detect Hepatitis B surface antigen (HBsAg).

Out of 90 butchers, the study found the sero-positivity represent 3/90(3.3%). The sero-positivity was more common in age group 20-29 years and there was 2(2.2%) between 20-29 years and 1(1.1%) between 40-49 years. Among the butchers infected with Hepatitis B, 2(2.2%) were single and 1(1.1%) were married, 2(2.2%) were in primary school level 1(1.1%) were university level, 2(2.2%) were had previous occupation butcher and 1(1.1%) were previous occupation other than butcher, 2(2.2%) were had an experience less than one years, 3(3.3%) had cut by shared knife, 1(1.1%) had surgical operation, 2(2.2%) had no history of blood transfusion, 1(1.1%) had previous history of jaundice and all positivity of hepatitis B were not vaccinated.

There were significant association between seropositivity of HBsAg and years of experience but there were no significant association between sero-positivity of HBsAg and age group, education level, occupation, marital status, previous history of jaundice, cut by shared knife, shared knives, previous surgical operation, blood transfusion and vaccination.

This study concluded that; hepatitis B infection was few among butchers in Khartoum State.

المستخلص

إلتهاب الكبد (ب) هو عدوى فيروسية تهاجم الكبد ويمكن أن تسبب أمراضًا حادة ومزمنةً. ينتقل الفيروس بشكل أكثر شيوعًا من الأم إلى الطفل أثناء الولادة، وكذلك من خلال ملامسة الدم أو سوائل الجسم الأخرى ، بما في ذلك ممارسة الجنس مع شريك مصاب ، وتعاطي المخدرات بالحقن الذي يتضمن مشاركة الإبر أو المحاقن أو معدات تحضير الأدوية وعصا الإبر أو التعرض للأدوات الحادة. أجريت هذه الدراسة المقطعية الوصفية خلال الفترة من يناير 2020 إلى ديسمبر 2020 والتي هدفت إلى الكشف المصلي للإصابة بفيروس التهاب الكبد ب وعوامل الخطر المرتبطة به بين الجزارين في ولاية الخرطوم. تم جمع تسعين (ن = 90) عينة دم من كل مشارك مؤهل ، واستخدم استبيان منظم لجمع البيانات منهم. تم استخدام تقنية الفحص المناعي المرتبط بالإنزيم (إليزا) للكشف عن مستضد إلتهاب الكبد ب السطحي.

من بين 90 جزاراً ، وجدت الدراسة أن الإيجابية المصلية تمثل 90/3(3.3٪). كانت الإيجابية المصلية أكثر شيوعاً في الفئة العمرية من 20–29 سنة وكان هنالك 2(2.2٪) بين 20–29 سنة و 1(1.1٪) بين 40–49 سنة. من بين الجزارين المصابين بإلتهاب الكبد(ب) ، كان 2(2.2٪) غير متزوجين و 1(1.1٪) متزوج ، 2(2.2٪) أنهو المرحلة الإبتدائية و 1(1.1٪) من خريجي الجامعات، 2(2.2٪) مهنتهم السابقه جزارين و1(1.1٪) لديهم مهنة سابقة بخلاف الجزار، 2(2.2٪) لديهم خبرة أقل من سنة واحدة، 3(3.3 ٪) أصيبوا بجرح سكين مشترك، 1(1.1 ٪) خضعوا لعملية جراحية ، 2(2.2 ٪) ليس لديهم تاريخ لنقل الدم، 1(1.1 ٪) كان لديهم تاريخ سابق من اليرقان وكل إيجابية إلتهاب الكبد (ب) لم تتعرض للتطعيم. كان هناك إرتباط كبير بين الموجبة المصلية لـ مستضد التهاب الكبد (ب) السطحي وسنوات الخبرة ولكن لم يكن هناك إرتباط كبير بين الإيجابية المصلية لـ مستضد التهاب الكبد (ب) السطحي والفئة العمرية، المستوى التعليمي، المهنة السابقة، الحالة الإجتماعية، الإصابة المسبقة باليرقان، القطع بالسكين، العمليات جراحية السابقة، نقل الدم والتطعيم.

خلصت هذه الدراسة إلى أن الإصابة بفيروس التهاب الكبد ب قليلة بين الجزارين في ولاية الخرطوم

TABLE OF CONTENTS

No.	Subject	Page No.
الآية		I
Dedication		II
Acknowledgement		III
Abstract (English)		IV
المستخلص		V
Table of contents		VI
List of tables		IX
List of abbreviations		X
CHAPTER I 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 II LITERUTURE REVIEW		
2.1	Hepatitis B virus	5
2.1.1	Classification	5
2.1.2	Genotype	5
2.1.3	Genome	6
2.1.4	Replication	6
2.1.5	Epidemiology	7
2.1.6	HBV transmission	8
2.1.7	Pathogenesis	8
2.1.8	Clinical presentation	8
2.1.9	Risk group for HBV	10
2.1.9.1	Health care workers	10

2.1.9.2	Sexual (heterosexual and homosexual) exposure	10
2.1.9.3	Hemodialysis patients.	11
2.1.10	Immunopathology	11
2.1.11	Laboratory diagnosis	12
2.1.11.1	Serologic and virologic markers	13
2.1.11.2	Molecular methods for HBV infection	13
2.1.12	Treatment	14
2.1.13	Prevention	14
2.1.14	HBV vaccines	15
2.2	Previous study	16
CHAPTER III **MATERIALS AND METHODS**		
3.1	Study design	17
3.2	Study area	17
3.3	Study duration	17
3.4	Study population	17
3.4.1	Inclusion criteria	17
3.4.2	Exclusion criteria	17
3.5	Ethical considerations	17
3.6	Sampling technique	17
3.7	Data collection	17
3.8	Laboratory processing	17
3.8.1	Collection of blood specimens	17
3.8.2	ELISA technique (method)	18
3.8.2.1	Procedure	18
3.8.2.2	Interpretation of results	18
3.8.2.3	Quality control	19
3.9	Data analysis	19
CHAPTER IV **RESULTS**		

	Results	20
CHAPTER V **DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS**		
5.1	Discussion	28
5.2	Conclusions	30
5.3	Recommendations	31
References		32
Appendices		38

LIST OF TABLES

No.	Legend	Page No.
4-1	Distribution of socio-demographic data of butchers	21
4-2	Frequency of HBsAg among study population	22
4-3	The association between HBsAg results and age group	22
4-4	The association between HBsAg results and marital status	23
4-5	The association between HBsAg results and education level	23
4-6	The association between HBsAg results and previous occupation	24
4-7	The association between HBsAg results and experience years	24
4-8	The association between HBsAg results and cutting by shared knives	25
4-9	The association between HBsAg results and history of surgical operation	25
4-10	The association between HBsAg results and history of blood transfusion	26
4-11	The association between HBsAg results and previous history of jaundice	26
4-12	The association between HBsAg results and expose to vaccination	27

LIST OF ABBREVIATIONS

3TC	Lamivudine
ACIP	Advisory committee on immunization practice
AHB	Acute Hepatitis B virus
ALT	Alanine aminotransferase
CD_4 T	T helper cells
CD_8 T	killer T cells
cccDNA	Covalently closed circular DNA
CDC	Centers for Disease Control and Prevention
CHB	Chronic Hepatitis B virus
CTL	Cytotoxic T-lymphocyte
DNA	Deoxyribonucleic acid
Ds	Double strand
ELISA	Enzyme linked immunosorbent assay
HBc	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
HCV	Hepatitis C virus
HCWs	Health care workers
HDV	Hepatitis delta virus
HIV	Human immunodeficiency virus
IDUS	Intravenous Drug Users
IFN	Interferon
NCs	Nucleocapsids
mRNA	Messenger RNA
PCR	Polymerase chain reaction
pgRNA	Progenomic RNA
Pre c	pre core

RNA	Ribonucleic acid
SS	Single_strand
STI	Sexual transmitted infection
WHO	World Health Organization

CHAPTER I

INTRODUCTION

CHAPTER I

1. INTRODUCTION

1.1. Introduction

Hepatitis B virus (HBV) infection is a severe public health burden and approximately one third of the world population has serological evidence of resolved or ongoing infection. It causes a broad spectrum of liver diseases ranging from acute to chronic HBV infection with no biochemical evidence of liver injury to progressive chronic HBV infection, which may advance to liver cirrhosis and liver failure (Yu *et al*., 2016).

It has been estimated that more than 2 billion people have been infected with HBV and 248 million of these people are chronically infected (Nelson *et al*., 2016) and approximately15%-25% of person with chronic HBV infection will die prematurely from cirrhosis or liver cancer since 2006 (WHO, 2011).

The majority of chronic carriers of HBV are found in South East Asia and sub-Saharan Africa (Musa *et al*., 2015). The prevalence of HBV among humans and the non-human primates may speed up evolution process due to high error rate of viral reverse transcriptase, and recombination among different genotypes of hepadnavirus strains from human and non- human primates (Shao *et al*., 2004).

The three main modes of transmission are via blood, during sexual intercourse and prenatally from mother to newborn (Levinson, 2014). A large number of workers do not wear protective clothing or gloves to reduce their exposure (Cui *et al*., 2013).

Viral infections are well known to be sustained by certain incubation for doctors, nurses and slaughterhouse workers (David *et al*., 2018).

Slaughter house personnel including butchers are at a higher risk of infections from cuts and blood-letting, with the possible risk of the transmission of blood-borne pathogens to their colleagues (Ola *et al.*, 2008). Slaughterhouse workers (SHW) are at a higher risk of infected hepatitis that can be multifactorial and should be evaluated for viral, bacterial and parasitic organisms (Tariq *et al.*, 2019).

Viral infections are commonly sustained by certain reservoirs e.g., the HBV has been found in gorillas, monkeys and cattle (Tariq *et al*., 2019).

Research on HBV-like viruses in domestic animals has been carried out since 1985 (Din *et al*., 2001). Liver of captive swine and chickens were found to be naturally infected

with HBV in China and these findings together with known ability of HBV to cross species barriers, suggested that human and non-human HBV variants might share hosts in nature (Yang *et al*., 2007).

Evidence of the existence of a novel member of the hepadnavirus family endemic in swine has also being established and temporarily this virus was designated as swine hepatitis B virus (SHBV) (Liu *et al*., 2010). A study from University of Ibadan has shown that butchers from Ibadan, Nigeria have a prevalence rate of HBV infection at 9.3% which may lead to the spread of infection in the community (Ola *et al.,* 2002).

1.2. Rationale

Butchers are at high risk for hepatitis B infection and transmission is likely occur throw cuts on the butcher's hands. Butchers and other persons who frequently receive lacerations in the workplace should be candidates for hepatitis B vaccination (Din *et al*., 2001).

Sharing of knives may be risk factor for transmission of HBV.

However, for my knowledge there were no data about the prevalence of HBV among butchers in Sudan, so the current study was designed to detect the frequency of HBV among butchers and the outcome of this study will serve as a baseline for future researches investigating the burden of HBV among butchers in Sudan.

The obtained results may be beneficial in adoption protocols that can be done by the Ministry of Health about the sharing of knives and give the clue about the percentage of infected butchers to treat them and to vaccinate the healthy one.

1.3. Objectives

1.3.1. General objective

To detet of HBV serologically among butchers in Khartoum State.

1.3.2. Specific objectives

1. To detect hepatitis B surface antigen (HBsAg) among butchers in Khartoum State using enzyme linked immunosorbent assay (ELISA).
2. To determine the frequency of HBV among butchers.
3. To determine the possible risk factors (e.g. sociodemographic, years of experience, cut injury by the shared knife previous, history of surgical operation and blood transfusion) associated with hepatitis B infection.
4. To determine the association between the positive HBsAg with previous jaundice.
5. To compare frequency of hepatitis B between vaccinated and non-vaccinated butchers.

CHAPTER II

LITERATURE REVIEW

CHAPTER II

2. LITERATURE REVIEW

2.1. Hepatitis B virus (HBV)

2.1.1. 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 hepadna virusis 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, 2013).

2.1.2. Genotyping

The viral population can be divided into nine genotypes (A to I) (Kramvis 2014) which differ in more than 7.5% of their nucleotide sequences (Pourkarim *et al*., 2014) and they are further subdivided into subgenotypes with a nucleotide divergence greater than 4% (Kramvis 2014). Genotypes A to H have long been accepted as individual genotypes, and two new genotypes (I and J) were proposed more recently (Tatematsu *et al*., 2009). Genotype I was first described in 2008 after isolation from a Vietnamese patient and constitutes a recombination of genotypes A, C and G (Huy *et al*., 2008).

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 E

infection 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, but hardly with geography. The origin and epidemiology of HBV-G remain unresolved, is also present in certain risk groups in Europe (Cornelissen, 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 (Velkove *et al.,* 2018).

Acute genotype A and D infection results in higher chronicity rates than B and C (Lin and Kao, 2015).

2.1.3. Genome

The genome of HBV is partially double-stranded circular DNA of about 3.2 kilobase (kb) pairs. The viral polymerase is covalently attached to the 5′ end of the minus strand (Liang, 2009)

The genome enclosed within an icosahedral capsid that is formed by 240 (or 180 in a minority population) copies of the same viral protein, the core or capsid protein (HBc), and is, in turn, coated with an outer envelope. Asparаretroviruses, hepadnaviruses assemble initially as immature nucleocapsids (NCs), packaging an RNA pregenome (pgRNA). These immature NCs undergo a process of maturation first to NCs containing a single-stranded (SS) DNA (still considered immature) and subsequently to mature NCs containing the DS DNA genome, via reverse transcription of pgRNA inside the maturing NCs. Only the mature NCs are then enveloped by the viral envelope or surface (HBs) proteins and secreted extracellularly (Ning *et al*., 2011)

2.1.4. Replication

The first step in the HBV life cycle is its attachment to the hepatocyte through the interaction of its envelope proteins with the host cell receptors. Then it penetrates in the hepatocyte, uncoating, and viral genome, organized as relaxed circular partially double stranded DNA, is sent to the nucleus and converted into covalently closed circular DNA (ccc DNA). The cccDNA acts as template for transcription of four co-terminal

hepatocyte, uncoating, and the viral genome, organized as relaxed circular partially mRNAs: 3.5 kb pre-core (pre-C) and progenomic RNA (pgRNA), 2.4 kb large surface mRNA, 2.1 kb middle and small surface mRNA and 0.7 kb X mRNA. pgRNA serves as template for the reverse transcriptase and, after being transported to the cytoplasm, encodes viral capside protein and viral polymerase, thus playing an important role in viral genome amplification and replication. The latter is transcripted into viral RNA gene products: HBV surface protein, structural core protein, non-structural core protein (secreted HBeAg), X protein and viral polymerase. After this step the viral assembly occurs (encapsidation by the core protein to form the viral nucleocapsid) (Caligiuri, 2016).

Hepadnaviruses are the only viruses that produce genome DNA by reverse transcription with mRNA as the template (note that this type of RNA-dependent DNA synthesis is similar to but different from the process in retroviruses, in which the genome RNA is transcribed into a DNA intermediate). Some of the progeny DNA integrates into the host cell genome, and this seems likely to be the DNA that maintains the carrier state. Progeny HBV with its HBsAg-containing envelope is released from the cell by budding through the cell membrane (Levinson, 2014).

2.1.5. Epidemiology

Worldwide estimates suggest that more than 2 billion people have been infected with HBV, and that 248 million of these people are chronically infected (defined as hepatitis B surface antigen [HBsAg] positivity). About 15% to 25% of persons with chronic HBV infection die from cirrhosis or liver cancer (Schweitzer *et al*., 2015).

The Global Burden of Disease study estimated that there were 686,000 deaths caused by hepatitis B in 2013 and a 5.9 per 100,000 age-standardized death rate globally (Abubakar *et al.,* 2015) of which 300,000 deaths were attributed to liver cancer and 317,400 deaths to cirrhosis of the liver secondary to hepatitis B (Mortal, 2015).

This rate represents a substantial global burden, with wide global geographic variation. Hepatitis B prevalence (HBsAg) is highest in the sub-Saharan African and western Pacific regions, considered high-intermediate to high endemicity countries (5% to ≥ 8% prevalence), and prevalence estimates exceed 15% in several countries. Low-intermediate regions (2%–4.99%) include the eastern Mediterranean and European regions. The

Americas and Western Europe regions are considered low endemicity, with HBsAg prevalence generally less than 2% (Franco *et al*., 2012).

There has been an overall decrease in HBsAg prevalence over time in most countries, but with notable increases in African and eastern European countries (Schweitzer *et al*., 2015).

2.1.6. HBV transmission

There are three important modes of transmission; parenteral transmission, perinatal transmission and sexual transmission (kumar *et al.,* 2013).

HBV is found in the blood during the last stages of a prolonged incubation period (4 to 26 weeks) and during active episodes of acute and chronic hepatitis. It is also is present in all physiologic and pathologic body fluids, with the exception of stool. HBV is a hardy virus and can withstand extremes of temperature and humidity. Thus, whereas blood and body fluids are the primary vehicles of transmission, virus also may be spread by contact with body secretions such as semen, saliva, sweat, tears, breast milk and pathologic effusions. In endemic regions, vertical transmission from mother to child during birth constitutes the main mode of transmission. In areas of low prevalence, horizontal transmission via transfusion, blood products, dialysis, needle stick accidents among health care workers, sharing of needles in intravenous drug use, and sexual transmission constitute the primary mechanisms for HBV infection (kumar *et al.,* 2013).

2.1.7. 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).

2.1.8. Clinical Syndromes

Hepatitis B virus is one of the most important causes of acute and chronic hepatitis. The clinical manifestations vary from subclinical hepatitis to symptomatic and icteric hepatitis. The incubation period varies from 6 weeks to 6 months and the clinical

manifestations of HBV infection depend on age of infection, immune status of the host, and the level of HBV (Parija, 2012).

The incubation period of acute hepatitis B (AHB) varies from 1 to 4 month post infection and clinical presentation varies from asymptomatic infection in two- thirds of patients to icteric hepatitis and, rarely fulminant liver failure. A serum-sickness-like illness, characterized by fever, arthralgias, and rash, may occur in the prodromal period, followed by constitutional symptoms, anorexia, nausea, jaundice, and right upper quadrant discomfort. Clinical symptoms coincide with biochemical abnormalities. The symptoms and jaundice generally disappear after 1– 3 months, although fatigue may persist for months in some patients, even after normalization of liver function tests (Burns and Thompson, 2014).

CHB infection the risk of chronicity in acute HBV infection is related to age at primary infection. Adults who become chronically infected during childhood have a 15–25% lifetime risk of dying from HBV–related cirrhosis or liver cancer. CHB is a dynamic disease that fluctuates over time, likely relating to interactions between the virus and the host immune system. The following five not-necessarily sequential-phases can be identified in chronic HBV infection (Feld and Janssen, 2015).

Immune-tolerant phase: characterized by high levels of serum HBV DNA, HBe Ag positivity, normal alanine aminotransferase (ALT) levels, and absent liver necroinflammation. Disease progression is minimal in patients who remain in this phase and patients are highly contagious in this phase. Immune-reactive phase (HBeAg-positive CHB): patients enter this phase after a variable time, linked to the age when HBV infection occurred. The immune system becomes more active and the infected hepatocyte are attacked. Characterized by highly fluctuating, but progressively decreasing, HBV-DNA levels, elevated ALT and hepatic necroinflammation (HBeAg-positive CHB). A prolonged immune-active phase with multiple ALT flares may result in progressive liver fibrosis, leading to cirrhosis. Immune-control phase (and inactive carrier state): transition into this phase as an outcome of the immune-active phase is marked by seroconversion from HBe Ag to anti-HBe positivity characterized by low (< 2000 IU/mL) or undetectable serum HBV DNA, normal ALT levels, and disappearance of liver necroinflammation (inactive carrier state) (Feld and Janssen, 2015).

Reactivation phase (HBeAg-negative CHB): despite HBe seroconversion, reactivation of HBV replication may occur due to the selection of HBeAg-defective HBV mutants and characterized by positive anti-HBe antibody levels, fluctuating HBV DNA and ALT levels, and a high risk of progression to severe hepatic fibrosis (HBeAg-negative CHB). Periodic ALT flares with intervening normalization may make it difficult to distinguish between HBeAg-negative CHB and inactive disease, and thus continued follow-up is required before patients with normal ALT and low HBV DNA levels are designated as inactive carriers. HBsAg-negative phase: after HBsAg loss, low-level HBV replication may persist, with detectable HBV DNA in the liver and rarely in the serum. In patients with-occult‖ HBV infection, persistence of effective HBV immunological control has been demonstrated significant immunosuppression may lead to HBV reactivation, with reappearance of HBsAg, known as -reverse seroconversion (Feld and Janssen, 2015).

2.1.9. Risk groups for HBV infecion

2.1.9.1 Health care workers

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 World Health Organization revealed that in 200, 66, 00 HBV infection among HCWs could have happened owing to their occupational exposure (Ganczak, 2019).

2.1.9.2. 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).

2.1.9.3. Hemodialysis patients

In recent decades, the use of hemodialysis has been expanded increasingly for end-stage renal disease. The modality of this treatment has led to the increased longevity of patients. It also predisposes these patients to some infections, mainly blood born viruses (Zahedi *et al.,* 2012).

HBV and HCV infections are the most causes of liver disease in hemodialysis patients and play an important role in serious morbidity and mortality among hemodialysis patients (Arababadi *et al.,* 2009).

2.1.10. Immunopathology

Unlike many other viruses, HBV infection is characterized by a delayed kinetics of viral replication and further uniqueness of HBV is its inability to trigger a classic innate immune response. Data *in-vitro* and *in-vivo* has shown the absence of activation of type I Interferon (IFN) genes during the logarithmic phase of HBV expansion and the absence of pro-inflammatory cytokines in the serum of patients in the early phases of acute infection. So, the virus is causes of inability to activate a classical innate immune response and whether HBV actively suppress innate immunity or only evade its recognition (Hong and Bertoletti, 2017).

It is able to 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 CD_4 T cells (T helper cells) and CD_8 T (cytotoxic T-lymphocyte) (CTL) cells are activated CD_4 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 CD_4 T cells have no direct effect on viral clearance and liver disease (Lu, 2011).

CD_8 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 (Molla, 2016).

Broadly reactive CD_4 T cells are predominantly detectable during acute infection, whereas their numbers decline during chronic infection. Both, CD_4 and CD_8 T cell responses are deterministic of whether an acute infection is resolved, or whether it progresses to chronic infection (Prieto and Dorner, 2017).

Depletion of CD_4 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, CD_4 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).

2.1.11. Laboratory Diagnosis

The specimen of choice for the diagnosis of HBV infection is blood. Serological tests for viral antigens and antibodies are typically used for diagnosis screening and can be performed on either serum or plasma. Both HBV antigens and antibody are stable at room temperature for days, at 4°C for months, and frozen at-20°C to -70°C for many years. Because modern testing involves automated enzyme immunoassays that depend on colorimetric or chemiluminescence signal measurement, care should be taken to avoid hemolysis of the sample because it may interfere with the ability of the assay to accurately detect marker. A number of nucleic acid-based tests, which have been the subject of recent reviews (Pawlotsky, 2002).

Are available directly detect HBV-DNA in serum or plasma. Care must be taken to avoid the degradation of viral nucleic acid in specimen, which can result in falsely low or no measurable viral load. Serum should therefore be removed from clotted blood within 4 hours of collection and stored at-20°C to -70°C (Krajden *et al.*, 2000). And can be

subjected to up to eight short-term freeze-thaw cycles without significant loss of detectable HBV-DNA (Krajden *et al.,* 2001).

2.1.11.1. Serologic and Virologic Markers

Serological markers for HBV infection consist of HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc IgM 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).

2.1.11.2. Molecular methods for HBV infection

The amount of virus in blood can be determined by quantitative genome assays using polymerase chain reaction (PCR) and related techniques. Knowing the virus load can help in following the course of chronic HBV infection and antiviral drug efficacy (Murray *et al*., 2013).

HBV DNA is a direct measurement of the viral load, which reveals the replication activity of the virus. It is detectable at the early stage of infection (1 month after HBV infection) and increases up to peak level (more than 10^8 copies/mL) approximately 3 months after the exposure to HBV and then gradually diminishes in chronic infection or disappears at the recovery from HBV infection. As the prevalence of serologically negative HBV infection (HBeAg-negative CHB and occult HBV infection) has increased. HBV-DNA detection has obtained more awareness in clinical medicine (Datta *et al.* 2014).

The detection of HBV DNA is a reliable marker of replication activity, and higher titers of HBV DNA are related to the more rapid disease progression and higher incidence of HCC (Chen*,* 2006). Furthermore, HBV DNA testing is useful in routine clinical setting to determine patients who need antiviral therapy and monitor them for suitable treatment (Chevaliez *et al.,* 2008).

There are two principles of techniques to identify and quantify HBV DNA: signal amplification such as hybrid capture and branched DNA technology; target amplification such as polymerase chain reaction (PCR) (Datta *et al.*, 2014). Real-time PCR can detect

wide dynamic range of viral load (lower range, 10–15 IU/mL; upper range, 10^7–10^8 IU/mL). For this reason, it come to be the standard method to detect and quantify HBV DNA in clinical setting. Furthermore, it can be fully automated and does not generate carry-over contamination (Bustin *et al*., 2005).

2.1.12. Treatment

Treatment should be considered for patients with rapid deterioration of liver function, cirrhosis or complications such as ascites, hepatic encephalopathy, or hemorrhage as well as those who are immunosuppressed. For chronic hepatitis B diseases, pegylated or regular interferon-α provides benefit in some patients. Lamivudine (3TC), a potent inhibitor of HIV reverse transcriptase, and other nucleoside analogs (entecavir, telbivudine) as well as certain nucleotide analogs (adfovir) are active against hepatitis B. These antivirals inhibit viral replication and may reduce viral load but do not cure HBV infection (Ryan *et al*., 2014).

2.1.13. 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 (Joshi and Kumar, 2001). Efficient HB vaccines have been available since the early 1980s (Joshi and Kumar, 2001).

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 positive 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 (Rajbhandari and Chung, 2016).

2.1.14. HBV vaccines

Commercial HBV vaccine supplies have been available for thirty years. HBV vaccine was the first vaccine against a chronic disease, the first vaccine to protect from a sexually transmitted infection and the first vaccine against a cancer (Lavanchy *et al*., 2012). Vaccination is the most effective measure to decrease the worldwide HBV incidence and its complications, including liver cirrhosis and HCC. Worldwide, immunization has been an essential strategy for many countries to decrease the burden of HBV infection (WHO, 2013). Economically, vaccination is an attractive option, both in terms of cost-effectiveness and benefit-cost ratios when compared with other health care interventions (Lyseng and Dhillon 2012). The choice of a vaccine type and a schedule for doses and route of vaccination varies between countries. An ideal HBV vaccine schedule should protect against infection in infancy when the risk of becoming a chronic HBV carrier is high and in adolescence with common, high risk behaviors such as sex and drug abuse (Mackie *et al.,* 2009). The CDC Advisory Committee on Immunization Practices (ACIP) recommends that all children should receive a birth dose of HBV vaccine and complete the vaccine series by 6-18 months of age. It also recommends that older children and adolescents who did not previously receive the HBV vaccine should be vaccinated (Holmberg *et al.,* 2012).

2.2. Previous studies

In Nigeria, Ola and her group studied the prevalence of hepatitis B infection in Nigerian butchers at 2009, they found the sero-prevalence rate in butchers and controls was 9.4% and 3.3 % respectively (Ola *et al*.,2009).

In Part Harcourt, Ibangan and his research group in 2015 studied the prevalence of HBV among butchers which was 13.71% (Ibangan *et al*., 2015).

In 2017, Kano State in Nigeria Kareem and his colleague found the sero-prevalence of HBV infection among butchers was 11.6% (kareem *et al*., 2017).

CHAPTER III

MATERIALS AND METHODS

CHSPTER III

3. MATERIALS AND METHODS

3.1. Study design

It is descriptive, cross-sectional, laboratory based study.

3.2. Study area

This study was conducted in Khartoum State (Alsahafa, Jabra, Almoasasa and Alarda).

3.3. Study duration

The study was carried out between January 2020 to February 2021.

3.4. Study population

Butchers who were working in Khartoum State.

3.4.1. Inclusion criteria

Butchers with different age groups and races.

3.4.2. Exclusion criteria

Butchers who was already been diagnosed with hepatitis B infection.

3.5. Ethical considerations

Ethical approval to carry out this study was obtained from the Scientific Research Committee, Collage of Medical Laboratory Science, Sudan University of Science and Technology. Written informed consent (appendix-1) was obtained from participants before collection of the blood specimens.

3.6. Sample size

Ninety (n=90) butchers were enrolled in this study due to the high cost.

3.7. Sampling Technique

Non-probability, convenience sampling technique was used.

3.8. Data collection

Data were collected through direct interview with each candidate. The interview instrument (Questionnaire) (appendix-2) consists of 10 questions was used.

3.9. Laboratory processing

3.9.1. Collection of blood specimens

Under sterile conditions, two ml of venous blood sample was withdrawn from each participant and then was waited until sample clotted. Serum was separated by

centrifugation at 5000 rpm for five minutes, and collected into plain containers by syringe and stored at -20°C until testing.

3.9.2. Enzyme linked immune sorbent assay (ELISA)

Sandwich ELISA was used to screen HBV surface antigen (HBsAg) which indicates to HBV infection.

3.9.2.1. Procedure

The steps were followed the manufacturing's instructions (For Tress, China) as follow: the reagent and samples were allowed to reach room temperature. Then 20ul of sample diluent was added to each well except the blank and mixed by toping the plate gently. 100ul of positive control and negative control and specimens were added to their respective wells. Then 50 ul HRP conjugate was added to each well except the blank and was mixed by tapping the plate gently then was incubated for 30 minutes in 37˚C. By the end of the incubation the plate cover was removed and discarded, after that each well was washed 5 times with diluted wash buffer. Each time was allowed the micro wells to soak for 45 seconds. After the five washing, 50ul of chromogen A and 50ul of chromogen B solutions were dispensed into each well including the blank and was mixed by tapping the plate gently, the plate was incubated at 37˚C for 15 minutes and the reaction was stopped by adding 50ul of stop solution into the each well and was mixed gently. The absorbance was measured at 450 nm and read within 5 minutes after the stopping the reaction. The cut-off value was calculated and the results were evaluated.

3.9.2.2. Interpretation of results

The results were calculated by relating each sample's optical density (OD) value to the cut-off value (C.O.) of plate.

Cut off value (C.O) = NC* x 2.1

NC*= If the mean OD value of the negative control is lower than 0.05, take it as 0.05 and if higher than 0.05 see the quality control range.

Negative result: sample gave an absorbance less than the cut-off 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 cut off value are considered initially reactive, which indicates HBsAg has been detected with this HBsAg ELISA kit.

Borderline

Sample with absorbance to cut off ratio between 0.9 and 1.00 are considered borderline, and another sample is recommended. Repeatedly positive sample can be considered positive for HBsAg.

3.9.2.3. Quality control

The test result will be valid if the quality control criteria are verified; a) if the OD value of the blank well, which contain only chromogens and stop solution, is less than 0.080 at 450 nm, b) the OD value of the positive control must be equal to or greater than 0.800 at 450/630nm, or at 450nm after blanking and c) the OD value of the negative control must be less than 0.100 at 450/630nm, or at 450nm after blanking.

3.10. Data analysis

Data were computed and analyzed by using Statistical Package for the Social Sciences (SPSS) software program version 20.0. Frequencies were expressed in form of tables and significant differences were determined using Chi-square test at *p-value* ≤ 0.05.

CHAPTER IV

RESULTS

CHAPTER IV

4. RESULTS

A total of ninety blood specimens were collected from butchers in selected areas, Khartoum State.

The age of the respondent ranged between 10 to 70 years, the mean age was 32.8±11.3 S.D and age were classified into 6 groups as follow: 7/90 (7.8%) in age group 10-19 years, 35/90 (38.9%) in age between 20 to 29 years, 24/90 (26.7%) in age group 30-39 years, 17/90 (18.9%) in age between 40 to 49 years, 4/90 (4.4%) in age group 50-59 years and 3/90 (3.3%) in age between 60 to 69 years.

There were 42/90 (46.7%) single, 42/90 (46.7%) married and 6/90 (6.7%) divorced.

Regarding to education level; there were 13/90 (14.4%) illiterate, 45/90 (50.0%) complete primary school level, 23/90 (25.6%) had secondary school level and 9/90 (10.0%) graduated from university.

There were 58/90 (64.4%) of respondents work just as butcher and 32/90 (35.6%) had previously occupied other occupations rather than butcher.

Regarding to years of experience; 8/90 (8.9%) were worked less than one year, 12/90 (13.3%) were worked between 1-5 years, 22/90 (53.3%) were worked between 6-10 years and 48/90 (53.3%) were worked more than 10 years as showed in the table (4-1).

Table 4-1: Distribution of socio-demographic data of butchers

Variables	**Frequency**	**Percentage**
Age groups/years		
10-19	7	7.8%
20-29	35	38.9%
30-39	24	26.7%
40-49	17	18.9%
50-59	4	4.4%
60-69	3	3.3%
Total	90	100%
Marital status		
Single	42	46.7%
Married	42	46.7%
Divorced	6	6.7%
Total	90	100%
Education level		
Illiterate	13	14.4%
Primary school level	45	50.0%
Secondary school level	23	25.6%
University level	9	10.0%
Total	90	100%
Previous occupation		
Butcher	58	64.4%
Other than butcher	32	35.6%
Total	90	100%
Years of experience		
< one year	8	8.9%
1year-5years	12	13.3%
6years-10years	22	24.4%
>10 years	48	53.3%
Total	90	100%

Out of 90 butchers, there were 3/90 (3.3%) positive for HBsAg as showed in table 4-2.

Table 4-2: Frequency of HBsAg among study population

HBsAg results	Frequency	Percentage
Yes	3	3.3%
No	87	96.7%
Total	90	100%

Concerning age groups, there were 2 (2.2%) HBsAg positive in age group 20-29 years and 1 (1.1%) in age between 40 to 49 years. There was insignificant association between HBsAg and age groups (*p.value* = 0.811) as explained in table 4-3.

Table 4-3: The association between HBsAg results and age groups

Age groups	HBsAg Results		Total	*P.value*
	Positive	Negative		
10-19 years	0 (0.0%)	7 (7.8%)	7 (7.8%)	0.811
20-29 years	2 (2.2%)	33 (36.7%)	35 (38.9%)	
30-39 years	0 (0.0%)	24 (26.7%)	24 (26.7%)	
40-49 years	1 (1.1%)	16 (17.8%)	17 (18.9%)	
50-59 years	0 (0.0%)	4 (4.4%)	4 (4.4%)	
60-69 years	0 (0.0%)	3 (3.3%)	3 (3.3%)	
Total	3 (3.3%)	87 (96.7%)	90 (100.0%)	

In the context of marital status, there were 2 (2.2%) single were HBsAg positive, 1 (1.1%) married was HBsAg positive and no divorced (0%) was HBsAg positive. There was meaningless association between HBsAg and marital status (*p.value*= 0.744) as presented the table 4-4.

Table 4-4: The association between HBsAg results and marital status

Marital status	HBsAg Results		Total	*P.value*
	Positive	Negative		
Single	2 (2.2%)	40 (44.5%)	42 (46.7%)	0.744
Married	1 (1.1%)	41 (45.6%)	45 (50.0%)	
Divorced	0 (0.0%)	6 (6. 6%)	6 (6. 6%)	
Total	3 (3.3%)	87 (96.7%)	90 (100.0%)	

According to education level, found 2 (2.2%) participants had primary school level were HBsAg positive and 1 (1.1%) graduated participant was HBsAg positive. There was non-significant association between HBsAg and marital status (*p.value* =0. 376) as displayed in table 4-5.

Table 4-5: The association between HBsAg results and education level

Education level	HBsAg Results		Total	*P.value*
	Positive	Negative		
Illiterate	0 (0.0%)	13 (14.4 %)	13 (14.4 %)	0.376
Primary school level	2 (2.2%)	43 (47.8%)	45 (50.0%)	
Secondary school level	0 (0.0%)	23 (25.6 %)	23 (25.6 %)	
University level	1 (1.1%)	8 (8.9%)	9 (10.0%)	

Total	3 (3.3%)	87 (96.7%)	90 (100.0%)	

In relation to previous occupation, there were 2 (2.2%) basically butcher were HBsAg positive and 1 (1.1%) worked other than butcher was HBsAg positive. There was insignificant association between HBsAg and previous occupation (*p.value* = 0.935) as indicated in table 4-6.

Table 4-6: The association between HBsAg results and previous occupation

Previous occupation	HBsAg Results		Total	*P.value*
	Positive	Negative		
Butcher	2 (2.2%)	56(62.3%)	58 (64.5%)	0.935
Other than butcher	1 (1.1%)	31(34.4%)	32(35.5%)	
Total	3 (3.3%)	87 (96.7%)	90 (100.0%)	

In this study, there were 2 (2.2%) were HBsAg positive and had an experience less than one year and others whom had longer experiences were negative. There was significant association between HBsAg and experience years (*p.value* = 0.005) as exhibited in table 4-7.

Table 4-7: The association between HBsAg results and experience years

Years of experience	HBsAg Results		Total	*P.value*
	Positive	Negative		
<one year	2 (2.2%)	6 (6.7 %)	8 (8.9%)	0.005
1-5 years	0 (0.0%)	12 (13.3%)	12 (13.3%)	
6-10 years	0 (0.0%)	22 (24.5 %)	22 (24.5 %)	

>10 years	1 (1.1%)	47 (52.2%)	48 (53.3%)	
Total	3 (3.3%)	87 (96.7%)	90 (100.0%)	

In this study, there were 2 (2.2%) were HBsAg positive and had shared knives with others. There was insignificant association between HBsAg and shared knives (*p.value* = 1.000) as showed in table 4-8.

Table 4-8: The association between HBsAg results and cutting by shared knives

Cutting by shared knives	HBsAg Results		Total	*P.value*
	Positive	Negative		
Yes	3 (3.3%)	85 (94.5%)	88 (97.7%)	0.791
No	0 (0.0%)	2 (2.2%)	2 (2.2%)	
Total	3 (3.3%)	87(96.7%)	90 (100.0%)	

The study found 1(1.1%) had a history of surgical operation and 2 (2.2%) without history of surgical operation were HBsAg positive. There was insignificant association between HBsAg and history of surgical operation (*p.value* = 0.360) (table 4-9).

Table 4-9: The association between HBsAg results and history of surgical operation

History of surgical operation	HBsAg Results		Total	*P.value*
	Positive	Negative		
Yes	1 (1.1%)	52 (57.8%)	53 (58.9%)	0.360
No	2 (2.2%)	35 (38.9%)	37 (41.1%)	
Total	3 (3.3%)	87 (96.7%)	90 (100.0%)	

Concerning history of blood transfusion, all HBsAg positive participants (3 (3.3%)) had no history of blood transfusion. There was meaningless association between HBsAg and history of blood transfusion (*p.value* = 0.669) (table4-10).

Table 4-10: The association between HBsAg results and history of blood transfusion

History of blood transfusion	HBsAg Results		Total	*P.value*
	Positive	Negative		
Yes	0 (0.0%)	5 (5.6%)	5 (5.6%)	0.669
No	3 (3.3%)	82 (91.1%)	85 (94.4%)	
Total	3 (3.3%)	87 (96.7%)	90 (100.0%)	

In this study, out of 3/90 (3.3%) HBsAg positive; there were 1(1.1%) had previous history of jaundice and 2(2.2%) hadn't history of jaundice. There was insignificant association between HBsAg and previous history of jaundice (*p.value* = 0.933) as showed in table 4-11.

Table 4-11: The association between HBsAg results and History of jaundice

History of jaundice	HBsAg Results		Total	*P.value*
	Positive	Negative		
Yes	1 (1.1%)	27 (30.0%)	28 (31.1%)	0.933
No	2 (2.2%)	60 (66.7%)	62 (68.9%)	
Total	3 (3.3%)	87 (96.7%)	90 (100.0%)	

In relation to vaccination, all HBsAg positive participants (3 (3.3%)) were non-vaccinated. There was insignificant association between HBsAg and vaccination (*p.value* = 0.407) (table 4-12).

Table 4-12: The association between HBsAg and vaccination

Vaccination	HBsAg Results		Total	*P.value*
	Positive	Negative		
Yes	0 (0.0%)	3 (3.3%)	3 (3.3%)	0.407
No	3 (3.3%)	84 (93.4%)	87 (96.7%)	
Total	3 (3.3%)	87 (96.7%)	90 (100.0%)	

CHAPTER V

DISCUSION, CONCLOSION AND RECOMMENDATIONS

CHAPTER V

DISCUSION, CONCLOSION AND RECOMMENDATIONS

5.1. DISCUSION

In this study 90 butchers workers were investigated for HBsAg at Khartoum State and only 3 (3.3%) were positive for HBsAg. This was lower than; Ola *et al*. (2008) (2009) in Nigeria*,* who found the seroprevalence rate in butchers and controls was 9.4% and 3.3% respectively, Ibanga *et al.* (2015) in Port Harcourt in which the hepatitis B infection among butchers was 13.71% and Kareem *et al*. (2017) in Nigeria which showed the prevalence rate 11.6 % among butchers. The differences might be due to the difference in sample size and habits (e.g. tattoo).

In this study; 2/3 (66.7%) were in age group between 20-29 years and 1/3 (33.3%) participant in age ranged between 40-49 years, this was agreed with Ola *et al.* (2009) in Nigeria in which 10.7% of positive HBsAg participants their age group between 20-29 years old and 10.0% their age group between 40-49 years old. This finding was disagreed with Kareem and his colleagues (2017) in Nigeria (Kano State) which showed that the most effective age group between 31-40 years (37.5%).

In this study, 2/3 (66.7%) positive HBsAg were single. It was differ from that done in Nigeria (Kano State) by kareem *et al*. (2017) which showed that married participants (62.5%) had higher positivity rate than single participants (37.5%).

So the most affected age group by HbsAg between 20-40 years in all studies. This result likely because this age group of hard working and have higher risk of exposure to contaminated materials by HBV. Also this age group have sexual activity which may be the cause of hepatitis B.

Regarding years of experience, 2/3 (66.7%) of positive HBsAg butchers had less than one year experience, it was mismatched with a study done in Port Harcourt by Ibanga and his group (2015) which showed that 60.0% of positive HBsAg butchers had 1-14 years experience and the rest of them had experience between 14-35 years. These result indicate that the positivity of HBsAg increase in inexperience butchers. This might be due to frequent cut injuries and sharing knives behavior between them.

In this study all of HBsAg positive participants 3/3 (100.0%) were cut by shared knives. Same result was seen in other studies done in Nigeria, by Ola *et al*. (2009) Kareem *et al.* (2017) which showed that all HbsAg positive butchers experience a cut by shared knives (81.7%), (100.0%), respectively.

Almost (2/3 (66.7%)) of HBsAg positive participants had no history of surgical operation, this result go with other one done in Port Harcourt, Nigeria which showed that only one participant of 96 positive HBsAg participants had history of surgical operation (Ibanga *et al.,* 2015). So, that mean there was good sterilization measures in Sudan during operation and blood transfusion.

Regarding to previous blood transfusion, all the participants in this study were HBsAg positive hadn't blood transfusion. This result looks like other one done in Port Harcourt, Nigeria which found positivity of HBsAg was higher in people who were not exposed to blood transfusion (98.9%) (Ibanga *et al.,* 2015). This may be due to good precaution measures which had been done in blood transfusion, including investigated bags for hepatitis B and other viruses and safe hygiene which have been done during blood transfusion.

In section of previous occupation ; 2/3 (66.7%) of case were started as butcher and had no history of other occupation and 1/3 (33.3%) had history of working in other occupation previously.

Regarding level to education 2/3 (66.7%) of positive HbsAg had primary school degree and 1/3 (33.3%) of them studying at university.

In this study all HBsAg positive participants weren't vaccinated and only one (33.3%) of them had previous history of jaundice.

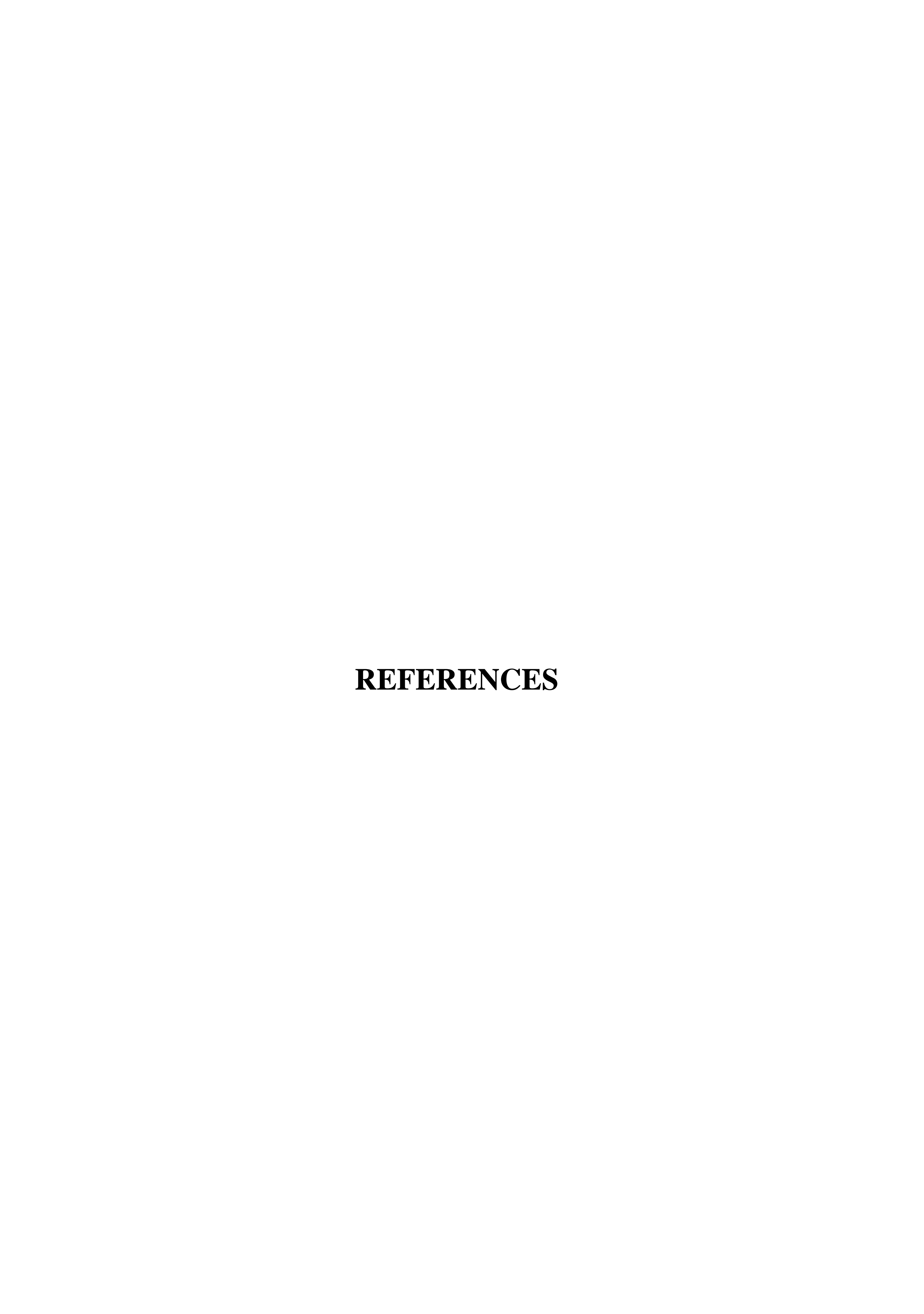
Other studies in literature did not disclose these areas.

5.2. Conclusion

This study concluded that; hepatitis B infection was few among butchers in Khartoum State and there were significant association between HBV infection and years of experience. But there were no significant association between HBV infection with socio-demographic data, cutting by shared knives, history of surgical operation, history of blood transfusion, history of jaundice and vaccination.

5.3 Recommendations

- Further studies with large sample size and more accurate techniques are recommended such as: anti-HBcAg and polymerase chain reaction (PCR).
- Vaccination is fundamental measures in prevention and protect butchers worker against HBV infection, should be mandatory for them.
- Continuous awareness to butchers worker should be reinforced to improve their knowledge in order to protect them.
- Other studies should be done to investigate the transfused people and compare the frequency of HBsAg positive with general population.



REFERENCES

References

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

Arababadi, M. K., Hassanshahi, G., & Yousefi, H. (2009). HBV-DNA in hemodialysis patients infected by HCV. *Saudi Journal of Kidney Diseases and Transplantation*, **20**(3): 398.

Badawi, M. M., Atif, M.S. and Mustafa, Y.Y. (2018) Systematic review and meta-analysis of HIV, HBV and HCV infection prevalence in Sudan. *Virology journal*, **15**(1):1-16.

Burns, G. and Thompson, A. (2014). Viral Hepatitis B: Clinical and Epidemiological Characteristics. *Cold Spring Harbor Perspectives in Medicine*, **4**(12): a024935-a024935.

Bustin, S. A., Benes, V., Nolan, T. and Pfaffl, M. W. (2005). Quantitative real-time RT-PCR–a perspective. *Journal of molecular endocrinology*, **34**(3): 597-601.

Caligiuri, P. (2016). Overview of hepatitis B virus mutations and their implications in the management of infection, *World Journal of Gastroenterolgy*, **22**(1): 145.

Chen, C. (2006). Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *Jama*, **295**(1): 65-73**.**

Chevaliez, S. and Pawlotsky, J.M. (2008). Diagnosis and management of chronic viral hepatitis: antigens, antibodies and viral genomes. *Best Practice & Research Clinical Gastroenterology*, **22**(6): 1031-1048.

Cornelissen, M. (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. *BMC Infectious Diseases*, **16**: 268.

Cui, Y., Jia, J. and Lok, A.S. (2013). Update of Epidemiology of Hepatitis B and C Globally. *Journal of Gastroenterological Hepatology*, **28**: 7-10

Datta, S., Chatterjee, S., & Veer, V. (2014). Recent advances in molecular diagnostics of hepatitis B virus. *World Journal of Gastroenterology,* **20**(40): 14615.

David, H., Teguh, W. and Rizalinda, S. (2018). Hepatitis B Virus Infection among Health Care Workers in Indonesia. *Euroasian Journal of Hepato-Gastroenterology*, **8** (1): 88–92.

Din, Z., Jin, N.Y., Chen, C.F., Zou, X.H. and Wang, C.Y. (2001). Study on S Gene Sequence Homologous Analysis between the Hepatitis B Virus from Sheep and Human. *Progress in Veterinary Medicine*, 22:54-58

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

Feld, J. and Janssen, H.L.A. (2015). Hepatitis B. *World Gastroenterology Organisation Global Guideline*, **2**:1-35

Franco, E., Bagnato, B., Marino, M.G., Meleleo, C., Serino, L. and Zaratti, L. (2012). Hepatitis B: Epidemiology and prevention in developing countries. *World journal of hepatology*, **4**(3): 74.

Ganczak, M. (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.

Holmberg, S.D., Suryaprasad, A. and Ward, J.W. (2012). Updated CDC recommendations for the management of hepatitis B virus–infected health-care providers and students. *Morbidity and Mortality Weekly Report: Recommendations and Reports*, **61**(3): 1-1x2.

Hong, M and Bertoletti, A. (2017). Tolerance and immunity to pathogens in early life: insights from HBV infection. *Seminars in Immunopathology*, **39** (6): 643-652.

Huy, T.T.T., Ngoc, T.T., & Abe, K. (2008). New complex recombinant genotype of hepatitis B virus identified in Vietnam. *Journal of virology*, **82**(11): 5657-5663.

Ibanga, E.R., Adikema, O.A., Adekema, O.A., Amala, S.E. and Emejuru, A.K. (2015). Prevalence of Hepatitis B virus among butchers in Port Harcort Metropolis, *Elixir Physiology and Anatomy*, **77**: 30651-30654.

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

Kareem A.M., Mohammed, Y. and Rogo, L.D. (2017). Prevalence of Hepatitis B surface Antigen (HBsAg) among butchers and slaughter cow in Kano Metropolis, Nigeria. *Journal of Medical Laboratory Science*, **2**(2): 105-109.

Krajden, M., Comanor, L., Rifin, O., Grigoriew, A., Minor, J.M and Kapke, G.F. (2000). Assessment of hepatitis B virus DNA stability in serum by the Chiron Quantiplex branched-DNA assay. *Journal of Clinical Microbiology*, **36**(2): 382-386.

Krajden, M., Minor, J.M., Rifin, O. and Comanor, L. (2001). Effect of multiple freeze-thaw ccles on hepatitis B virus DNA and hepatitis C virus RNA quantification as meassued with branched-DNA technology. *Journal of Clinical Microbiology*, **37**(6): 1683-1686.

Kramvis, A. (2014). Genotypes and genetic variability of hepatitis B virus. *Intervirology*, **57**(3-4): 141-150.

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

Lavanchy, D. (2012). Viral hepatitis: global goals for vaccination. *Journal of Clinical Virology*, **55**(4): 296-302.

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

Liang, T.J. (2009). Hepatitis B: The Virus and Disease. *Hepatology*, **49**(5): 13–21.

Lin, C. and Kao, J. (2015). Hepatitis B Virus Genotypes and Variants. *Cold Spring Harbor Perspectives in Medicine*, **5** (5): a021436-a21436.

Liu, H., Wengui, L., Ruiping, S., Liqiang, L., Hua, Y. and Jun, Y. (2010). Prevalence of a Virus Similar to Human Hepatitis B Virus in Swine. *Journal of virology*, 23:133-146.

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

Lyseng-Williamson, K. A and Dhillon, S. (2012). DTPa-HBV-IPV/Hib vaccine (Infanrix hexa™): a guide to its use in infants. *Pediatric Drugs*, **14**(5): 337-343.

Mackie, C.O., Buxton, J.A., Tadwalkar, S. and Patrick, D.M. (2009). Hepatitis B immunization strategies: timing is everything. *Canadian Medical Association Journal*, **180**(2): 196-202.

Mahmood, M. (2016). Distribution and clinical significance of hepatitis B virus genotypes in Pakistan. *Bio Med Central Gastroenterology*, **16**(1): 104.

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.

Molla, S., (2016). Review on Humman Immune Response against Hepatitis B Virus Infection. *Journal of Emerging Diseases and Virology,* **2**(2).

Mortal, G.B.D. (2015). Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*, **385**(9963): 117-171.

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

Musa, B., Bussel, S., Boroda, M.M., Samaila, A.A., and Femi, O.L. (2015). Prevalence of Hepatitis B Virus Infection in Nigeria. *Nigeria Journal of Clinical Practice*, 18:163-172.

Nelson, N.P., Easterbrook, P. and McMahon, B. (2016). Epidemiology of Hepatitis B Virus Infection and Impact of Vaccination on Disease. *Clinical Liver Disease*, **20** (4): 607–628

Ning, X., Nguyen, D., Mentzer, L., Adams, C., Lee, H., Ashley, R. and Hu, J. *et al*. (2011). Secretion of genome-free hepatitis B virus–single strand blocking model for virion morphogenesis of para-retrovirus. *PLoS pathogens*, **7**(9):1002255.

Ola, S.O., Otegbayo, J.A., Odaibo, G.N., Olaleye, O.D and Olubuyide, I.O. (2002). Serum hepatitis C virus and hepatitis B surface antigenaenia in Nigerian patients with acute icteric hepatitis. *West African Journal of Medicine*, 21:215-217.

Ola, S.O., Otegbayo, J.A., Yakubu, A., Odaibo, G.N., Olaleye, D.O. (2008). Risk of hepatitis B virus in the slaughter house. *Tropical doctor*, **38**(4):249-250.

Ola, S.O., Otegbayo, J.A., Yakubu, A., Odaibo, G.N. and Olaleye, D.O., (2009). Nigerian butchers and hepatitis B virus infection. *Tropical Gastroenterology*, **29**(1): 32-34.

Olusola, B.A., Gometi, E.A., Ogunsemowo, O., Olaleye, D.O. and Odaibo, G.N. (2017). High rate of Hepatitis B virus infection among hairdressers in Ibadan, Nigeria. *Journal of Immunoassay and Immunochemistry*, **38**(3): 322-332.

Parija, C.C. (2012). Textbook of Microbiology and Immunology. Second Edition. India: Elsevier, p.p. 552.

Powlotsky, J.M. (2002). Molecular diagnosis of viral hepatitis. *Gastroenterology,* **122**(6): 1554-1568.

Pourkarim, M. R., Amini-Bavil-Olyaee, S., Kurbanov, F., Van Ranst, M. and Tacke, F. (2014). Molecular identification of hepatitis B virus genotypes/subgenotypes: revised classification hurdles and updated resolutions. *World Journal of gastroenterology*, **20**(23): 7152.

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.

Ryan, K. J., Ray, C.G., Ahmad, N., Pottinger, P., Drew, W.L., Reller, L.B., Lagunoff, M. and Sterling, C.R. *et al.* (2014). Sherris Medical Microbiology. Sixth Edition. United States: McGraw-Hill, p.p. 234.

Schweitzer, A., Horn, J., Mikolajczyk, R.T., Krause, G. and Ott, J. J. (2015). Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. *The Lancet*, **386**(10003): 1546-1555.

Shao, X.A., Xu, W., Wang, Y. and Xiang, S.D. (2004). HBsAg-like Protein Detected in the Bovine Serum. *Fudan Universal Journal of Medical Science*, 31: 585-587.

Shuping, T. (2013). Hepatitis B virus genetic variants: biological properties and clinical implications. *Emerging Microbes and Infections*, **2** (3): 10.

Sondlane, T. H., Mawela, L., Razwiedani, L.L., Selabe, S.G., Lebelo, R.L., Rakgole, J. 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.

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

Tariq, H., Kamal, M.U., Makker, J., Azam, S., Pirzada, U.A., Mehak, V., Kumar, K. *et al.* (2019). Hepatitis in slaughterhouse workers. *World journal of haplology*, **11**(1):37.

Tatematsu, K., Tanaka, Y., Kurbanov, F., Sugauchi, F., Mano, S., Maeshiro, T. and Mizokami, M. *et al*. (2009). A genetic variant of hepatitis B virus divergent from known human and ape genotypes isolated from a Japanese patient and provisionally assigned to new genotype J. *Journal of virology*, **83**(20): 10538-10547.

Velkov, S., Ott, J., Protzer, U. and Michler, T. (2018). The Global Hepatitis B Virus Genotype Distribution Approximated from Available Genotyping Data. *Genes*, **9** (10):495.

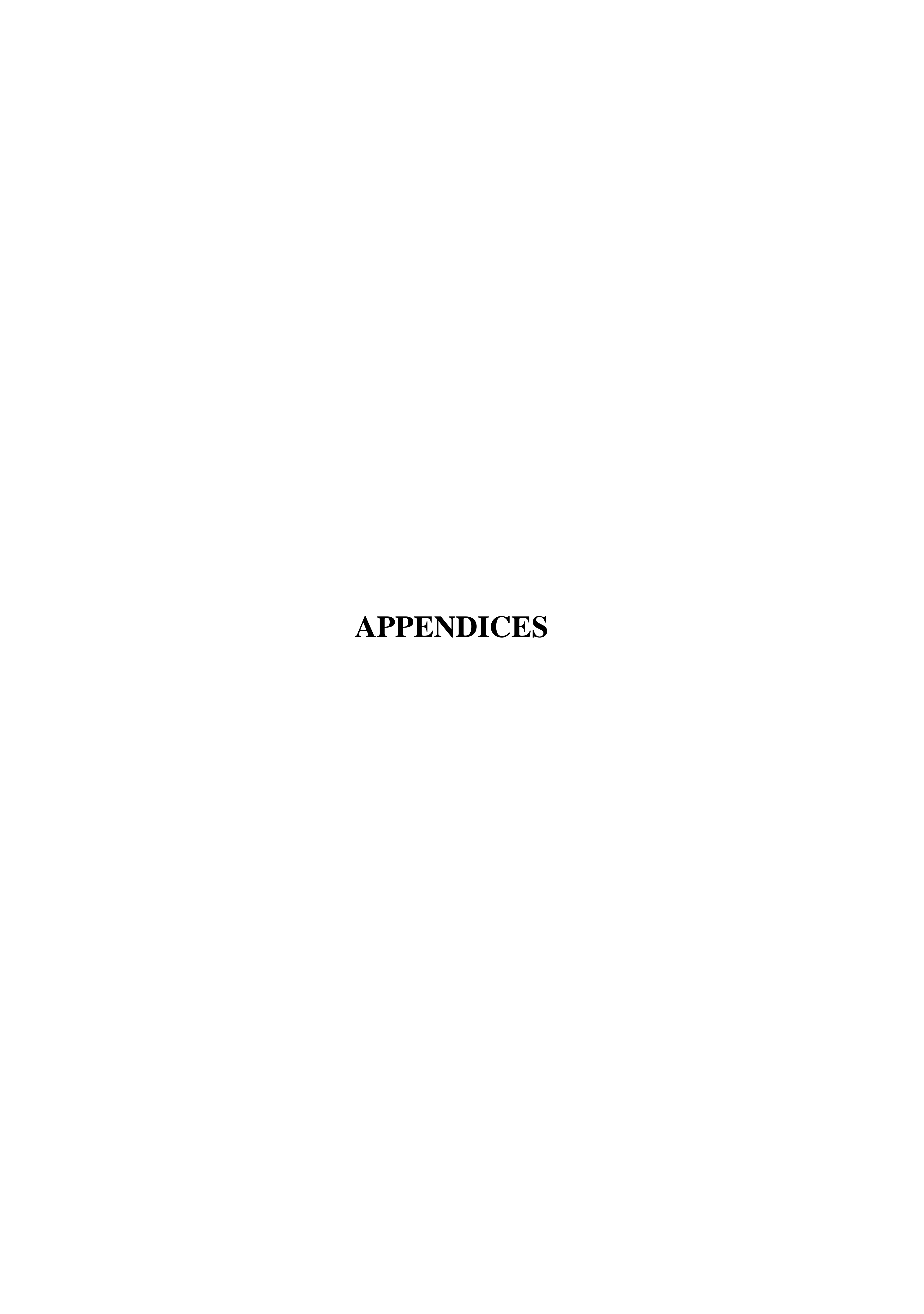
World Health organization (WHO) (2011). Introduction of Hepatitis B vaccine into childhood immunization services. http://www.who.int/vaccine-documents (accessed on 17-3-2019-9:00 p.m.).

World Health Organization (2013). Global policy report on the prevention and control of viral hepatitis in WHO Member States. http://www.who.int/vaccine-documents (accessed on 17-3-2019-9:00 p.m.).

Yang, J., Xi, Q., Deng, R., Wang, J., Hou, J. and Wang, X. (2007). Identification of Inter species recombination among hepadnaviruses infecting cross-species hosts. *Journal of Medical Virology,* 79:1741-1750

Yu, X., Zheng, Y., Deng, Y., Li, J., Guo, R., Su, M. and Ming, D. (2016). Serum Interleukin (IL)-9 and IL-10, but not T-Helper 9 (Th9) Cells, are Associated with Survival of Patients with Acute-on-Chronic Hepatitis B Liver Failure. *Medicine Journal*, **95** (16): 1-8.

Zahedi, M.J., Moghaddam, S. D., Alavian, S.M., & Dalili, M. (2012). Seroprevalence of hepatitis viruses B, C, D and HIV infection among hemodialysis patients in Kerman Province, South-East Iran. *Hepatitis monthly*, **12**(5), 339.



APPENDICES

Appendix -1

Informed consent

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا

<u>وثيقة موافقة للمشاركة فى بحث علمي</u>

عنوان البحث: الكشف المصلي لفيروس التهاب الكبد الوبائي (النوع ب) وسط الجزارين في ولاية الخرطوم

الباحث:الريان عبدالغفار عثمان.

مقدمة : التهاب الكبد (ب) هو عدوى فيروسية تصيب الكبد ويمكن أن تتسبب في أمراض حادة ومزمنة على حد سواء. أكثرطرق إنتقال الفيروس شيوعاً تكون من الأم إلى الطفل أثناء الولادة فضلا عن انتقاله من خلال ملامسه دم الشخص المصاب او سوائل جسمه الاخرى. وتؤدي الإصابة بإلتهاب الكبد الوبائي إلى زيادة خطر فشل الكبد أو سرطان الكبد أو تليف الكبد

الهدف من الدراسة: تهدف هذه الدراسة للكشف عن وجود فيروس الكبد الوبائي (ب) في الجزارين العاملين بولاية الخرطوم. حيث يمكن أن يكون الجزار لديه المرض بدون أعراض ظاهرة عليه ويمكن ان ينقل المرض للآخرين. تتطلب مشاركتك في هذه الدراسة إجراء بعض الإختبارات لعينة الدم التي لا يزيد مقدارها عن 3 مل. و ستفحص هذه العينات في المعمل المركزي وجامعة السودان للعلوم والتكنولوجيا- الجناح الغربي. وستحفظ العينات بالمعمل حتى إكتمال مشروع البحث.

المخاطر : قد يتعرض المشارك في البحث لألم بسيط نتيجة لوخز الحقن وسوف تتخذ كل تحوطات السلامة المعملية لأخذ العينة.

البدائل : البديل للمشارك في الدراسة هو عدم المشاركة ولك كل الحرية المطلقة لإختيار المشاركة أو عدم المشاركة في هذه الدراسة.

إنهاء المشاركة : سيتم إنهاء المشارك فى الدراسة إذا قررت الإنسحاب من الدراسة او إذا قرر الباحث بأنك غير مستوفيه لشروط المشاركة فى البحث.

المشاركة التطوعية : المشارك في هذه الدراسة طوعية وإذا قررت عدم المشاركة فإنك لن تتعرضى لأى مضايقات.

السرية : كمشارك فى الدراسة ستكون هويتك ومحتويات الإختبارات المعملية سرية فى جميع المنشورات المتعلقة بنتائج الدراسة ويمكن الإطلاع عليها من قبل الباحثين ولجان الكلية فى حدود النظم والقوانين المطبقة بهذا الخصوص. هذه الدراسة بغرض الحصول على درجة الماجستير في الأحياء الدقيقة بكلية المختبرات الطبية. كل المعلومات المتحصلة سرية للغاية وعند الإنتهاء من الفحص المعملي سوف يتم إبلاغك عن النتائج.

الأشخاص الذين يمكن الإتصال بهم للإستفسار عن نتائج البحث : يمكن الإتصال بالباحث على رقم الموبايل:

0924372730

الجزء الثاني

أنا .... أوقع علي هذه الموافقة بعد ان شرح لي الباحث انني سأشارك في بحث علمي وأجاب علي كل تساؤلاتي بخصوص هذا البحث.

وبتوقيعي هذا أقر بأننى موافق علي اخذ العينة (الدم) لغرض البحث.

المشارك في البحث أو من يوقع عنه :	الباحث:
الإسم :	الإسم :
التوقيع أو البصمة :.................	
	التوقيع : ....
التاريخ :....................	التاريخ : ....
صلة القرابة :......................	
(اذا كان الموقع غير المشارك)	

Appendix -2

<u>Questionnaire</u>

Sudan University of Science and Technology

Frequency of Hepatitis B Virus among Butchers in Khartoum State

ID. Number: …………………………

Contact number: ……………….…… (If you want to know your result)

Age: …………………………………..

Educational level: Illiterate ( ) Primary school ( ) Secondary school ( )

University ( ) Post graduate ( )

Marital status: Married ( ) Single ( ) Divorced ( )

What was your occupation before being butcher? Bucher ( ) Other than butcher ( )

Years of experiences: < one year ( ) 1-5 years ( ) 6-10years ( )

> 10 years ( )

<u>Medical History:</u>

Do you have previous history of jaundice? Yes ( ) No ( )

Have you ever been exposed to cauterize? Yes ( ) No ( )

Was you cut by shared knives? Yes ( ) No ( )

Do you have history of blood transfusion? Yes ( ) No ( )

Do you have history of surgical operation? Yes ( ) No ( )

Are you vaccinated against HBV? Yes ( ) No ( )

<u>Investigation results:</u>

HBsAg: +ve ( ) -ve ( )

Appendix -3

Leaflet

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 microtiter 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 peroxidise 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 labeled in accordance with the existing 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.

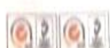
1. Do not exchange reagents from different lots, or use reagents from other commercially available kits. The components of the kit are precisely matched as to achieve optimal performance during testing.
2. Make sure that all reagents are within the validity indicated on the kit box and are of the same 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 reagents as to avoid cross-contaminations. Never 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 assure 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 hypochloite, 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 distilled or deionized water. Use only clean vessels to dilute the

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



BXE0742A HbsAg (HS) Revision No 12 APR/14 Page 1 of 2

buffer.

Step2 Numbering Wells:
Set the strips needed in strip-holder and sufficient number of wells including three Negative controls (e.g. B1, C1, D1), two Positive Controls (e.g. E1, F1) and one Blank (e.g. A1, neither samples nor HRP Conjugate should be added into the blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.

Step3 Adding Sample Diluent:
Add 20ul of Sample Diluent to each well except the Blank, and mix by tapping the plate gently.

Step4 Adding Sample:
Add 100ul of Positive control, negative control and specimen into their respective wells. Note: Use a separate disposable tip for each specimen, Negative control and Positive control to avoid cross contamination.

Step5 Incubation:
Cover the plate with the plate cover and incubate for **60 minutes at 37°C**. It is recommended to use thermostat-controlled water tank to assure the temperature stability and humidity during the incubation. If dry incubator is used, do not open the door frequently.

Step6 Adding HRP Conjugate:
Add 50ul HRP Conjugate to each well except the Blank and mix by tapping the plate gently.

Step7 Incubation II:
Cover the plate with the plate cover and incubate for 30 minutes at 37°C as in Step 5.

Step8 Washing:
At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Wash buffer. Each time, allow the microwells to soak for 30-60seconds. After the final washing cycle, turn the plate down onto blotting paper or clean towel, and tap it to remove any remainders.

Step9 Colouring:
Dispense **50µl** of Chromogen A and **50µl** Chromogen B solution into each well including the **Blank**, and mix by tapping the plate gently. Incubate the plate at **37°C for 15minutes avoiding light**. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue colour in Positive control and HBsAg positive sample wells.

Step10 Stopping Reaction:
Using a multichannel pipette or manually, add **50µl** Stop Solution into each well and mix gently. Intensive yellow colour develops in Positive control and HBsAg positive sample wells.

Step11 Measuring the Absorbance:
Calibrate the plate reader with the blank well and read the absorbance at **450nm**. If a dual filter instrument is used, set the reference wavelength at **630nm**. Calculate the Cut-off value and evaluate the results. (**Note**: read the absorbance within **5** minutes after stopping the reaction)

Interpretation of Results
Each microplate should be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each sample's optical density (OD) value to the Cut-off value (C.O.) of the plate. If the Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well OD value from the print report values of samples and controls. In case the reading is based on dual filter plate reader, do not subtract the Blank well OD from the print report values of samples and controls.

1. **Calculation of Cut-off value**

Cut-off value (C.O.) = *Nc × 2.1

*Nc = the mean absorbance value for three negative controls.
Important: If the mean OD value of the negative control is lower than 0.05, take it as 0.05. If higher than 0.05 see the Quality control range.

Example:
1. Calculation of Nc:

Well No:	B1	C1	D1
Negative controls OD value	0.02	0.012	0.016

Nc= 0.016 (the Nc Value is lower than 0.05 so take it as 0.05)
Calculation of Cut-off value: Cut off(C.O)=0.05×2.1=0.105

If one of the Negative control values does not meet the Quality control range specifications, it should be discarded and the mean value is calculated again using the remaining two values. If more than one negative control OD value does not meet the Quality control range specifications, the test is invalid and must be repeated.

2. **Quality control range:**
The test results are valid if the Quality Control criteria are verified. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed.
1. The OD value of the Blank well, which contains only Chromogens and Stop solution, is less than 0.080 at 450 nm.
2. The OD value of the Positive control must be equal to or greater than 0.800 at 450/630nm, or at 450nm after blanking.
3. The OD value of the Negative control must be less than 0.100 at 450/630nm or at 450nm after blanking.

3. **Interpretations of the results:**
(S = the individual absorbance (OD) of each specimen)

Negative Results (S/C.O. <1): samples giving an absorbance less than the Cut-off value are considered negative, which indicates that no hepatitis B surface antigen has been detected with this HBsAg ELISA kit, therefore the patient is probably not infected with hepatitis B virus.

Positive Results (S/C.O≥1): samples giving an absorbance greater than or equal to the Cut-off value are considered initially reactive, which indicates that HBV surface antigen has probably been detected with this HBsAg ELISA kit. Any initially reactive samples should be retested in duplicates. Repeatedly reactive samples could be considered positive for HBsAg, therefore the patient is probably infected by HBV and the blood unit should not be transfused.

Borderline: Samples with absorbance to Cut-off ratio between 0.9 and 1.00 are considered borderline samples and retesting is recommended. Repeatedly positive samples can be considered positive for HBsAg.

Fortress HBsAG 3rd Gen Performance:

Clinical Specificity: The clinical specificity of this assay was determined by a panel of samples obtained from 4476 healthy blood donors and 6344 hospitalized patients.

	Sample	-	+	Specificity
Donors	4476	4471	5	99.89%
Patients	6344	6340	4	99.94%

Clinical Sensitivity: 1) A panel of 40 serum samples including 26 positive confirmed samples.

PANEL	BACKGROUND	FORTRESS HBSAG 3RD GEN	
		+	-
CDC	+	26	0
	-	0	14
DETECTION RATE		100 %	

2. A panel of 108 samples sequenced by PCR method.

BACKGROUND		NUMBER	FORTRESS HBSAG 3RD GEN
adr(+)	Wildtype	35	33
	4 mutations	5	4
adw(+)	Wild type	37	34
	16 mutations	25	24
ayw(+)	Wild type	2	2
	2 mutations	2	2
ayr(+)	2 mutations	2	2
TOTAL		108	101

3. Two seroconversion panels from BBI

CODE	DAYS	FORTRESS HBSAG 3RD GEN
PHM909	0	0.03
	4	0.07
	7	0.16
	9	1.64
	14	5.09
	18	17.17
	21	27.10
PHM920	0	0.04
	5	0.03
	26	2.46
	35	27.62
	37	29.00
	42	28.40

Analytical Specificity:
1. No cross reactivity observed with samples from patients infected with HAV, HCV, HIV, CMV, and TP.
2. No interference from rheumatoid factors up to 2000U/ml observed.
3. No high dose hook effect up to HBsAg concentrations of 200000ng/ml observed during clinical testing.
4. Frozen specimens have been tested too to check for interferences due to collection and storage.

Analytical Sensitivity (lower detection limit):The sensitivity of the assay has been calculated by a panel of series of dilutions of WHO reference standard. The assay shows that lower detection limit reaches 0.1 IU/ml.

CONCENTRATION LEVEL	FORTRESS HBSAG 3RD GEN
0.5 IU/ml	+
0.2 IU/ml	+
0.1 IU/ml	+
0.05 IU/ml	+/-
0.025 IU/ml	-

Limitations:
1. Non-repeatable positive result may occur due to the general biological and biochemical characteristics of ELISA assays. The test is design to achieve very high performance characteristics of sensitivity and specificity. However, in very rare cases some HBV mutants or subtypes can remain undetectable. Antigens may be undetectable during the early stages of the disease and in some immunosuppressed individuals.
2. If, after retesting of the initially reactive samples, the assay results are negative, these samples should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step.
3. Any positive results must be interpreted in conjunction with patient clinical information and other laboratory testing results.
4. Common sources for mistakes: kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add samples or reagents, equipment, timing, volumes, sample nature and quality.
5. The prevalence of the marker will affect the assay's predictive values.
6. This kit is intended ONLY for testing of individual serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.
7. This is a qualitative assay and the results cannot be use to measure antigens concentrations.

Indications of Instability or Deterioration of the Reagents:
1. Values of the Positive or Negative controls, which are out of the indicated Quality control range, are indicator of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the samples must be retested. In case of constant erroneous results classified as due to deterioration or instability of the reagents, immediately substitute the reagents with new ones.
2. If after mixing of the Chromogen A and B solutions into the wells, the, the colour of the mixture turns blue within few minutes, do not continue carrying out the testing and replace the reagents with fresh ones.

Reference:

1. Stevens, C. E., P. E. Taylor, and M. J. Tong. 1988. Viral hepatitis and liver disease. Alan R. Liss, New York, N.Y. 142. Stevens, C. E., P. E. Taylor, M. J. Tong, P. T. Toy, G. N. Vyas, P. V. Nair.
2. J.Y. Weissman, and S. Krugman. 1987. Yeast-recombinant hepatitis B vaccine. Efficacy with hepatitis B immune globulin in prevention of perinatal hepatitis B virus transmission. JAMA 257:2612-2616. 143. Stevens, C. E., P. T. Toy, P. E. Taylor, T. Lee, and H. Y. Yip. 1992. Prospects for control of hepatitis B virus infection: implications of childhood vaccination and long term protection. Pediatrics 90(Suppl.):170-173.
3. Hurie, M. B., E. E. Mast, and J. P. Davis. 1992. Horizontal transmission of hepatitis B virus infection to U.S. born children of Hmong refugees. Pediatrics 89:269-273.

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

Appendix -4

Color plates

4-1 ELISA Kit **4-2 ELISA reader**

4-3 Microtiter plate