SEROPREVALENCE OF HEPATITIS B VIRUSAND PARVOVIRUS B19 INFECTIONS AMONG BLOOD DONORS IN SELECTED HOSPITALS IN JIGAWA STATE, NIGERIA

BY

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FACULTY OF LIFE SCIENCE

AHMADU BELLO UNIVERSITY,

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OCTOBER, 2016

DEDICATION

This dissertation is dedicated to Almighty God for His wisdom and grace towards me.

DECLARATION

I declare that the work in this thesis entitled: "**Seroprevalence of Hepatitis B Virus and Parvovirus B19 Infections among Blood Donors in selected Hospitals in Jigawa State, Nigeria**" has been performed by me in the Department of Microbiology under the supervision of Prof. J. B. Ameh and Dr. M. Aminu-Mukhtar. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

Name of Student	Signature	Date

CERTIFICATION

This dissertation entitled "**Seroprevalence of Hepatitis B Virus and Parvovirus B19 Infections among Blood donors in selected hospitals in Jigawa State, Nigeria**" by Benjamin Onyekachi AJAH meets the regulations governing the award of the degree of M. Sc. Microbiology of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Hepatitis B virus (HBV) and Human Parvovirus B19 (HPVB19) infections have been implicated in blood transfusions. This study was conducted to determine the prevalence of HBV and HPVB19 infections and the possible potential risk factors among blood donors in selected hospitals in Jigawa State, Nigeria. The study was conducted between March and August, 2013. Blood samples were collected from blood donors attending Jahun General Hospital, Jahun; Rasheed Shekoni Specialist Hospital, Dutse; and Federal Medical Centre, Birnin Kudu. The study population comprised of 421 blood donors seen at the blood banks of the three hospitals. Structured questionnaires were administered to assess the demographic data and the possible risks factors that might be associated with the viral infections. The blood samples collected were screened for Hepatitis B Surface Antigen using Determine™ HBsAg kit, The positive HBsAg samples were confirmed using HBV ELISA kit and further tested for HBsAg, anti-HBs, HBeAg, anti-HBe and anti-HBc using HBV multi-5 test kit. Screening for both IgG and IgM antibodies to HPVB19 was performed on 266 of the blood samples using Parvovirus B19 ELISA Kit. A prevalence of 15.7% (66/421) was obtained for HBV. The age group 46-54 years had the highest prevalence (22.2%: 2/9) while those aged 25-31 and 32-39 years had the least (14.3%). The relationship between history of scarification andsharing sharp objects were statistically significant risk factors for HBV infection(P=0.021; 0.015). Other studied demographic parameters were not statistically associated with Hepatitis B infection (P>0.05). For HPVB19, prevalence was 72.2% (192/266) and 63.5% (169/266) for IgM and IgG antibodies respectively. There was an association between having multiple sex partners and presence of IgG antibodies to HPVB19 (P=0.04). Prevalence of 15.7% for HBV infection among blood donors in Jigawa State is very high compared to other states in Nigeria, which emphasizes the need for the Government and other relevant agencies to conduct HBV vaccination in the study area. There is also need for enforcement of HPVB19 awareness campaign programs in the study area.

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LIST OF ABBREVIATIONS

ABU	Ahmadu Bello University
ACIP	Advisory committee on immunization practices
ALT	Alanine transaminase
Anti-HBc	Antibodies to the HBV core antigen
Anti-HBe	Antibodies to the HBV e antigen
Anti-HBs	Antibodies to the HBV surface antigen
Anti-Parvovirus B19	Antibodies to Parvovirus B19
CO	Cut-off
CTL	Cytotoxic T-lymphocyte
°C	Degree Celsius
DNA	Deoxyribonucleic acid
ds	Double stranded
EDTA	Ethylene diamine tetra-acetic acid
ELISA	Enzyme-linked immunosorbant assay
FMC BK	Federal Medical Centre, Birnin Kudu
HBcAb	Hepatitis B virus core antibody
HBeAb	Hepatitis B virus e antibody
HBsAb	Hepatitis B virus surface antibody
HBcAg	Hepatitis B virus core antigen

HBeAg	Hepatitis B virus e antigen
HBsAg	Hepatitis B virus surface antigen
HBV	Hepatitis B Virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HIV	Human Immunodeficiency virus
HPVB19	Human Parvovirus B19
HRP	Horseradish peroxidase
Ig	Immunoglobulin
IgM	Immunoglobulin M
IgG	Immunoglobulin G
ISGs	Interferon inducible genes
JGH	Jahun General Hospital
kb	Kilobase
kg	Kilogram
mg	Milligram
nm	Nanometer
NUCs	Nucleotide analogues
OD	Optical Density
PCR	Polymerase Chain Reaction
RSSH	Rasheed Shekoni Specialist Hospital

SGPT	Serum glutamic-pyruvic transaminase
STD	Sexually transmitted disease
SVR	Sustained virological response
ss	Single stranded
TTIs	Transfusion Transmissible Infections
µl	Microliter
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Transfusion transmissible infections (TTIs) are still major concerns in blood transfusion in Nigeria and the world all over making the practice of safe blood transfusion increasingly difficult. Hepatitis B is one of the most infectious diseases in the world and a major public health problem (Aminu *et al.,* 2013).Hepatitis B virusis one of the infections implicated in blood transfusion all over the world (Shittu *et al.,* 2014).It is the most serious viral hepatitis, leading to liver cirrhosis and liver cancer with chronic cases.Subsequently, donor screening procedures and tests are implemented to ensure the safety of the blood (Goodnough and Shander,2008).Human Parvovirus B19 virus is a newly recognized agent of blood transfusion transmitted diseases(Kishore*et al.,* 2011).

About 5% of the world's populations are asymptomatic carriers and 350 million people worldwide are chronic carriers of hepatitis B virus (Aminu *et al.,* 2013).Nigeria is one of the African countries with high endemic HBV infection (Olotu *et al.,* 2016). Infections with this virus have been associated with long-term morbidity and mortality due to complications like cirrhosis, portal hypertension, chronic liver diseases and hepatocellular carcinoma (Drosten*et al.,* 2004).The systematic practice of testing for HBV in blood donations started in 1986 (Rivera-Lopez *et al.*, 2004) and since then, laboratory screening of potential blood donors has relied on the use of evolving immunoassays to detect viral antibodies or antigens.

The complete Hepatitis B virus, about 42nm in diameter, is a double stranded DNA spherical particle with a double shell. It is a non-cytopathic virus that causes a spectrum of liver diseases ranging from acute hepatitis (including fulminanthepatic failure) to chronic hepatitis, cirrhosis, and hepatocellular carcinoma (Fields *et al.*,2001).Hepatitis B surface antigen (HBsAg), Hepatitis B core antigen (HBcAg) and Hepatitis B e antigen (HBeAg) are the three forms of antigens associated with hepatitis B infection (Shittu *et al.,* 2014). The levels of all these three antigens rise during infection. HBsAg level remains high both in acute and chronic phases of viral infection.This virus is responsible for 80% of all cases of primary liver cancer, which is one of the leading causes of death in Asia and Africa (Emechebe*et al.,* 2009).The likelihood of progression to chronic infection is inversely related to age at infection. Around 90% of infants infected perinatally become chronic carriers, unless vaccinated at birth. The risk for chronic HBV infection decreases to 30% of children infected between ages 1 and 4 years and to less than 5% of persons infected as adults (Ott *et al.*,2012). Deaths resulting from HBV yearly stand at about 500,000 to 1.2 million worldwide. Most of these are due to sequelae of chronic HBV infection such as cirrhosis, liver failure and hepatocellular carcinoma (Olotu *et al.*, 2016).

Transmission of hepatitis B virus can result from exposure to infected blood or body fluid. Possible forms of transmission includes unprotected sexual contact, blood transfusion, reuse of contaminated needles and syringes and vertical transmission from mother to child during childbirth without intervention(El-ishaq and Liman, 2015). A mother who is positive for the hepatitis B surface antigen has a 20% risk of passing the infection to her offspring at the time of birth. Also, HBV can be transmitted between members within households, possibly by skin contact or mucus membrane with secretion or saliva containing HBV.This virus has been detected in peripheral and mononuclear cells, tissues of pancreas, spleen, kidney, skin and fluids

like saliva, semen, sweat, breast milk, tears, urine, vaginal secretion (Chen*et al.,* 2009) and faeces (Willey *et al.,* 2011).

Human parvovirus B19 (HPVB19) is a newly emerging DNA virus discovered by an Australian virologist, Yvonne Cossart in the 1970's while working in London, when she was testing donor sera for HBV. She found the B19 virus in the sera, numbered 19 in row B; hence, she named it B19. Later, the B19 virus was placed in the genus *Erythrovirus* of the family *Parvoviridae*. Human parvovirus B19 is the only member of the *Parvoviridae* family known to be pathogenic to human. It is a non-enveloped small single stranded (ss) DNA virus that infects and destroys human erythroid progenitor cells.These erythroid progenitor cells in the bone marrow and spleen, when infected, undergo lysis. There is a resulting decline in the erythrocyte count as well as in lymphocyte, granulocyte, and platelet counts(Alao *et al.*,2010).

Immaturecells in the erythroid lineage are principal target forhuman B19 parvovirus. Hence viral replicationcauses cell death, interrupting red cell productionand has since then beenrecognized as an important human pathogencausing significant morbidity and mortality invarious patient population groups (Jegede*et al.,* 2014). This virus can be found in the blood andrespiratory secretion of infected patients and can be transmittedthrough respiratory secretions, transplacentally and by transfusion of blood or blood products (Bukar*et al.,* 2013).

Parvovirus B19 infection can occur throughout the year, in all age groups, with many showing subclinical manifestations. Estimates of attack rates in susceptible contacts range from 20 to 50%(Brooks *et al.,* 2007). About 30 to 60% of blood donors have antibodies to parvovirus B19(Kaur and Basu, 2005). Acute infection is associated with a viremic phaseshortly followed by IgM antibody production (10 – 14days post-infection). This is followed by IgG

antibodyproduction against the viral capsid. Viraemia declineswith IgM production; IgM declines after a fewmonths but IgG persists longer to convey immunityagainst reinfection(Iheanacho*et al.,* 2014).

1.2 Statement of the Research Problem

Hepatitis B is one of the most infectious diseases in the world and a major public health problem (Aminu *et al.,* 2013).It is estimated that about two billion people who are alive today have at some time been infected with HBV and about 350 million people are chronic carriers (O'Connell *et al.,* 2000). The viral infection may go unnoticed because greater than 50% of the cases are subclinical. Infected people therefore either unconsciously contribute to the transmission of the virus or do not seek medical attention with a resultant danger of liver cirrhosis or hepatocellular carcinoma (Pennap *et al.,* 2011). Hepatitis B virus is responsible for the death of over one million people annually (Ziraba *et al.*,2010), thus making it a growing public health concern. The virus is widely referred to as a silent killer because many carriers do not realize they are carrying the virus (Libbus and Phillips, 2009).It accounts for 400 million chronic infections worldwide, and is hyperendemic in sub-Saharan Africa and Asia (Olokoba *et al.,* 2000).In sub-Saharan Africa, close contact amongtoddlers is considered to be instrumental to the high rate of infection among the population (Dienstag *et al.,* 2005).

In Nigeria, the transmission of hepatitis B virus occurs mainly during childhood and all the risk factors (like blood transfusion, sexual promiscuity, and lower socioecomic status) implicated elsewhere in the spread of the virus in the general population also play a role in Nigeria (Emechebe*et al.,* 2009). Nigeria is one of the several countries with high endemic HBV infection (Sirisena *et al.,* 2002; Mbaawuaga *et al.,* 2008; Aminu *et al.,* 2013). Mbamara and Obiechina

(2010) estimated that about 12% of Nigerians are chronic HBV carriers. Further studies in Nigeria have shown that the prevalence of HBV infection differs among various populations. Aminu *et al.,* (2013) found a prevalence of 12.5% HBV infection among tertiary institution studentsin Kaduna State while Alao *et al.,* (2008) observed 20% positivity for HBsAg among blood donors in Benue State.

Hepatitis B virus was among the first virus known to be transmitted by blood and blood products. The infection from blood transfusions became rare after the introduction of the HBsAg test in early 1970, but remained of the serious complications of blood transfusion in the tropical countries (Afolabi*et al.*,2013). The residual transmission risk of HBV through blood transfusion remains high(Dodd*et al.*,2002). This is attributed to the interval between initial HBV infection and the detection of HBsAg, resulting in a long window period during which the virus is transmissible (Stroffolini*et al.*,2000).

Parvovirus B19 is the causative agent of erythema infectiosumor fifth disease in children, and is associated with a wide range of clinical diseases (Kishore *et al.,* 2011). Some of these diseases include acute or chronic arthritis in adults, transient anaemia amongst healthy adults, aplastic crises in infected persons with an underlying blood disorder and prolonged anaemia in immunocompromised persons such as AIDS and organ transplant patients. Also, during pregnancy, the virus can be transmitted from the mother to the foetus, which can result in hydrops foetalis, spontaneous abortion or intrauterine foetal death (IUFD), most commonly during the second trimester of pregnancy (Tolfvenstam *et al.,* 2001). However, a persistent infection may be observed in immunocompromised patients unable to produce neutralizing antibodies and to clear the virus leading to chronic B19 carriage with or without anaemia

(LaMonte *et al.,* 2003). Transmission of the virus occurs mainly through the respiratory droplets of patients, transfusion of B19 infected blood and blood products, and transplacentally during maternal B19 infection (Kishore and Kapoor, 2000). In Nigeria, 14.3% prevalence rate of anti-Parvovirus B19 IgM was observed among non-sickle cell anemia subjects in Lagos (Iwalokun *et al.,* 2013), while Girei *et al.,* (2010) reported 13.2% anti-Parvovirus B19 IgM prevalence among pregnant women in Jos.

1.3 Justification of the Study

Hepatitis B virus and Parvovirus B19 have been implicated to be transmitted through transfusion of improperly screened blood (Kishore and Kapoor,2000; Adekanle*et al.,* 2010). Although screening of Hepatitis B surface Antigen prior to blood donations in blood banks is a common routine,current blood banking practice does not require routine screening for human Parvovirus prior to blooddonation (Lefrère*et al.,* 2006). It is important to determine the prevalence of HBV and Parvovirus antibodies among blood donors since blood transfusion has been identified as one of the major means of transmitting these infections.The study therefore is aimed at determining the seroprevalence of HBsAg as a surrogate of HBV infection and IgG and IgM antibodies to Parvovirus B19 among blood donors in some parts of Jigawa State in order to provide information on the immune status of the screened population to the viruses and possible risk factors for acquiring these viruses, with the hope of aiding in the control and spread of these infections.

1.4 Aim of the Study

The aim of this research was to determine the sero-prevalence of hepatitis B virusand human parvovirus B19 infections among blood donors in some hospitals in Jigawa State.

1.5 Objectives of the Study

The objectives of this study were to:

1. Determine the sero-prevalence of Hepatitis B surface antigenamong blood donors in some selected hospitals in Jigawa State.
2. Determineother HBV infection serological markers in the HBsAgpositive samples.
3. Determine the sero-prevalence of human parvovirus B19 infection among blood donors in some selected hospitals in Jigawa State.
4. Determine some risk factors that might be associated with the spread of these infections among the blood donors.

1.6 Research Questions

i. What are the sero-prevalence of HBV and human parvovirus B19 infections among blood donors in Jigawa State?

ii. Is there any difference among the blood donors in relation to these diseases according to their gender and other demographic data?

iii. What are the other risk factors common among the blood donors that could contribute to the spread of hepatitis B virus and human parvovirus B19 infections?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Hepatitis B Virus

Hepatitis B infection is caused by Hepatitis B virus, an enveloped, double-stranded circular DNA virus of complex structure. It is classified as an *Orthohepadnavirus* within the family *Hepadnaviridae*.The virus is composed of a 27nm nucleocapsid core (HBcAg), surrounded by an outer lipoprotein coat (also called envelope) containing the surface antigen (HBsAg). The surface antigen is generally produced in vast excess, and its presence in body fluids is an indication of hepatitis B infection (Willey *et al.,* 2011).The inner core of the virus contains HBcAg, HBeAg, a single molecule of partially double-stranded DNA, and a DNA-dependent DNA polymerase (Figure 2.1). Although replication takes place in the liver, the virus spreads to the blood where virus-specific proteins and their corresponding antibodies are found in infected people. Blood tests for these proteins and antibodies are used to diagnose the infection (Brooks *et al.,* 2007). Hepatitis Bsurface antigen can be identified in serum 30 to 60 days after exposure to HBV and persists for variable periods (Mahoney, 1999).

Hepatitis B is a viral infection that affects the liver and can cause both acute and chronic disease. It is encouraging that majority of patients do recover from the acute infection, however, those that progress to chronic disease state are at great risk of developing complications such as hepatocellular carcinoma, cirrhosis and liver failure. This viral infection can be influenced by many factors such as host immune status, age at infection, and level of viral replication (Pan and Zhang, 2005). Both acute and chronic hepatitis can be either symptomatic orasymptomatic.

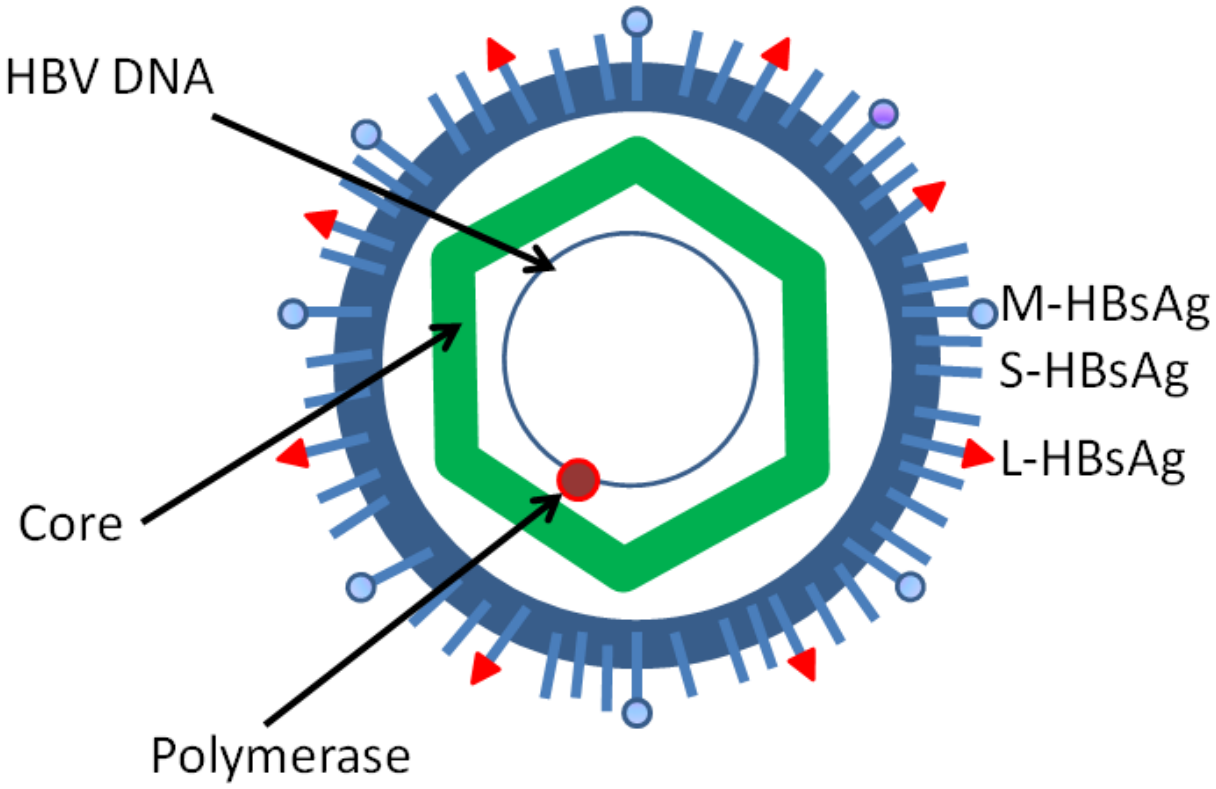


Figure 2.1: The structure of Hepatitis B Virus

Source: Locarnini, (2004)

Key: M-HBsAg = Middle Hepatitis B surface Antigen; S-HBsAg = Small Hepatitis B surface Antigen; L-HBsAg = Large Hepatitis B surface Antigen; HBV DNA = Hepatitis B Virus Deoxyribonucleic Acid.

Most primary infections are self-limited with clearance of virus and development of immunity. However, an estimated 3 to 5% of adults and up to 95% of children develop chronic HBV infection (Pan and Zhang, 2005).

Age-specific prevalence of HBV infection varied by geographical region with the highest endemicity levels found in sub-Saharan Africa (Ott *et al.,*2012). Seventy to 95% of adults in the Sub-Sahara have at least one marker of HBV.Most people become infected at birth or during childhood, or by sexual contact, and up to 20% become chronic carriers, particularly those infected between 1-5 years (Cheesbrough, 2010). Chronic carrier rate is 20% in these children.Studies done in Nigeria showed HBV carriage rate in the range of 9 to 39% (Fakunle*et al.,* 1981;Ezegbudo *et al.,* 2004; Uneke*et al.,* 2005).

Hepatitis B virus is a common viral pathogen that infects an estimated 4 million people worldwide, including 400 million who have chronic infection(McMahon,2005).Many of these people appear healthy but can spread the virus to others and 1 million persons die each year from HBV-related chronic liver disease.In the past decade, significant progress in the understanding of the molecular virology and pathogenesis of HBV infection has been made. In addition, effective treatment modalities have been developed for persons with chronic infection. Worldwide, prevention of HBV transmission has become a high priority. In 1992, the Global Advisory Group to the World Health Organization recommended that all countries integrate hepatitis B vaccine into national immunization programs by 1997. Many countries have reported dramatic reductions in the prevalence of chronic HBV infection among children born since the hepatitis B vaccine was introduced into infant immunization schedules (Mahoney, 1999).

2.1.1 Acute Hepatitis B Infection

The consequences of acute HBV infection are highly variable. The incubation period ranges from 6 weeks to 6 months, and development of clinical manifestations is highly age dependent. Newborns generally do not develop any clinical signs or symptoms, and infection produces typical illness in only 5 to 15% of children between 1 to 5 years of age(McMahon, 1985).Older children and adults are symptomatic in 33 to 50% of infections. Symptomatic infections vary in severity from mild to fulminant forms. Clinical signs and symptoms of acute HBV infection include fever, anorexia, nausea, malaise, vomiting, jaundice, dark urine, clay-colored or pale stools, and abdominal pain. Fulminant hepatitis occurs in about 1 to 2% of persons with reported acute disease and has a case-fatality ratio of 63 to 93% (Mahoney, 1999).

2.1.2 Chronic Hepatitis B Infection

Chronic HBV infection is defined as the presence of HBsAg in serum for at least 6 months or the presence of HBsAg and the absence of anti-HBc immunoglobulin M (IgM). Persons with chronic HBV infection are at a lifelong risk of developing hepatocellular carcinoma (HCC) cirrhosis, or both. Many persons with HBV are unaware that they carry the infection (McMahon,2005).The risk of developing chronic infection varies inversely with age and is highest (up to 90%) for infants infected in the perinatal period. Between 25 and 50% of children infected between 1 and 5 years of age develop chronic infection, compared to 6 to 10% of acutely infected older children and adults (Hyams *etal.,* 1989). Patients with chronic infection may be divided into those with evidence of active replication, typically associated with abnormal transaminase levels and higher viral loads, and those in the nonreplicative state, associated with decreased markers of liver inflammation and damage and lower viral loads.

The presence of HBeAg in serum reflects active viral replication, and the clinical outcome of infection is correlated with HBeAg status. Conversion to HBeAg-negative and HBeAb-positive status in patients with chronic hepatitis B typically leads to decreased inflammation, with normalizing transaminase levels and decreased levels of HBV DNA in serum, which is the inactive carrier or non-replicative state. The e antigen marker is also absent in patients with core or precore mutants. Using conventional hybridization assays, HBsAg carriers do not have detectable HBV DNA in serum. Testing for HBV DNA with more sensitive techniques, such as the polymerase chain reaction (PCR) assay, however, usually demonstrates low levels of viral DNA in serum in these carriers. Chronically infected patients without active liver disease or viral replication (inactive carriers) generally have a benign course, with a smaller likelihood of progressing to cirrhosis. Patients who continue to have active viral replication with high levels of HBV DNA and HBeAg in serum have progressive liver injury, and cirrhosis and end-stage liver disease can develop (Hsu*et al.,* 2002).

Chronic HBV infection is associated with a ten-fold increase in the risk of developing hepatocellular carcinoma. This risk is further magnified in the setting of ongoing inflammation. In patients with both HBsAg and HBeAg, the risk increases to 60-fold compared with the general population (Yang *et al.,* 2002). Older men with cirrhosis and those coinfected with hepatitis C are at greatest riskhepatocellular carcinoma.

2.1.3 Pathogenesis of Hepatitis B Virus

Hepatitis B virus primarily interferes with the functions of the liver by replicating in the liver cells, known as hepatocytes (Yan *et al.,* 2012). Chronic HBV infections can progress to life-threatening complications such as cirrhosis and hepatocellular carcinoma Worldwide, more than

350 million people are chronically infected with HBV. More than one-third of these individuals will die fromserious liver diseases such as cirrhosis and HCC if their conditions are left untreated.Therefore, it is important to improve our understanding of HBV pathogenesis, which will lead to the development of better treatments for the infection (Lu, 2011).

During theacute infection while using Chimpanzees as models, hepatic injury and the body's immune response were evaluated. Results showed that HBV is not directly cytopathic for the infected hepatocytes(Chisari, 2010). During the early phaseof HBV infection, or before HBVspecific T lymphocytesenter the animal's liver, 100% of the hepatocytes may be infected without histological or biochemical evidence of liverdisease. Damage to the liveroccurs only after the initiation ofthe immune response to clear the virus,which suggests that HBV does not directly cause hepatic injury. Further study suggested that damage to the liver is associated with the infiltration of activated T lymphocytes in HBV-infected liver.Moreover, if the cellular immune response mediated by T-lymphocytes is deficient or pharmacologically suppressed, HBV can replicate at high levels in the liver of patients in the absence of cytological abnormalities or inflammation.Theseresults further support the hypothesis that hepaticinjury is triggered by the body's immune response during clearance of virus-infected cells(Lu, 2011).

In chronic HBV infection, usually only slight or no liver damage occurs, although active viral replication is found that may be due to the absence of anactive immune response or to the fact that the immune system is overwhelmed.The body's immune response usually includes innate and adaptive immune responses. Unlike the adaptive immune response, the innate immune response is a cellular responseto the virus or other pathogen. Innate immune response is an immediate, nonspecific response that often results in a rapid induction of interferon alpha/beta by

the infected cell, which triggers the transcriptional expression of a large number of interferoninducible genes (ISGs) (Alexopoulou*et al*., 2001).The ISGs in turn initiatea variety ofintracellular antiviral mechanisms that have the potential tominimize pathogenetic processes by limiting viral production and spread.Surprisingly, intrahepatic gene expression profiling acutely infected cell with HBV revealed that it acts like a stealth virus early after infection because it does not induce any cellular gene expression including ISGs asit spreads through the liver. In chronic hepatitis, no evidence indicates that HBV replication leads to apoptotic death of hepatocytes and is mediated by aninnate immune response (Lu, 2011).

The clearance of viral infected cells during acute and probably also in chronic HBV infection is due to the body's adaptive immune response, which usually prompts the death ofinfected hepatocytes leading to hepatic injury and damage (Thimme *et al.*,2003).Serum glutamic-pyruvic transaminase (SGPT), known alsoas alanine transaminase (ALT), is one of the markers for diagnosing liver damage. Dead hepatocytes release ALT,increasing its level in the blood. In acute HBV infection, the death of hepatocytes is caused by an attack from the T lymphocytes, the intention of which is to remove virus-infected cells. In this immune response, both CD4T cells (Thelper cells) and CD8(cytotoxic T-lymphocyte [CTL])cells are activated.CD4 T cells are robust producers of cytokines and are required for the efficient development ofCTLs and B cells, which produce anti-HBV antibody to reduce the levels of circulating virus.Studies of HBV-infectedchimpanzees suggest that CD4 T cells have no direct effect on viral clearance and liver disease (Thimme *et al.*,2003).

Depletion of CD4 Tcells at the peak of HBV infection in chimpanzees does not affect the rate of viral clearance or the extent of liver damage, thereby supporting the fact that the death of

infected hepatocytes were due to the body's adaptive immune response.However, CD4 T cells may be necessary to instruct and maintain anti-HBV CTLs. The specific CTL responseplays a significant role in viral clearance and the pathogenesis of liver damage. In acuteHBV 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 onsetof liver damage and viral clearance in chimpanzees.The association of CTLs with liver injuryis also observed in patients with acute viral hepatitis whosuccessfully clear HBV(Ferrari, 2003). In patients with chronic HBVinfection, CTLs seem to be suppressed, although low levelsof CTLs exist in the infected liver.Reactivation of thekilling mediated by CTLs usually leads to the clearance of HBV in patients with chronic infection (Ferrari, 2003).

2.1.4 Clinical Features of Hepatitis BVirus Infection

In approximately 30 to 50% of adults, infection causes symptomatic acute hepatitis, but in neonates and young children, particularly those <1 year of age, initial infection is usually asymptomatic (Shepard *et al.,* 2006). The incubation period is usually 45 to 180 days and the period of communicability extends from several weeks before the onset of acute illness usually to the end of the period of acute illness. Acute illness is clinically indistinguishable from other forms of hepatitis, and symptoms include fever, jaundice, malaise, anorexia, nausea and vomiting, abdominal pain (especially in the right upper quadrant), myalgia, and the passage of dark-coloured urine and light-coloured stools. Jaundice may be preceded by an acute febrile illness with arthralgia or arthritis and rash. During recovery, malaise and fatigue may persist for many weeks. Fulminant hepatitis occurs in up to 1% of acute cases(Mast and Ward, 2008).

Following acute infection, approximately 1 to 10% of persons infected in adulthoodbut up to 90% of those infected in early infancy, become chronically infected with hepatitis B (Edmunds*et al.*,1993). Persons chronically infected with HBV are identified by the long-term presence (longer than 6 months) of circulating HBsAg (Koziel and Thio, 2010). Those with occult infection may reactivate HBV infection if they become immunocompromised.Persons with chronic HBV infection are capable of transmitting the disease, including mother-to-child peripartum transmission, though they often remain asymptomatic and may not be aware that they are infected. Most of the serious complications associated with hepatitis B occur in the context of chronic HBV infection, which is associated in up to 25% of cases with premature mortality due to cirrhosis and/or hepatocellular carcinoma (Mast and Ward, 2008).

2.1.5 Hepatitis B VirusSerological Markers

Three distinct anti-body systems associated with hepatitis B virus infection have now been established: hepatitis B surface antigen (HBsAg) and its antibody (anti-HBs); hepatitis B core antigen (HBcAg) and its antibody (anti-HBc); and the more recently discovered hepatitis B e-antigen (HBeAg) and its antibody (anti-HBe). The presence of HBsAg in serum has been used as a marker for HBV infection(Karayiannis, *et al.*, 2009).Anti-HBs is observed most frequently in convalescent serum (i.e. serum from patients that recently recovered from a disease), whereas the presence of anti-HBc indicates past or current infection with HBV. HBcAg is not detectable in serum, but can be detected in liver tissue in persons with acute or chronic hepatitis B infection. (Ling*et al.*,1979).When HBsAb is positive (antibodies are present), it usually means that the patient had recovered from hepatitis B virus infection and have some immunity, or that the patients once received the hepatitis B vaccination and are immune. The hepatitis e antigen, or

HBeAg, is a marker of an actively replicating HBV infection. Those with a positive HBeAg have active replication of the virus in their liver cells, more of the virus circulating in their blood, and as a result, they are more infectious, with a higher likelihood of transmitting HBV to others. More often, when a person is HBeAg positive, they tend to be HBeAb negative and vice-versa. This active, replicating phase may go on for weeks, as in the case of an acute infection, or for years, or even decades in those chronically infected. Thus, HBeAg is a marker of high infectivity and appears after HbsAg. An uninfected person vaccinated against hepatitis B will generate an immune response, or surface antibody (HBsAb, or anti-HBs) to the HBV vaccine.Serologic assays are available commercially for all markers except HBcAg, because no free HBcAg circulates in blood(Japhet*et al.,* 2011).

2.1.6 Transmission of Hepatitis B Virus

Hepatitis B virus is transmitted by percutaneous (i.e., puncture through the skin) ormucosal contact with infectious body fluids(e.g. semen and saliva), by sexual contact with an infected person, and perinatally from an infected mother to her infant. Other means are drug injections that involve sharing of needles, syringes, or drug-preparation equipment, transfusion of improperly screened blood and blood products, contact with blood or open sores of an infected person, needle sticks or sharp instrument exposures, sharing items such as razors or toothbrushes with an infected person. The virus incubation period is 90 days on average, but can vary from about 30 to 180 days(Mahoney, 1999; Olokoba*et al.,* 2010).

2.1.7 Hepatitis B Virus Vaccines

Hepatitis B virus vaccines are produced with the use of yeast (*Saccharomyces cerevisiae*) and recombinant techniques to generate the hepatitis B surface antigen (HBsAg) protein. In 1991, the Advisory Committee on Immunization Practices (ACIP) recommended a comprehensive strategy to eliminate HBV transmission in the United States, which included universal vaccination of infants. In 1995, routine immunization of adolescents was added. In 1999, the strategy was further modified to include immunization of all persons up to 18 years of age. This strategy has reduced the overall annual incidence of acute HBV infections in the United States, from 8.5 cases per 100,000 persons in 1990 to 2.8 per 100,000 in 2002, a decrease of 67 percent (Polandand Jacobson,2004).

According to Hepatitis B Foundation(2014), the following list is a general guide for people to be vaccinated for HBV: all infants at birth and all children up to 18 years, health care professionals and emergency personnel, sexually active teens and adults, men who have sex with men, sex partners or close family/household members living with an infected person, families considering adoption, either domestic or international, travelers to countries where hepatitis B is common (Asia, Africa, South America, the Pacific Islands, Eastern Europe, and the Middle East), patients with kidney disease or undergoing dialysis, residents and staff of correctional facilities and group homes, any person who may fall into a high risk group due to occupation or lifestyle choices, and people living with type 1 or type 2 diabetes (Hepatitis B Foundation, 2014).

The vaccine is generally administered in three doses, with the second dose given one month after the first dose and the third dose given six months after the first dose and it is not necessary to restart the series if there have been prolonged intervals between doses.According to Fisman*et al.*

(2002), the antibody response to HBV vaccination declines with increasing age. Patients older than 30 years have an increased risk of nonresponse to HBV vaccine, as compared with younger persons.Thus, immunization during childhood or adolescence offers the greatest potential for protectionand provides lifelong immunity. Ninety percent of healthy adults and 95 percent of infants, children, and adolescents have protective serum anti-HBs antibody concentrations after the vaccine series has been complete (Hepatitis B Foundation, 2014).

2.1.8 Diagnosis of Hepatitis B Virus Infection

Testing for HBV offers the opportunity to provide treatment and prevention advice to those who are positive (and their contacts) and recommend vaccination to those who are negative but at continuing risk. The hepatitis B surface antigen is the most frequently used to screen for the presence of this infection. It is the first detectable viral antigen to appear during infection. However, in an early infection, this antigen may not be present and it may be undetectable later in the infection as the diagnosis of HBV infection requires the evaluation of the patient's blood for HBsAg, hepatitis B surface antibody (HBsAb), and hepatitis B core antibody (HBcAb) (Karayiannis, *et al.,* 2009).HBsAb indicates recovery and immunity from HBV infection or successful immunization against HBV infection. HBcAb appears at the onset of acute HBV infection, but may also indicate chronic HBV infection (Wilkins *et al.*, 2010).

According to Hepatitis B Foundation(2014),common hepatitis B blood test results are:

- HBsAg - This refers to the outer surface of the hepatitis B virus that triggers an antibody response. A "positive" or "reactive" HBsAg test result means that the person is infected

with the hepatitis B virus. This can be an "acute" or a "chronic" infection. Infected people can pass the virus on to others through their blood.

- HBsAboranti-HBs - This refers to the protective antibody that is produced in response to an infection. It appears when a person has recovered from an acute infection and cleared the virus (usually within six months) or responded successfully to the hepatitis B vaccine shots. A "positive" or "reactive" HBsAb (or anti-HBs) test result indicates that a person is "immune" to any future hepatitis B infection and is no longer contagious**.** This test is not routinely included in blood bank screenings.

- HBcAbor anti-HBc - This refers to an antibody that is produced in response to the core-antigen, a component of the hepatitis B virus. However, this is not a protective antibody. In fact, it is usually present in those chronically infected with hepatitis B. A "positive" or "reactive" HBcAb (or anti-HBc) test result indicates a past or present infection, but it could also be a false positive. The interpretation of this test result depends on the first two test results. Its appearance with the protective surface antibody (positive HBsAb or anti-HBs) indicates prior infection and recovery. For chronically infected persons, HBcAb and HbsAg are usually present(Hepatitis B Foundation, 2014).

2.1.9 Treatment of Hepatitis B Virus Infection

The aim of treatment of chronic hepatitis B infection is toachieve sustained suppression of HBVreplication and re-mission of liver disease. The ultimate goal is to preventcirrhosis, hepatic failure and hepatocellular carcinoma. Parameters used toassess treatment response include normalization of serumALT, decrease in serum HBV DNA level, loss of HBeAgwith or without

detection of anti-HBe, and improvementin liver histology (Lok and McMahon, 2007). Long-term treatment is often required, although some individuals maintain a low or undetectable HBV DNA level >6 months after stopping treatment, which is classed as a 'sustained virological response' (SVR). Complete eradication of HBV infection does not occur, due to the persistence of HBV DNA within the nuclei of host hepatocytes (Di Marco andCraxì, 2009).

Treatment should be considered for patients in either the HBeAg-positive chronic hepatitis B or the HBeAg-negative chronic hepatitis B phases and for those with cirrhosis, irrespective of HBeAg status (Jafri and Lok, 2010). Treatment is not indicated for individuals in the immune tolerant phase, where liver damage has not yet occurred. The phase of infection or extent of liver damage may be difficult to assess, in which case a liver biopsy can be helpful.

There are currently seven drugs available for the treatment of chronic hepatitis B: five nucleotide analogues (NUCs) (lamivudine, adefovir, entecavir, tenofovir and telbivudine) and two interferon-based therapies (conventional interferon and pegylated interferon alpha). The NUCs suppress viral replication by inhibiting HBV viral polymerase, whereas interferon therapy works by enhancing the host immune response (Di Marco and Craxì, 2009).

Nucleotide analogues therapy has a good side-effect and safety profile but there is a considerable risk of viral resistance (Liaw andChu, 2009). Long-term studies of lamivudine therapy have shown that treatment reduces the incidence of cirrhosis and HCC compared to untreated controls. The advantages of interferon therapy are the absence of viral resistance, the finite course of treatment (normally 48 weeks) and an increased chance of sustained virological response (SVR) and HBeAg and HBsAg clearance compared with those taking NUC therapy.

2.1.10 Prevention of Hepatitis B Virus Infection

Immunization is one of the mainstrategiesto prevent hepatitis B infection. Vaccination programs usually target individuals with increased risk of HBV exposure, including infants at particular risk of infection at birth. Effective strategies to prevent HBV infection include the avoidance of high-risk behaviors, the prevention of exposure to blood and bodily fluids, the screening of women in late pregnancy for the presence of HBV, active immunization with the hepatitis B vaccine, and passive immunization with hepatitis B immune globulin before or after exposure. Optimal protection is achieved with a completed series of hepatitis B vaccine before exposure occurs (Poland and Jacobson, 2004).

2.2 Parvovirus B19

Parvovirus B19 is a newly emerging DNA virus discovered by an Australian virologist, working in London, when she was testing donor sera for hepatitis B virus, but found the B19 virus in the sera, numbered 19 in row B, hence, she named it B19. It was placed in the genus *Erythrovirus* of the family *Parvoviridae*. Human Parvovirus B19 is the causative agent of erythema infectiosum or fifth disease in children, and is associated with a wide range of clinical diseases (Kishore and Kapoor, 2000).It may cause transient anaemia amongst healthy adults, aplastic crises in infected persons with an underlying blood disorder and prolonged anaemia in immunocompromised persons such as AIDS and organ transplant patients(Jegede*et al.,* 2014). In addition, during pregnancy the virus may be transmitted to the foetus which can result in hydrops foetalis, spontaneous abortion or intrauterine foetal death (IUFD), most commonly during the second trimester of pregnancy (Tolfvenstam *et al.,* 2001).

It was first discovered in the sera of healthyblood donors and patients, one of whom had acutehepatitis (Cossart *et al.,* 1975).Immature cells in the erythroid lineage are principal target for human B19 parvovirus. Hence viral replication causes cell death, interrupting red cell production (Jegede*et al.,* 2014).Parvovirus B19 can be found in the blood andrespiratory secretion of infected patients.

Parvovirus B19 constitutes a non-envelopedhuman DNA virus of world-wide distribution whosesingle-stranded genome comprises 5.6kb (Shade *et al.,* 1986). Human parvovirus B19 virus is a newly recognized agent for transfusion transmitted diseases.As a non-enveloped DNA virus, itescapes viral inactivation methods(Kishore,*et al.,*2011).However, parvovirus B19 screening of whole blood donations has not been a high priority because of the benign and/ or transient nature of most parvovirus diseases, the availability of effective treatment for chronic haematologic sequelae and the extreme rarity of reports of parvovirus B19 transmission by blood components (Kaur and Basu, 2005). Therefore, the issues on safety of blood and blood products were raised(Alter *etal*.,2007).

2.2.1 Epidemiology of Parvovirus B19 Infection

Infection with parvovirus is very common and occurs worldwide. Seroprevalence of human B19 virus is age related.Acquisition is often during childhood and continues at lower rates throughout adulthood such that between 70 and 85% of adults show serologic evidence of past infection *(*Kelly *et al.,*2000). In USA, seropositivity rate is 5-10% among young children (aged 2-5 years), increasing to 50% by age 15 years and 60% by 30 years of age. A small percentage of adults acquire infection every year, resulting in an incidence of approximately 90% in adults older than 60 years (Servey *et al.,*2007).

2.2.2 Pathogenesis of Parvovirus B19 Infection

The virus replicates in human erythroid cells of the bone marrow and blood,which carry the erythrocyte blood group P antigen (Lefrère*etal*.,2006). Viremia in early infection occurs with extremely high titersup to 1,011 to 1,013 genome equivalents/ml(Frickhofen and Young,1989).At this point the patients tend to be asymptomatic,and B19 parvovirus infected blood donors easily go unrecognized.The high-titer viremia lasts for only about 5 to 10 days,until expression of specific antibodies.Although the virus is thought to be cleared from blood,its DNA at low levels can be detected by PCR in a declining proportion for months or,less frequently years after infection (Cassinotti and Siegl, 2000).

This viral infection displays a broad spectrum of clinical manifestations such as erythema infectiosum in children, aplastic crisis in patients with hemolytic anemia, chronic bone marrow failure in immunocompromised hosts, and hydrops fetalis after intrauterine infection or spontaneous abortion(Tolfvenstam *et al.*, 2001).

After initial replication, probably in the respiratorytract, the virus enters its target cells in thebonemarrow, erythroid precursor cells, through its receptor,the blood group P antigen (van Elsacker-Nieleand Kroes, 1999). Ensuing viral replication leads toan arrest in erythropoiesis which usually lasts approximatelyone week. At this stage, the patient under‘erythropoietic stress’ can experience an aplastic crisis.Viremia ceases as virus-specific antibodies appearin thepatient’s serum, which may trigger potentiallyimmune-mediated symptoms such as a rash or arthralgia. In immunologicallynormal individuals, the infection is cleared withinseveral weeks by humoral immune response, withdetectable specific IgG conferring lifelong immunityto re-infection. In patients with dysfunctional oraltogether absent humoral immunity, persistent

infection resulting in chronic suppressionof erythropoiesis with chronic anemia canoccur (van Elsacker-Niele and Kroes, 1999).

About 30 to 60% of blood donors have antibodies to parvovirus B19. This is indicative of immunity rather than chronic persistent infection (Luban, 1994). The virus has been found regularly in clotting factor concentrates and has been transmitted to persons with hemophilia. Rare transmission through cellular blood components and plasma, but not intravenous immunoglobulin and albumin, has been reported (Prowse*etal.*, 1997).Parvovirus B19 can infect and lyse red cell progenitors in the bone marrow. This may result in sudden and severe anemia in patients with underlying chronic hemolytic disorders. Patients with cellular immunodeficiency, including those infected with HIV, are at risk for chronic viraemia and associated hypoplastic anemia (Arnold *etal.*, 2005).

2.2.3 Transmission of Parvovirus B19 Infection

A common mode of transmission is through personal contact via aerosol or respiratory secretions. Iatrogenic transmission via contaminated blood products, such as clotting factor concentrates also occurs (Elnifro *et al.,* 2009). Also, the virus can be transmitted by blood transfusions (Jegede*et al.,* 2014).Transplacental transmission of parvovirus B19 during pregnancy is one of the leading causes of non-immune foetal hydrops, spontaneous abortion or intrauterine foetal death, which occur more during the second trimester (Emiasegen*et al.,* 2011).

2.2.4 Diagnosis of Parvovirus B19 Infection

Based on the condition of the individual's immune system, the parvovirus infection can be diagnosed in three major ways: the detection of the pronormoblasts, the detection of the B19

virus and the detection of antibodies. For people whose immune systems have been suppressed or compromised, the detection of the presence of the human parvovirus will be carried out. For people with competent immune systems, the detection of the presence of antibodies will indirectly indicate the presence of the human parvovirus.Modern diagnostic analysis of parvovirus B19 infection usually includes measurement of parvovirus B19 IgG and IgM antibodies in blood samples and parvovirus B19 DNA in blood or tissue samples and especially in bone marrow samples by quantitative PCR (Reed*etal.*, 1999). Morphologically, bone marrow aspirate samples do not show mature erythroid precursors but do show characteristic giant pronormoblasts at the time of acute infection (Brolide*etal.*, 2006).

2.2.5 Treatment of Parvovirus B19 Infection

Specific antiviral therapy is not available to treat parvovirus B19 infection. The approach to therapy of infection depends on host factors such as immune status, underlying conditions, and manifestations of infection. Most cases of infection in immunocompetent hosts do not need treatment because the symptoms are transient, although nonsteroidal anti-inflammatory agents may be helpful in cases of arthropathy. Patients with transient aplastic crisis may need supportive therapy with blood transfusions until neutralizing antibody response can clear the virus and hematopoiesis is restored(Waldman and Kopp, 2007).

There are several options for the treatment of pure red cell aplasia and persistent infection in immunocompromised patients in whom B19-specific antibody response is absent or minimal. Commercial Ig (IVIG), a significant source of anti-B19 antibodies, has proved to be efficacious, although no controlled studies have been carried out (Moudgil*etal.*,1997;Egbuna*etal.*, 2006). Various regimens have been reported with favorable outcomes, but on the basis of the pooled

data, 400 mg/kg per dose of IVIG for 5 to 10 consecutive days seems to be clinically useful in most cases. Although clinical response is common as evidenced by reticulocytosis, increased hemoglobin levels, and decline in serum viral DNA, complete eradication of viremia may not occur in some patients, particularly in transplant patients, who are highly immunosuppressed. Thus, relapses of anemia can occur up to several months after completion of treatment. Repeated administration of IVIG may be helpful, but some patients experience multiple relapses (Eid *et al.*, 2006). Reduction of immunosuppressive medication is often recommended in addition to IVIG (or without IVIG in less severe cases) to allow the patient's own immune response to mature and neutralize the virus (Geetha*etal.*, 2000).

2.3 Common Modes of Transmission of Hepatitis B Virus and Parvovirus B19 Infections

Mahoney (1999) wrote that Hepatitis B is transmitted perinatally from an infected mother to her infant and transfusion of improperly screened blood and blood products. Jegede*et al.*, (2014), on the other hand, wrote that Parvovirus B19 is transmitted though transfusion of Parvovirus B19 infected blood and blood products, and transplacentally from mother to child.This study is aimed at finding the seroprevalence of Hepatitis B and Parvovirus infections among blood donors.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Jigawa State,created out of the old Kano State in August 1991. It has a population of 4,348,649 according to National Population Census(2006).The state is situated in the north-western part of the country between latitudes 11.00°N to 13.00°N and longitudes 8.00°E to 10.15°E with 27 Local Government Areas. Kano and Katsina States border Jigawa to the west, Bauchi State to the east and Yobe State to the Northeast. To the North, Jigawa shares an international boundary with Zinder Region in The Republic of Niger.The State enjoys vast fertile arable land to which almost all tropical crops could adapt. Most parts of Jigawa lie within the Sudan Savannah with a vegetation zone that constitutes vast grazing lands suitable for livestock production (Jigawa State - Wikipedia).

The three hospitals selected for this study were selected because they operated blood bank systems. The hospitals were located in Birnin-Kudu, Jahun and Dutse Local Government Areas (Figure 3.1).

3.2 Study Design

The three hospitals used in the study were Jahun General Hospital, Jahun; Rasheed Shekoni Specialist Hospital, Dutse; and Federal Medical Centre, Birnin Kudu. These hospitals were selected based on the facts that they operate blood banking system. These hospitals were located in three different Local Government Areas with Dutse LGA in between Jahun LGA and Birnin

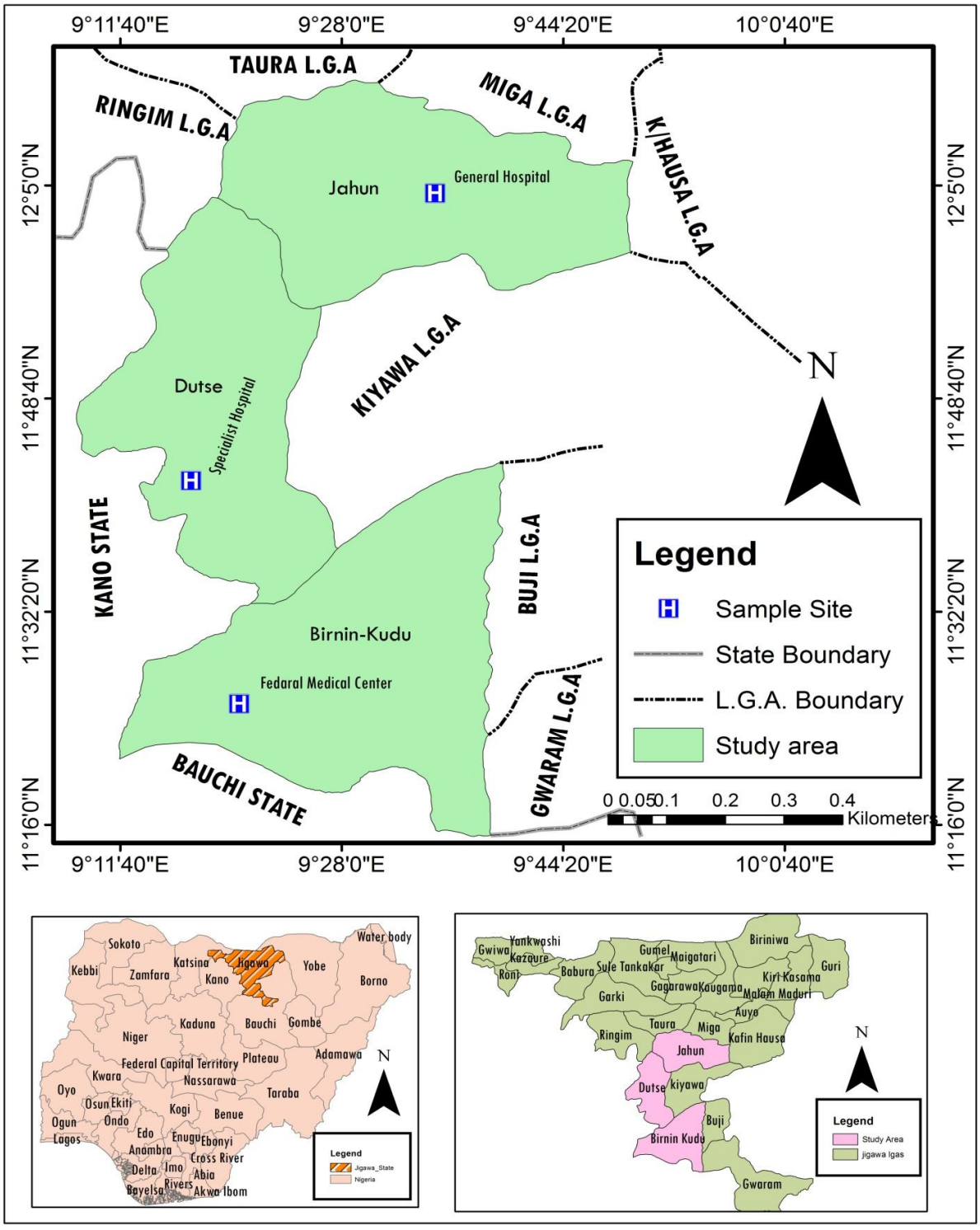


Figure 3.1. A Map of Jigawa State showing Birnin-Kudu, Jahun and Dutse Local Government Areas – The Study Area.***Source:*** Geographic Information System Laboratory, Department of Geography, Ahmadu Bello University, Zaria

Kudu LGA. This study was carried out using random sampling method in which every other blood donor was selected randomly and samples were collected every other day to minimize bias among the tested population.The blood donors had to first consent to participate in the study.

3.3 Study Population

This study was conducted among blood donors that presented themselves in Jahun General Hospital, Jahun; Rasheed Shekoni Specialist Hospital, Dutse; and Federal Medical Centre, Birnin Kudu. The blood donors that participated in this study were voluntary blood donors that attended blood donation for their patients and consented for the study. Commercial blood donors, who get paid for blood donations, were not involved in this study. Majority of the donorswere self-employed with agriculture as their main occupation.

3.4 Inclusion and Exclusion Criteria

Inclusion: Blood donors who gave their consent to participate in the study irrespective of age and gender.

Exclusion: Blood donors who did not give their consent and those who were not fit for blood donation.

3.5 Ethical Approval and Consent

Ethical approval (Appendix I) was obtained from the Jigawa State Health Research Ethics Committee of the Ministry of Health, before the work commenced. An informed consent form was signed by each the blood donor (Appendix II).

3.6 Sample Size

A sample size was determined using the equation below as described by Naing *et al.*(2006) and a prevalence rate of 14.9% of HBsAg in Jos, Plateau State, Nigeria.

$$\boldsymbol{n = \frac{z^2 p_0 (1 - p_1)}{d^2}}$$

Where

$\boldsymbol{n}$= sample size,

$\boldsymbol{z}$= statistic for a level of 95% confidence interval =1.96

$\boldsymbol{p_0}$ = Prevalence (in proportion of one; if 14.9%, *P*= 0.149),

$\boldsymbol{p_1}$= Prevalence rate of HBV infection= 0.149 (Egesie*et al.*,2008) and

$\boldsymbol{d}$= precision (in proportion of one; if 5%, *d* = 0.05).

Therefore

$$\boldsymbol{n = \frac{1.96^2 \, X \, 0.149(1 - 0.149)}{0.05^2}}$$

$\boldsymbol{n}$ **= 194.84**

=195 samples

For the sample size of Parvovirus B19, the same equation below as described by Naing *et al.*(2006) was used with a prevalence rate of 85.4% obtained in Zaria, Kaduna, Nigeria.

$$\boldsymbol{n = \frac{z^2 p_0 (1 - p_1)}{d^2}}$$

Where

$\boldsymbol{n}$ = sample size,

$\boldsymbol{z}$= statistic for a level of 95% confidence interval =1.96

$\boldsymbol{p_0}$ = Prevalence (in proportion of one; if 85.4%,*P*= 0.854),

$\boldsymbol{p_1}$= Prevalence rate of HPVB19 infection = 0.854 (Ujo *et al.*, 2012) and

$\boldsymbol{d}$= precision (in proportion of one; if 5%, *d* = 0.05).

Therefore

$$\boldsymbol{n = \frac{1.96^2\ X\ 0.854(1-0.854)}{0.05^2}}$$

$\boldsymbol{n}$ **= 191.59**

=192 samples

The calculated sample sizes were195 and 192samples for HBsAg and Parvovirus B19. However, in order to have a more reliable result, 421samples werecollected from the three hospitals.

3.7 Questionnaire Administration

A structured questionnaire (Appendix III) was administered to the consenting blood donors in order to obtain information on their socio-demography, clinical data and potential risk factors that might be associated with HBV and Parvovirus infections.

3.8 Sample Collection and Processing

The samples were collected with the assistance of the laboratory personnel in each of the chosen hospitals. About 3-5 ml of blood was collected from eachapparentlyhealthy and consenting blood donorinto an EDTA tube. The samples were transported to the laboratory where they werecentrifuged at 1000rpm (revolution per minute) for 10 minutes to separate the plasma from

the packed cells (Cheesbrough, 2010).Aliquots of the plasma were be transferred intolabeled tubes and kept in a deep freezer at -20°C until analyzed.

3.9 Detectionof HBV Infectionin Sera Using HBV Rapid Test Strips

Blood samples were screened for HBsAg as a serological marker for HBV infection using a one Step Hepatitis B Surface Antigen Test Strip kit(Abon Biopharm Co., Ltd., Hangzhou, China). This is a rapid chromatographic immunoassay for the qualitative detection of Hepatitis B Surface Antigen in serum or plasma.

3.9.1 Principle of HBsAgRapid Test Strips

Abon one Step HBsAg test is an antibody sandwich immunoassay. The membrane is pre-coated with anti-HBsAg antibodies on the test line region of the strip. During testing, the serum or plasma specimen reacts with the particle coated with anti-HBsAg antibody. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-HBsAg antibodies on the membrane and generate a colored line. The presence of this colored line in the test region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

3.9.1.1 Assay Procedure and Interpretation for HBsAg Test

The test strips and labeled samples were equilibrated to room temperature (25-30°C) prior to testing. The test strip was removed from the sealed pouch and labeled accordingly. Fifty microliter (50µl) of the plasma was dispersed in the specimen pad on one end of the strip, placed on a non-absorbent flat surface and the result read after 15 minutes.

A positive sample was indicated by two distinct red lines in the control region (C) and test region (T) region. The result was interpreted as negative when one red line appeared in the control region (C) with no apparent red or pink line on the test region (T). When the line failed to appear in the control region (C), the result was interpreted as invalid and was repeated.

3.10 Screening for HBsAg using ELISA

The samples that tested positive for HBsAg using the HBsAg rapid test strips were subjected to further testing using AccuDiag^{TM}HBsAg ELISA (Diagnostic Automation/Cortez Diagnostics, Inc., California, USA).

3.10.1 Principle of HBsAg ELISA

The HBsAg ELISA Test kit employs an antibody sandwich ELISA technique where monoclonal antibodies unique to HBsAg are coated on polystyrene microwell strips. The serum or plasma sample is added together with a second antibody, the Conjugate (horseradish peroxidase) and directed against a different epitope of HBsAg. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produced a blue color in HBsAg positive sample. Throughout the time of incubation, specific immunocomplex that may have formed (indicating presence of HBsAg) is captured on the solid phase. After washing to eliminate serum proteins and unbound HRP-conjugate, chromogen solutions containing tetramethyl-benzidine (TMB)and urea peroxide are added to the wells. Next, the colorless chromogens are hydrolyzed by the bound HRP-conjugate to a blue-colored product while in the presence of antibody-antigen-antibody (HRP) sandwich immunocomplex. Halting the reaction with sulfuric acid, the blue color then turns yellow. The color intensity is measured using spectrophotometer and is proportional to the

amount of antigen captured in the wells. The wells remain colorless if the HBsAg result is negative.

3.10.2 Test Procedure for HBsAg ELISA

All the seropositive samples for HBsAg were equilibrated to room temperature (25-30°C) priorto ELISA testing. The stock wash buffer was diluted 1 in 20 with distilled water. The wells of the microtiter plate were numbered, including three for Negative controls (B1, C1, and D1), two for the Positive controls (E1 and F1) and one for Blank (A1). Neither sample nor HRP-Conjugate were added into the Blank well. Fifty microliters of the Positive control, Negative control, and sample was added into their respective wells. Fifty microliters of HRP-Conjugate were added to each well except the Blank, and mixed by tapping the plate gently. The plate was covered with the plate cover and incubated for 60 minutesat 37°C. The content of the plated was poured off, washed five times with the Wash buffer and blot dried on an absorbent paper. Fifty micro literseach of Chromogen A and Chromogen B were dispensed into each well including the Blank and mixed by tapping the plate gently. The plate was incubated at 37°C for 15 minutes. A multichannel pipette was used to add 50µl Stop Solution into each well and mixed gently. Intensive yellow color developed in the Positive control and HBsAg positive sample wells. The plate reader was calibrated with the Blank well and the absorbance was read at 450nmusing GF-M3000 Microplate Reader photometer.

3.10.3 Interpretation of the HBV ELISA Results

The results were calculated by relating the value of the optical density (OD) of each sample value to the Cut-off value (CO) for the respective plate. The value of the OD of the Blank well was subtracted from that of the samples and controls. The mean absorbance value of the three negative controls was multiplied by 2.10 to get the Cut-off value in order to determine the seropositivity of the samples.The optical density value of the samples after subtracting the value of the blank wellwere related to the Cut-off value. Those values greater than or equal to the Cut-off value were interpreted as positive while those lower than the Cut-off were interpreted as negative. The positive result revealed that the blood donor might have been infected with HBV while negative result indicated that there was no detected HBsAg in the sample.

3.11 Detection of HBV Markers

All samples that were reactive to HBsAg were further screened for other serological markers of HBV infection.Hepatitis B virus multi-5 test kit manufactured by BioApex Diagnostic Inc., Canada, was used to detect the following HBV markers: hepatitis B surface antigen, hepatitis B surface Antibody, hepatitis B “e” antigen, hepatitis B “e” antibody and hepatitis B core antibody.

3.11.1 Principles of HBV Marker Detection

The HBV marker kit uses the colloidal gold and membrane chromatography technology, and measures HBsAg, HBeAg in serum/plasma with dual-antibody sandwich method, and measures HBsAb with dual-antigen sandwich method, and measures HBeAb and HBcAb with neutralization competitive inhibition method.

3.11.2 Test Procedure and Interpretation for HBV Markers

The test kit was removed from the pouch and labeled according to the samples to be tested. The samples were equilibrated to room temperature (25-30°C). Four drops of the plasma sample (150–200µl) were pipetted into each of the five sample wells of the test board. The result was observed and recorded after 15 minutes (Plate 1).

Using the sandwich method, a clear pink control band and a detectable test band appearing on the C and T regions indicated that the test result was positive for HBsAg, HBsAb and HBeAg while a pink colored band appearing only on the control region indicated a negitive result. If a pink colored band appeared only on the T region or no band appeared on both regions, it showed the test result was invalid. For HBeAb and HBcAb using the neutralization competitive inhibition method, the result was positive if one red line appeared only at the control region while two clear pink colored bands in both control and test regions indicated negative result.

3.12 Detection of Parvovirus B19 Infection

Samples from the blood donors were screened for the presence of IgG and IgM antibodies to Parvovirus B19 using SERION ELISA *classic*Parvovirus B19 IgG/IgM (Virion/Serion, Germany). Before running the test, the samples were diluted with dilution buffer. However, the dilution methods differed for the Parvovirus B19 IgG and Parvovirus B19 IgM.

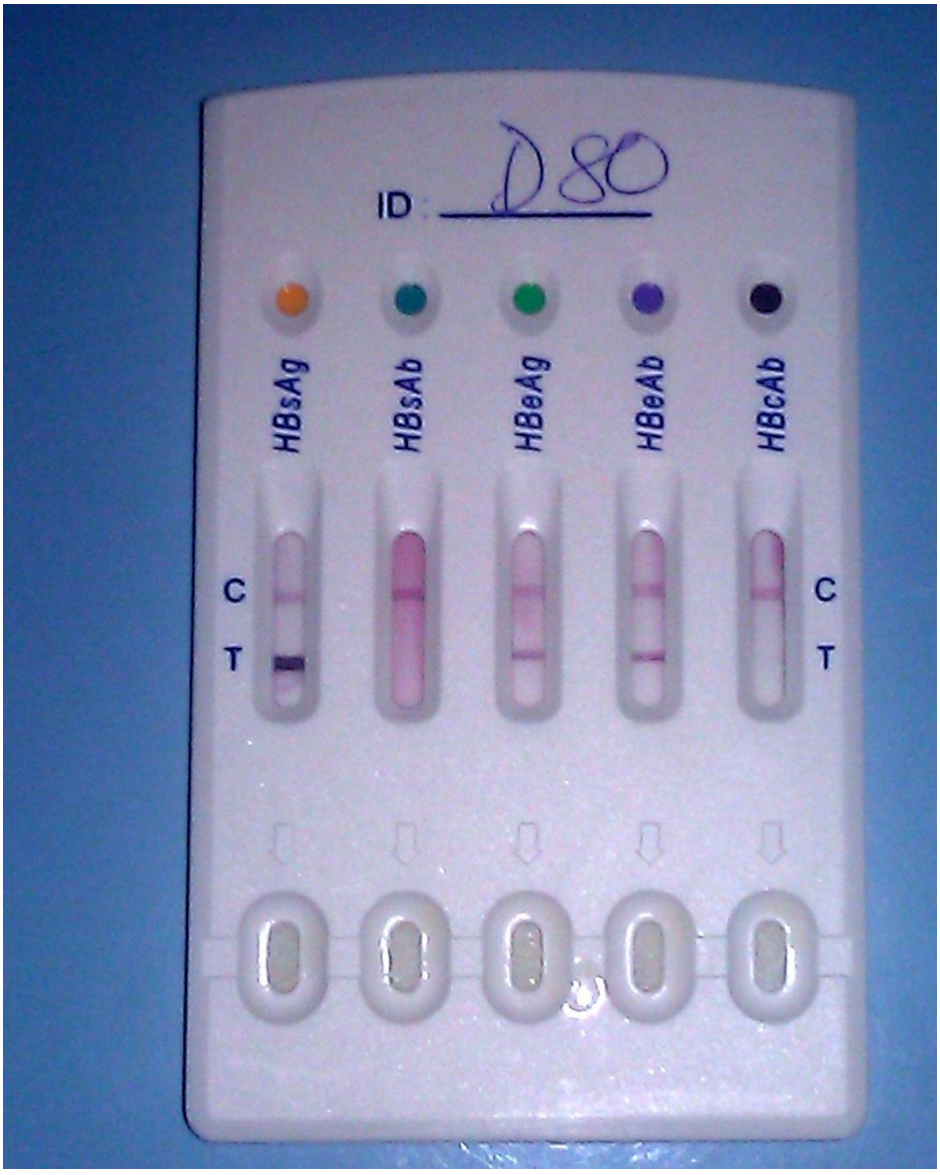


Plate I: Test Kit for detecting 5 Viral Hepatitis B Markers

3.12.1 Dilution of Samples for Parvovirus B19 IgG and IgM Screening

For parvovirus B19 IgG, 1µl of the donor's sample was added to the 100µl of the dilution buffer in a microtiter well and mixed thoroughly to produce a homogenous solution.Due to the presence of Rhaumatoid factors or non-specific IgM antibodies in the samples, which can lead to false-positive results in the IgM assay, the samples were pretreated with rhemautoid factor-absorbens prior to IgM detection. Therefore, before running Parvovirus B19 IgM assay, 20µl of rhemautoid factor-absorbent each was added 80µlof the dilution buffer. Then, 1µl of the patient's sample was diluted in 100µl of this Rf-dilution buffer.

3.12.2 Principle of Parvovirus B19 ELISA

The Parvovirus B19 ELISA is an immunoassay, which is based on the specific interaction of antibodies with their corresponding antigens. The test strips of the SERION ELISA *classic* microtiter plate are coated with specific antigens of the Parvovirus B19. If there are antibodies in the patient's plasma sample, they bind to the fixed antigen. A secondary antibody, which has been conjugated with an enzyme alkaline phosphatase, detects and binds to the immune complex. The colorless substrate p-nitrophenylphosphate is then converted into the colored product p-nitrophenol. The signal intensity of this reaction product is proportional to the concentration of the analyte in the sample and is measured photometrically.

3.12.3 Test Procedure and Interpretation for Parvovirus B19 IgG/IgMDetection

A protocol sheet was prepared and labeled according to the labeled samples to be tested. Well A1 was spared for substrate blank; well A2 was for Negative Control; A3 and A4 presented the two Standard sera while the donors' samples started from A5.

With the exception of the Substrate blank well, 100µl of the diluted samples and ready-to-use controls were pipetted into the appropriate wells of the microtiter test strips and incubated ina moist chamber for 60 minutes at 37 °C. After the incubation, all wells were washed with washing solution using an automated washer. The plate was aspirated and each well filled with 300µl of the washing solution. The washing buffer was aspirated and the washing procedure was repeated for three more times. Then, the microtiter plate was drained by tapping the plate on a paper towel. One hundred microliters of the ready-to-use IgG/IgM conjugate was added to the appropriate wells (except substrate blank) and incubated for 30 minutes at 37 °C in a moist chamber. After the incubation, the wells were washed and drained as previously. Then, 100µl of ready-to-use substrate solution was added to each well, including the substrate blank well and further incubated for 30 minutes at 37 °C in a moist chamber. One hundred microliters of stopping solution was added to each well and gently agitated. The optical density of the samples was read after 60 minutes at 450nm against the substrate blank.

The results were calculated by relating the value of the optical density (OD) of each sample value to the Cut-off value (CO) for the respective plate. The value of the OD of the Blank well was subtracted from that of the samples and controls. The cut-off ranges of each of the plates were determined by multiplying the mean value of the measured standard OD with the numerical data of the quality control certificates (included in the kits). Those values greater than or equal to the Cut-off value were interpreted as positive while those lower than the Cut-off were interpreted as negative. The positive result revealed that the blood donor indicated seropositivity to IgG antibodies to HPVB19 while negative result indicated that there was no IgG antibodies to HPVB19 in the sample.

3.13 Data Analysis

The data obtained from the questionnaire and the result of the laboratory tests were analyzed using SPSS 20 (Statistical Package for Social Science version 20) and the results obtained were presented in tables. The prevalence of the each viral infection (HBV and Parvovirus B19) was obtained from the proportion of seropositive individuals in the total population under consideration and expressed as a percentage. The Pearson chi-square test was employed to determine the relationships between the sociodemographic data and clinical information with HBV and Parvovirus B19 infection. P value of ≤ 0.05 was considered significant at 95% confidence interval. Odd Ratio was also used to compare two variables.

CHAPTER FOUR

4.0 RESULTS

4.1 Analysis of the Study Population

During the study, data were collected from the blood donors using questionnaires. Analysis of the donors by age (Table 4.1) revealed that they were in the age group 23-31 years had the highest frequency (48.2%: 203/421) while those in age group of 39 years and above constituted the least (10.5%: 44/421). The distribution of the donors by their level of education revealed that a good number of them had obtained secondary level of education (29.7%: 125:421) while only 8.3% (35/421) had attended tertiary education. Majority of the donors were married (72.2%: 304/421) while the single men were 117 (27.7%). While majority of the donors were from monogamous families (60.3%: 253/421), the analysis showed that 39.7% (167/421) were from polygamous families. The distribution of donors by their occupations showed that majority were farmers (43.4%: 183/421) while the unemployed had the least frequency of 11.4% (48/421).

4.2 Sero-prevalence of HBV Infection

To check the sero-prevalence of HBsAg, 421 blood samples were analyzed using HBsAg strip. Sixty six (66) of the study participants were found to be HBsAg-positive, giving an overall sero-prevalence of 15.7%(66/421) (Table 4.2).Relating the HBV infection with the three hospitals where the samples were collected revealed that those from Federal Medical Centre, Birnin Kudu, had the highest prevalence of 20.3, while those from Rasheed Shekoni Specialist Hospital, Dutse, had prevalence of 14.8% and those from Jahun General Hospital, Jahun, had 13.5% prevalence.

Table 4.1: Distribution of Blood Donors by Demographic and Social Characteristics

Feature	Number Examined	Percentage (%)
Age (Years)		
18-24	111	26.4
25-31	203	48.2
32-38	63	14.9
39 and above	44	10.5
Educational status		
Informal	55	13.1
Primary	119	28.2
Secondary	125	29.7
Tertiary	35	8.3
Quranic	87	20.7
Marital Status		
Married	304	72.2
Single	117	27.8
Family type		
Monogamous	254	72.2
Polygamous	167	27.8
Occupation		
Farmers	183	43.4
Civil servants	60	14.3
Businesspersons	130	30.9
Unemployed	48	11.4

Table 4.2: Prevalence of Hepatitis B Virus among Blood Donors attending some Hospitals in Jigawa State

Hospital	Number examined	Number positive	Prevalence (%)
JGH	261	38	13.5
FMC BK	79	16	20.3
RSSH	81	12	14.8
Total	421	66	15.7

Key: JGH = Jahun General Hospital; FMC BK = Federal Medical Centre, Birnin Kudu: RSSH = Rasheed Shekoni Specialist Hospital.

Socio-demographic data from the questionnaires were analyzed and the results were presented. From this study, the age of the blood donors ranged from 18 to 52 years with a mean of 28.6 years.There was a relatively high sero-prevalence of HBsAg amongdonors within the age group 39 and above (18.2%: 8/44) (Table 4.3). The results also revealed those donors within the age group 25-31 years had a sero-prevalence of 14.3% (29/203). Also, there was a prevalence of 14.3% among those donors between the age 32-38 (9/63). However, the difference observed was not statistically significant (P=0.88). Therefore, there was no relationship between age differences and hepatitis Bvirusinfection among the blood donors.

Blood donors that attended only primary school education had the highest sero-prevalence of HBsAg(17.6%: 21/119). Individuals who had no formal education had the least sero-prevalence of HBsAg (9.1%: 5/55)(Table 4.3).However, the difference in sero-prevalence was not statistically significant(χ^2=2.232, df=4, P=0.693), which means that there was no relationship between hepatitis B infection and educational status.

The distribution of HBsAg according to marital status, as shown in Table 4.3, demonstrated that was no relationship between hepatitis B virus infection and marital status of blood donors (P=0.688, OR=1.130, 95% CI=0.621-2.056). However, the blood donors who were married had a higher sero-prevalence of 16.1% while those that were unmarried had a sero-prevalence of 14.5%. Table 4.2 also revealed that there was an insignificant relationship between HBV infection and patients' family type (χ^2=1.257, df=1, P=0.257). The sero-prevalence of HBV infection among those donors who were in monogamous relationships was 17.4% (43/247) as against 13.2% among those in polygamous marriages.

Table 4.3: Some Socio-Demographic Factors Associated with Hepatitis BVirusInfection among Blood Donors in Selected Hospitals in Jigawa State

Variable	Number Examined	Number Positive	Prevalence (%)	P-value
Age (Years)				
18-24	111	20	18.0	0.88
25-31	203	29	14.3	
32-38	63	9	14.3	
39 and above	44	8	18.2	
Educational status				
Informal	55	5	9.1	0.693
Primary	119	21	17.6	
Secondary	125	20	16.0	
Tertiary	35	6	17.1	
Quranic	87	14	16.1	
Marital Status				
Married	304	49	16.1	0.688
Single	117	17	14.5	
Family type				
Monogamous	254	44	17.3	0.257
Polygamous	167	22	13.2	
Occupation				
Farmers	183	26	14.2	
Civil servants	60	8	13.3	0.675
Businesspersons	130	25	19.2	
Unemployed	48	7	14.6	

As demonstrated in Table 4.3, the highest sero-prevalence of hepatitis B virus in relation to occupation was obtained among the businesspersons (19.2%), followed by people who were unemployed (14.6%), while the civil servants had the least prevalence (13.3%).However, the difference in sero-prevalence was not statistically significant (χ^2=2.33, df=4, P=0.675), which means that there was no relationship between hepatitis B virus infection and occupation.

The result was analyzed according to the possible risk factors that might be associated with HBV infection and the results are presented in Table 4.4. There was no significant association between HBsAg infection and history of blood donation (χ^2=0.068, df=1, P=0.794). However, infection was higher in those who had not donated blood before now (15.8%: 60/379) than among those who had donated blood before (14%: 6/42). Similarly, there was no observed statistical significance among those who use condom and those who do not use condom (χ^2=1.132, df=1, P=0.287,OR=2.885; CI=0.374-22.22). However, the sero-prevalence of HBsAg was higher in those who did not use condom (16.1%: 65/403) than those who used condom (5.5%: 1/18).However, the people who did not use condom were at 2.885 times higher odds of being HBsAg positive than people who used condom.

Blood donors who had multiple sex partners had a higher sero-prevalence of HBsAg infection (18.5%: 5/27) while those with single sex partners had a lower sero-prevalence (15.5%: 60/388) (Table 4.4). There was no significant association between the HBV infection and number of sexual partners, but those that multiple sex partners were at 1.242 times statistically significant higher odds of being HBsAg positive than those who had single sexual partners (χ^2=0.178, df=1, P=0.673; OR=1.242; 95% CI=0.453-3.409). Although there was a high sero-prevalence of

Table 4.4: Some Risk Factors Associated with Hepatitis BVirusInfection among Blood Donors in Selected Hospitals in Jigawa State

Variable	Number Examined	Number Positive	Prevalence (%)	P-value	Odds Ratio	Confidence Interval (95%)
History of transfusion						
Yes	3	0	0.0	0.540	Undefined	Undefined
No	418	66	15.8			
History of donation						
Yes	42	6	14.3	0.794	0.886	0.358-2.199
No	379	60	15.8			
History of surgery						
Yes	3	0	0.0	0.454	Undefined	Undefined
No	418	66	15.8			
History of HBV vaccination						
Yes	5	2	40.0	0.132	0.275	0.045-1.665
No	416	64	15.4			
Use of condom						
Yes	18	1	5.5	0.287	2.885	0.374-22.22
No	403	65	16.1			
Multiple sex partner						
Yes	30	5	18.5	0.673	1.242	0.453-3.409
No	391	60	15.5			
History of scarification						
Yes	25	8	32.0	0.021*	2.742	1.131-6.647
No	396	58	14.6			
Practice personal drug injection						
Yes	4	1	25.0	0.606	1.805	0.185-17.62
No	417	65	15.6			
Share sharp object						
Yes	62	16	25.8	0.015*	2.197	1.154-4.184
No	359	50	13.9			
History of smoking						
Yes	22	3	13.6	0.787	0.842	0.242-2.931
No	399	63	15.8			

Key: *=significant difference exists at 95% Confidence Interval

HBsAg among those who said to have been vaccinated for HBV (40%: 2/5), there was no significant difference observed (χ^2=2.265, df=1, P=0.132; OR=0.275; 95% CI=0.045-1.665).

While none of those who had been transfused previously was infected, there was high sero-prevalence of HBsAg infection among those who had not been transfused (15.8%: 66/418)(Table 4.3). However, there was no significant difference in the sero-prevalence observed (χ^2=0.375, df=1, P=0.540). Similarly, there was high sero-prevalence of HBV infection among donors who had no history of surgery (15.8%: 66/418). However, there was no statistical significance (χ^2=0.562, df=1, P=0.454).

Although there was only 1.2% (5/421) blood donors that attested to have hadHBV vaccination, there was no statistical difference observed (χ^2=2.265, df=1, P=0.132, OR=0.275, CI=0.045-1.665)(Table 4.4)with higher sero-prevalence of 40.0 (2/5) observed among those vaccinated before against 15.4% (64/416)among those who had not vaccinated.

The distribution of HBsAg in relation to having scarification was of utmost significant statistically(χ^2=5.357, df=1, P=0.021, OR=2.742, CI=1.131-6.647)(Table 4.4). The highest sero-prevalence was recorded among those who had scarification (32.0%: 8/25) while those without scarification had HBsAg sero-prevalence of 14.6% (58/396). People who had recent scarificationwere at 2.742 times higher odds of being HBsAg positive than people who do have scarification.

Analysis of the presence of HBsAg and sharing of sharp objects revealed that those that shared sharp objects had a higher prevalence (25.8%: 16/62) than those who did not share sharp objects (13.9%: 50/359). There was a significant association between HBV and sharing sharp objects (χ^2=5.958, df=1, P=0.015; OR=2.197; CI=1.154-4.184)(Table 4.4). People who shared sharp

objects were at 2.197 times higher odds of being HBsAg positive than people who did not share sharp objects.

There was no significant association between infection with HBsAg and history of smoking (χ^2=0.073, df=1, P=0.787; OR=0.842; CI=0.242-2.931). Sero-prevalence of HBsAg was higher in those that had no history of smoking (15.8%: 63/399) than among smokers (13.6%: 3/22). Infection with HBsAg was more prevalent among those that practiced personal drug injection (25.0%: 1/4) than those who did not (15.6%: 65/417). Even though, there was no significant association in the sero-prevalence, those that practiced personal drug injection were at 1.805 higher odds of acquiring HBV infection than those who did not(χ^2=0.266, df=1, P=0.606; OR=1.805; CI=0.185-17.62).

All the samples that tested positives for HBsAg by the rapid tests were also positives for HBsAg when HBV multi-5 test kitwas used. The HBV marker screening results revealed that none of the HBsAg positive sample was positive for HBsAb (Table 4.5). However, a sero-prevalence of 94% (4/66) was recorded for HBcAb; HBeAb was 76% (16/50) while HBeAg prevalence was found to be 18% (12/66).

Table 4.5: Prevalence of HBV Infection Markers among the HBsAg Positive Blood Donors

HBV markers	Number Examined	Number Positive	Number negative	Prevalence (%)	Interpretation
HBsAb	66	0	66	0.0	Presence of HBsAb indicates immunity which can occur via vaccination or at the end of active infection.
HBeAg	66	12	54	18.2	A positive HBeAg confirms actively replicating virus.
HBeAb	66	50	16	75.8	A positive HBeAb usually means no replication is occurring.
HBcAb	66	62	4	93.9	HBcAb appears one month after HBV infection and usually present for life after an active infection.

4.3 Sero-prevalence of HPVB19 Infection

The sero-prevalence of IgM and IgG antibodies to Human Parvovirus B19 among the blood donors was determined in this study. A total of 266 samples were selected from the 421 blood donor samples and screened for IgM and IgG antibodies to HPVB19, due to inadequate funds for the ELISA kits and some experimental errors. Immunoglobulin M antibodies to the virus were detected with a sero-prevalence of 72.2% (192/266) and 63.5% (169/266) for IgG antibodies (Table 4.6).

Table 4.7 showed the different parameters analyzed according to antibodies to HPVB19. Individuals who were in the age group 25-31 years had the highest sero-prevalence of 75.4% (95/126) while a sero-prevalence of 64.7% (22/34) was detected in the age group 39 years old and above. However, there was no significant difference between the age group and the viral antibody prevalence(χ^2=1.83, df=4, P=0.77). Similarly, the distribution of IgG antibodies to HPVB19 in relation to age was also not statistically significant (χ^2=5.88, df=4, P=0.21) (Table 4.6). However, individuals who were within the age group of 25-31 years had the highest sero-prevalence (69.1%: 87/126) while the sero-prevalence rate was lowest among individuals within the age group of 32-38 years (52.8%: 19/36).

There was no significant difference in the distribution of IgM antibodies to HPVB19 by educational status (χ^2=1.59, df=4, P=0.811) (Table 4.7). However, blood donors that had no formal education were found to have the highest sero-prevalence of IgM antibodies(77.8%: 28/36), followed by individuals with a tertiary level of education (77.3%: 17/22). Those that attended only primary school had the least prevalence (67.9%: 55/81). Likewise for IgG antibody with respect to educational status, the blood donors that had informal education had the

Table 4.6: Prevalence of Human Parvovirus B19 among Blood Donors.

Issues Examined	**No. Examined**	**No. Positive**	**Prevalence (%)**
IgM antibodies to HPV	266	192	72.2
IgG antibodies to HPV	266	169	63.5

Table 4.7: Some Socio-demographic Factors Associated with Human Parvovirus B19 Infection among Blood Donors in Selected Hospitals in Jigawa State

Variable	Number examined	Number positive	IgM Prevalence (%)	P-value	Number Positive	IgG Prevalence (%)	P-value
Age (Years)							
18-24	70	49	70.0	0.77	42	60.0	0.32
25-31	126	95	75.4		87	69.1	
32-38	36	26	72.2		19	52.8	
39 and above	34	22	64.7		21	61.8	
Education							
Primary	81	55	67.9	0.81	46	56.8	0.50
Secondary	76	55	72.4		50	65.8	
Tertiary	22	17	77.3		13	59.1	
Quranic	51	37	72.5		34	66.7	
Informal	36	28	77.8		26	72.2	
Marital Status							
Single	65	47	72.3	0.979	40	61.5	0.70
Married	201	145	72.1		129	64.2	
Family Type							
Monogamy	154	110	71.4	0.772	97	63.0	0.94
Polygamy	112	76	67.8		66	58.9	
Occupation							
Farmer	110	87	79.1	0.174	68	61.8	0.86
Civil servant	40	29	72.5		24	60.0	
Businessmen	88	55	62.5		57	64.7	
Unemployed	28	20	71.4		19	67.8	

highest sero-prevalence (72.2%: 26/36), followed by those with Quranic education (66.7%: 34/51) while those that attended only primary school had the least (56.8%: 46/81).

There was no statistically significant association between the occurrence of IgM antibodies to HPVB19 and marital status (χ^2=0.001, df=1, P=0.979; OR=1.008; CI=0.540-1.883)(Table 4.7). However, those who were not married had a sero-prevalence of 72.3% (47/65) against 72.1% (145/201) among the married blood donors. Also, the distribution of IgM antibodies to the virus according to family type revealed that those from polygamous families had a lower sero-prevalence (67.8%: 76/112) when compared to those from monogamous families (71.4%: 110/154). However, there was no significant association between the IgM antibodies and family type (χ^2=0.084, df=1, P=0.772; OR=0.921; CI=0.528-1.607).

The distribution of IgG antibodies to HPVB19 in relation to marital status showed no statistically significant association (χ^2=0.001, df=1, P=0.979; OR=1.008; CI=0.540-1.883) (Table 4.7). However, those who were married had a higher sero-prevalence (64.2%: 129/201) than the unmarried (61.5%: 40/65).Similarly, the distribution of IgG antibodies according to family type revealed that those from monogamous families had a higher sero-prevalence (63.0%: 97/154) compared to those from polygamous families (58.9%: 66/112)(χ^2=0.01, df=1, P=0.94; OR=0.98; CI=0.59-1.64).

When analyzed according to the occupation of the blood donors, sero-prevalence of IgM antibodies was highest among farmers (79.1%: 87/110)(Table 4.7) while those engaged in business were found to have the least sero-prevalence (62.5%: 55/88)(χ^2=6.36, d=4, P=0.174). Similarly, the distribution of IgG antibodies with respect to occupation revealed the highest sero-prevalence among health workers (77.8%: 7/9) (Table 4.7) while the least was among civil

servants (60.0%: 24/40). There was no statistically significant association in the prevalence obtained (χ^2=1.29, df=4, P=0.86).

The result was analyzed according to the possible risk factors that might be associated with IgM and IgG antibodies toHPVB19(Table 4.7). There was no significant association between IgM antibody occurrence to HPVB19 and history of blood transfusion(χ^2=0.39, df=1, P=0.53). The only blood donor said to have been transfused before, was positive for IgM antibodywhile among those who had been transfused before, the prevalence was 72.0% (190/264).There was no significant association between the prevalenceand history of blood transfusion (χ^2=0.58, df=1, P=0.45). Similarly, those who had not been transfused before had a prevalence of 63.0% (167/265) for IgG antibody.

The sero-prevalence of IgM antibodies to HPVB19 was higher among blood donors who had donated blood before (82.1%: 23/28) than in those who had not donated blood before (71.0%: 169/238) (χ^2=1.55, df =1, P=0.21; OR=1.88; CI=0.68-5.14) with those had donated blood before at 1.88 higher odds of having IgM antibodies than those who had not donated before. Similarly, there was no significant association between IgG antibody occurrence and history of blood donation (χ^2=0.11, df=1, P=0.74; OR=0.87; CI=0.39-1.95). Those that had not donated blood before had a sero-prevalence of 63.9% (152/238) while 60.7% (17/28) was recorded among those who have donated blood before.None of the participant had a history surgery.

Although condom use was not significantly association with IgM antibody prevalence (χ^2=0.54, df=2, P=0.76), those who used condom had higher prevalence (78.6%: 11/14)than those who did not use condom (71.8%: 181/252) (Table 4.8). Likewise, there was no significant association with IgG antibodies and the use condom (χ^2=3.23, df=2, P=0.19), where those who did not use

Table 4.8: Some Risk Factors Associated with Human ParvovirusB19 Infection among Blood Donors in Selected Hospitals in Jigawa State

Variable	Number examined	Number positive	IgM Prevalence (%)	P-value	Number Positive	IgG Prevalence (%)	P-value
History of transfusion							
Yes	1	1	100.0	0.53	1	100.0	0.45
No	265	190	71.7		167	63.0	
History of donation							
Yes	28	23	82.1	0.21	17	60.7	0.74
No	238	169	71.0		152	63.9	
History of surgery							
Yes	0	0	0.0	Undef.	0	0.0	Undef.
No	266	192	72.2		169	63.5	
Use of condom							
Yes	14	11	78.6	0.76	11	78.5	0.19
No	252	181	71.8		157	62.3	
Multiple sex partner							
Yes	20	17	85.0	0.18	17	85.0	0.04*
No	246	173	70.3		151	62.1	
History of scarification							
Yes	24	18	75.0	0.75	18	75.0	0.22
No	242	174	71.9		151	62.4	
Practice personal drug injection							
Yes	1	1	100.0	0.68	0	0.0	0.19
No	265	191	72.1		169	63.8	
Share sharp object							
Yes	50	35	70.0	0.91	29	59.2	0.50
No	216	156	72.2		139	64.4	
History of smoking							
Yes	15	11	73.3	0.92	9	60.0	0.77
No	251	181	72.1		160	63.7	

Key: *=significant difference exists at 95% Confidence Interval

condom had a sero-prevalence of 62.3% (157/252) compared to 78.5% (11/14) among those who used condom.

The distribution of IgM antibodies to HPVB19 in relation to having multiple sex partners was not statistically significant (χ^2=1.76, df=1, P=0.18; OR=2.29; CI=0.65-8.07) (Table 4.8).However, those who had multiple sex partners had a higher sero-prevalence (85.0%: 17/20) and at 2.29 statistically higher odds of been sero-positive than those who did not (70.3%: 173/246). Conversely, with respect to IgG antibodies, it was statistically significant (χ^2=4.19, df=1, P=0.04; OR=3.45; CI=0.99-12.10). Those who had multiple sex partners had a higher sero-prevalence (85.0%: 17/20) than those with single partner (62.1%: 151/243). Those with multiple sex partners were 3.45 time higher odds of being positive to IgG antibodies than those with single partner.

Sero-positivity with IgM antibodies to HPVB19 was higher among those with history of scarification(75.0%: 18/24) than those who did not have scarification(71.9%: 174/242) (Table 4.8). Although there was no significant difference in the prevalence obtained, those donors with history of scarification are 1.173 higher odds of been sero-positive to IgM antibodies (χ^2=0.10, df=1, P=0.75; OR=1.173; CI=0.45-3.08). Similarly, individuals who had scarificationhad a higher prevalence of IgG antibodies to the virus (75.0%: 18/24) than those who had none (62.4%: 151/242)(χ^2=1.50, df=1, P=0.22; OR=1.81; CI=0.69-4.72).Meanwhile, those donors who had history of scarification are 1.81 higher odds of been sero-positive IgG antibodies than those who did not.

A seroprevalence of 72.1% (191/265) IgM antibodies was detectedamong those who do not practice self-drug injection (χ^2=0.78, df=2, P=0.68) while the only blood donor that practiced self-drug injective was seropositive for IgM antibodies but sero-negative for IgG antibodies.

Sharing of sharp objects was not found to be associated with viral infection such that both IgM and IgG antibodies were higher among those who did not that share sharp objectsthan in those who shared sharp objectives (72.2%:156/216 vs 70.0%: 35/50 and 64.4%: 139/216 vs 59.2%: 29/50 respectively

Prevalence of IgM antibodies was higher among blood donors who were smokers (73.3%: 11/15) than in those who were not smokers (72.1%: 181/251) (Table 4.7). There was no statistical difference in the prevalence obtained (χ^2=0.01, df=1, P=0.92; OR=1.06; CI=0.33-3.45).Likewise, there was no statistical difference in the prevalence of IgG antibodies, but non-smokers had a higher prevalence of 63.7% (160/251) against 60.0% (9/15) detected among the smokers.

CHAPTER FIVE

5.0 DISCUSSION

This study was conducted to determine the sero-prevalence of HBsAg (a marker of hepatitis B virus infection) and Human Parvovirus B19among blood donors in some selected hospitals in Jigawa State. The results obtained from this study showed that 15.7% (66/421) of the donors were seropositive for HBsAg. Also, IgM and IgG antibodies were found to be 72.2% and 63.5% respectively in 266 of these donors.

The presence of HBsAg among 15.7% of the donors indicates that most blood donors are potentially infectious (WHO, 2012). According to the WHO classification of assessing the severity of HBsAg infection in HBV endemic countries, 15.7% sero-prevalence rate of HBV determined in this study is regarded as high prevalence. WHO defines low prevalence to be <2%, moderate prevalence as 2-8%, and high prevalence as >8% HBsAg positivity (WHO, 2010).

The high sero-prevalence of HBsAg obtainedin this study (15.7%) suggests that hepatitis B virus infection is commonamong blood donors in Jigawa State. It washigher than the 14.3%prevalence obtained in Jos among blood donors (Uneke *et al.,* 2005).However, the prevalence obtained in this study was lower than the 21.3% prevalencedetected among blood donors in Ibadan (Otegbayo *et al.,*2003). Also, in another study, there was 12.5% prevalenceearlier reported amongst asymptomatic tertiary students in Zaria, Kaduna State (Aminu *et al.,* 2013).

All the donors in this study were males. However, Egah *et al.* (2004) observed that 95% of the donors were males in Jos; Muktar *et al.* (2005) found that 98% of their donors were males in

Zaria while Nwokediuko *et al.* (2007) in their study in Enugu, South-eastern, Nigeria found that 91.8% of their donors were males.

From this study, the age range of the blood donors werebetween 18 and 52 years, with a mean of 28.6 years.This issimilar to the donors in Jos, North-central, Nigeria, who were within the age range of 21 to 50 years according to Egah *et al.* (2004). However, the age range in this study was different from the findings of Muktar *et al.* (2005) in Zaria, Northwestern,Nigeria, in which their donors had a mean age of 33 years even though their age ranged from19 to 42 years. Olokoba*et al.* (2010) reported an age range of 18 to 61 years with a mean of 31.3 years among voluntary blood donors in Yola, North-Eastern Nigeria.

This study revealed that,among the blood donors, the highest prevalence of HBsAg occurred in the 46-54 years age group (22.2%: 2/9) while those of 18-24 years had a prevalence of 18.0%. The result is similar to the finding of Okonko *et al.* (2012), which found higher prevalence in those above 40 years. This could also be because older people might have acquired the infection in their younger age and become chronic carriers (Pennap *et al.*, 2011).Also, the high prevalence of seropositivity among the 18-24 years age group could be associated with lifestyles that predispose them, such as sexual activity and intravenous drug use (Lwoff, 2007).

According to the study, there was no significant association between having multiple sexual partners and HBV infection. This contradicts with works of Hou*et al.*(2005), who reported that sexual transmission of hepatitis B virus is a major source of HBV infection in all areas of the world, especially in the low endemic areas.

Hepatitis B infections were not associated with marital status of the blood donors though seemingly higher prevalence was found among those who were married than the unmarried

donors.This result is similar to the report of Aminu*et al.* (2013) where HBsAg was detected with higher prevalence among married students. In the relation to family type, HBsAg sero-positivity was higher among in monogamy as compared to those in polygamous relationship.

It was noted from this study that having scarificationand sharing of sharp objects were statistically significant factors to HBsAg infection. There was a 2-fold risk of getting infected among those with scarificationand sharing of sharp objects according the study. This may be dueto the mode of transmission of hepatitis B virus, which is via contact with all bodilyfluidsandlack of knowledge of these modes of transmission of HBV. This is in line with the findings of Aminu *et al.* (2013) who reported high prevalence of HBsAg among tertiary students in Zariawithone third of the students lacking inthe knowledge of hepatitis B virus infection.

This study revealed that HBV infection is prevalent among the population studied and higher than those found in blood donors in some other towns and cities in Nigeria, such as Lagos (8.2%), Ibadan (5.10%), Zaria (11.40%), Benin (10.40%), Enugu (3.70%) and Port Harcourt (1.57%) (Smith, *et. al.*,1972; Tribedi, 1974; Udeozo and Ele, 1974; Abiodun, *et. al.*,1985; Ejele and Ojule, 2004; Akinbami *et. al.,* 2012). Meanwhile, Benue State (Otukpo) showed a higher prevalence of 20% to HBsAg among blood donors (Alao *et al.,* 2008).

The study revealed that there was varying prevalenceof other HBV markers among the HBsAg seropositive blood donors. The antibody to the core antigen (HBcAb)had the highest sero-prevalence of 94.0% among the HBsAg sero-positive blood donors. This implies that majority of blood donors analyzed for HBV serological markers showed past infection and were currently infected with HBV. Also, HBcAb only occurs after HBV infection and does not develop in

persons whose immunity to HBV is from vaccine (Dolin, 1999).The 94.0% HBcAb obtained in this study is, however, higher than that which was reported by Belo (2002), who found HBcAb prevalence rate of61.7% among HBsAg seropositive surgeons in Lagos.

There was 18.0% sero-prevalence of hepatitis B "e" antigen detectedamong the donors in this study.The HBeAg is a marker of high infectivity and appears after HbsAg.SimilarlyRufai*et al.* (2014) reported 13.3% prevalence of HBeAg among blood donors in Ghana. A much lower prevalence of 8.9% of HBeAg was reported by Japhat *et al.* (2011) among blood donors in Osun State, Nigeria while a higher prevalence of 19% was reported among 42 Nigerian adults with HBsAg sero-positivity in Ibadan (Ola*et al.*,2009).

This study also revealed a high prevalence of HBeAb (76.0%), among whom the immune system had produced antibodies against the HBeAg and thus no active viral replication. This result is in line with the findings of Ola *et al.*(2009) who detected 64% sero-prevalence of HbeAb among the HBsAg sero-positive Nigerian adults. However, thesero-prevalence rate observed in this study was higher than the 26.7% reported by Japhat *et al.* (2011).

None of the HBsAg sero-positive blood donor was positive for hepatitis B surface antibody (HBsAb). The presence of HBsAb indicates recovery and immunity from HBV infection or successful immunization against HBV (Wilkins*et al.,* 2010). Thus, none of the HBsAg seropositive donors manifested sero-positivity due to HBV vaccination or recovery from HBV infection.

This study determined the sero-prevalence of Human Parvovirus B19 among 266 blood donors. The result obtained showed 72.2% (192/266) and 63,5% (169/266) sero-prevalence to B19 IgM and IgG antibodies respectively. The presence of B19 IgM antibodies among 72.2% of the donors indicates a high prevalence of B19 IgM antibodies among blood donors in Jigawa State,

Nigeria. It is higher than the prevalence obtained among other blood donors in some countries. Nabae *et al.*(2014) reported a sero-prevalence of 1.2% among blood donors in Japan while Kooistra*et al.*(2011) reported 24% among Dutch blood donors.

In terms of parvovirus B19 infection burden, Iwalokun*et al.*(2013) observedanti-parvovirus B19 IgM prevalence of 14.3% among154non-sickle cell anemia healthy subjects in Lagos while Girei*et al.*(2010)reported 13.2% anti-parvovirus B19 IgM prevalence among pregnant women in Jos.The prevalence observed in this study is, however, higher than the 32% and 32-35% found in non-hepatitis controls and hepatitis B and C patients in Oshunrespectively (Opaleye*et. al.*, 2011). Therefore, the data from this study suggest a possible variation in parvovirus B19 prevalence in different parts of the country. Lower prevalence of anti-parvovirus B19 IgM ranging from 3.9% to 11.1% have been reported in Saudi-Arabia, USA and Tunisia(Smith-Whitley*et. al.*, 2004;Regaya*et. al.*, 2007; Obeid, 2011).

Immunoglobin G testing indicates previous infection and immunity.The overall prevalence of HPVB19 IgG antibodydetermined in this study was63.5%.These IgG-positive donors could be at the tail end of resolvingtheir B19 viraemia after having been exposed to the viral infection.At present, interventions for preventing human parvovirus B19 transmission fromblood components have not been implemented in the vast majority of developed countries, due in part to the prevailingview that blood components with low levels of parvovirus B19 will not transmit B19 infection(Kumar *et al.*, 2013).The 63.5% prevalence ofB19 IgG antibodies obtained in this study was higherthan the prevalence of 41.5% detected in Kano (Jegede*et al.*, 2014) and 39.5% reported in Jos (Girei *et al.*, 2010). However, a higher prevalence of 85.4% was reported from Zaria (Ujo *et al.*, 2012). These figures showed that the prevalence of Human Parvovirus B19

differ in different parts of Nigeria. Otherstudies reported 55.4% and 16.3% prevalence in China and in Singapore respectively (Matsunaga*et al.,* 1994).

It has been established that Parvovirus B19 sero-positivity is significantly associated with age, with increase in sero-positivity associated with increase in age (Gilbert*et. al.,* 2005). This study showed no consistency with increase in sero-prevalence as the age increased. However, sero-prevalence of B19 IgM antibodies was highest among the young donors between 25-31 years of age (75.4%: 95/126) and lowest among those aged39 years old and above (64.7%: 22/34). Akyala *et al.*(2012) reported the highest occurrence of B19 IgM antibodies of 16.7% in the age group 15-20, while the lowest was observed in the age group 36-40years among pregnant women in Nasarawa State, Nigeria. However, the result in this study showed no statistical significance in age differences with B19 IgM antibodies.

Also, age has been consistently shown to be a major determinant of anti-parvovirus B19 IgG sero-positivity (Ooi *et al.*,2002; Mazyar and Abdolvahab, 2007; Alao *et al.*,2009).It was not demonstrated in this study that sero-positivity significantly increased with age. The present studyshowed that by the age of 24, about 60% of the population had been infected by HPVB19.However, the age groups of 39 years old and aboveshowed decrease in prevalence of IgM antibodies to HPVB19 than the younger blood donors in this study. Even though this difference contrasts with some studies (Alao *et al.*,2009; Girei*et al.*,2010), itwas in agreement with the results obtained by Ujo and colleagues whose studyreported that sero-positivity did not increase with age (Ujo *et. al.,* 2012).The highest prevalence in this studywas found among the age group32-38 years and was higher than the peak age of 35-45 years reported by Wei *et al.* (2006).

The distribution of parvovirus B19 IgG antibodies according to age in this study also revealed that the highest IgG antibodies was in the age group 25-31 years (61.8%) and the lowest parvovirus B19 IgG antibodies was in the age group 32-38 years (52.8%). Akyala *et al.* (2012), on the other hand, reported that the highest IgG antibodies to HPVB19 was observed in the age group 36-40 years, while the lowest prevalence of Parvovirus B19 IgG antibodies was observed in the age group 15-20 years.

It was also found that there was no significant difference in the occurrence of parvovirus B19 virus and educational level, although it differed according to their level of education.The distribution of parvovirus B19 antibodies according to educational level revealed that the highest prevalence IgM antibodies (77.8%) and IgG antibodies (72.2%) antibodies were observed in those without formal education, while the lowest Parvovirus B19 IgM antibodies and IgG antibodies were observed among those that attended only primary education.

In this study, the donors' occupation, which may be directly related to their income and therefore their general health status, was assessed.Sero-positivity of the IgMantibodies to HPVB19 was highest among the farmers (79.1%) and lowest among the businessmen (63.2%). There was no significant difference in the sero-prevalence of the IgM and occupational status (P=0.174). People who were unemployed had the highest prevalence (67.8%) for HPVB19 IgG antibodies while the civil servants had the lowest prevalence (60.0%). This finding confirms with the work of Kishore *et al.* (2010) among blood donors in India where high positivity was observed among unskilled laborers.

This study however has not demonstrated any association between marital status and sero-positivity of parvovirus as the difference in the prevalence was not significantly different

between married and unmarried individuals. This same observation reported in India where the authors noted that the route of transmission of the virus is not through sexual means (Kishore *et al.,* 2010). Similarly, family type was not associated with IgM and IgG sero-positivity, although a slightly higher sero-positivity of IgM antibodies was observed in individuals from polygamous families than in those from monogamous families.

There was a high positivity of IgG occurrence observed among the men with multiple sexual partners,with a 4 fold risk of acquiring the infection. This could be due to the fact that their female sexual partners are always in contact with children both at home and outside home. Although Kishore and colleagues (2010) reported that the route of transmission of parvovirus is not through sexual means, however, exposure to more sexual partners could increase the chances of the acquiring the infection since women are often in contact with children.

It is now recognized that patients with dual seropositivity pose a great threat in the dissemination of parvovirus B19 in their environment, making their surveillance and exclusion from non-infected patients very important in order to halt viral transmission and reduce the risk of an outbreak (Iwalokun*et al.,* 2013).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The findings in this study confirmed the presence of hepatitis B virus and human parvovirus B19 in Jigawa State and their sero-prevalence are comparable with those in various States in Nigeria and countries of the world. The prevalence of HBsAg in this study was found to be 15.7% among the study population. History of scarification and sharing of sharp objects proved to be significantly associated with HBV infection (P=0.021 and P=0.025 respectively).

Serological markers of HBV infection among the HBsAg positive samples showed prevalence of 94%, 76% and 18% for HBcAb, HBeAb and HBeAg respectively. The high prevalence of HBcAb and HBeAb imply that those individuals were currently infected and did not develop immunity through vaccination respectively. This knowledge can, thus, be utilized in establishing strategies to curtail HBV infection in the study area.

Sero-prevalence of HPVB19 antibodies in the study population was 72.2% and 63.5% for IgM and IgG antibodiesrespectively. This signifies that the majority of the blood donors are susceptible to HPVB19 infection. Having multiple sexual partners proved to be significantly associated with HPVB19 infection (P=0.04).

Prevention is the most important key to combatingthe burden of these infections.This can be achieved through vigilant blood donor selection, health education and awareness campaign.Immunization of people at risk particularly health care workers, colleagues, family members of the infected persons, accurate identification and clinical management of the blood

donors withHBV and HPVB19 infections will reduce the probability of transmission and prevent further dissemination of the infections.Also, this research will help to establish health policies in Jigawa State and Nigeria, in general, that will generate preventive and control measures to combat these infections. It is also expected that the findings of the study will help educate the public on the danger associated with these infections.

6.2 Recommendations

1. The prevalence of 15.7% for HBV obtained in this study is appreciable; as such it is recommended that adequate screening of blood donors should be instituted in all the hospitals in State with establishment of adequate public health measures in order to reduce the transmission of the virus

2. With 1.2% HBV vaccination coverage amongst the study population, HBV vaccination will undoubtedly reduce the spread of the virus in among populace in the State.

3. Screening kits for human parvovirus B19 should be provided at the various hospital settings, especially for screening of blood donors.

4. Screening of inpatients for HBV should be a routine practice in our hospital for early identification and clinical management.

5. Sensitization and awareness campaign of these infections should be incorporated into the State's public announcements in Television and radio jingles periodically.

6. Further studies should be carried out to identify other factors associated with the spread of these viruses in Nigeria.

6.3 Limitation of the Study

1. Some of the participants might have withheld some vital information concerning their health status as well as their behavioral patterns due to their socio-cultural beliefs which could have affected the analysis of some of the results obtained

2. There was lack of fund to increase the sample size of the population, and to include more hospitals in Jigawa State in the study, which could give a more general prevalence of these infections in the State.

3. There was lack of fund to screen all the samples for HPVB19 and also check coinfection among the tested population.

4. Molecular studies could have been carried out among the seropositive donors to determine the predominant type, if there were more funds available.

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APPENDIX I: ETHICAL APPROVAL

JIGAWA STATE HEALTH RESEARCH ETHICS COMMITTEE

STATE MINISTRY OF HEALTH

MOTTO: FOSTERING CHANGE THROUGH HEALTH RESEARCH

Registration number NHREC/10/11/2011

GHSB/ET/COM/2013/7/35 Friday, March 05, 2013

ATTENTION

Mr. Ajah, Benjamin Onyekechi

ETHICAL CLEARANCE TO CONDUCT RESEARCH ON SERO PREVALENCE OF HEPATITIS B VIRUS PAVOVIRUS INFECTIONS IN BLOOD DONORS IN SOME SELECTED HOSPITALS IN JIGAWA STATE OF NIGERIA

With reference to your research protocol you submitted to the office of the Director General on the above topic, and pursuant to your request for Ethical approval to conduct the study in some selected hospitals in the state. I wish on behalf of the JIHREC to notify you that it was observed that you intend to conduct a research study as part of partial fulfillments of an academic programme.

Following your initial request, and the subsequent recommendation there in GHSB/ET/COM/2/25 of 05/10/2012 and your earlier response on the study. I wish to convey clearance on behalf of the JHREC for you to conduct the study as outlined in your original protocol submitted before the Committee.

Please note that JHREC reserve the right to conduct monitoring visits to your research sites without prior notification and to have copy of the study findings at the end of the research. I wish you a successful and hitch free exercise in the state.

Thank you and Best Regards.

Pharm. Ibrahim Hassan MNIM, FCAI

For: Chairman, Health Research Ethics Committee

APPENDIX II: CONSENT FORM

I, Ajah Benjamin Onyekachi, a post graduate student of Ahmadu Bello University, Zaria in the Department of Microbiology, will be undertaking a research work on the topic: Seroprevalence of Hepatitis B Virus and Parvovirus B19 among Blood Donors in selected Hospitals in Jigawa State, Nigeria.

The purpose of the research is to determine the seroprevalence of Hepatitis B virus and Parvovirus infections among blood donors in some selected hospital in Jigawa State and the risk factors associated with these infections using some of the commonly used diagnostic kits. This will provide information to aid Medical Practitioners to ensure adequate screening of blood for transfusion. This will also provide base line data for future researches and recommendations that will be helpful to the state and public on the prevention and control of these infections.

Your partition will involve collection of 5mls of blood from you using a needle and syringe. Your sample will be screened for Hepatitis B Surface Antigen and Parvovirus. I will appreciate your participation in this study.

The information obtained will be kept confidential and use only for the purpose of the research.

Participation is voluntary. Non participation will not hinder you from getting full medical services from the hospital. All investigationsare free. Participates that tested positives to the tests will be referred to the medical doctor of the hospital for appropriate medical. In case of injury to the participant during sample collection, the participate will be treated free of charge.

Kindly fill the space below if you have consent to participate.

I .....(Reference No.) give my consent to participate in this study. I have been duly informed of the process and have given my free will and not under pressure.

Signature /Thumbprint of subject Date.....

.....

Signature of witness Date.....

.....

Signature of the investigator

..... Date.....

APPENDIX III: QUESTIONNAIRE

Seroprevalence of Hepatitis B Virusand Parvovirus B19 Infections Among Blood Donors In Selected Hospitals in Jigawa State, Nigeria.

1.Reference No:……..

2. Sex: Male ( ) Female ( )

3.Age: 18-24( ) 25-31( ) 32-38( ) 39 and above

4. Address……..……

5. State of origin: ……

6.Educational level: Tertiary () Secondary ( ) Primary ( )Quranic ( )none ( )

7.Marital status: Single ( ) Married ( ) Widower/Widow ( ) Separated ( ) Divorced ( )

8.Type of family:Monogamous ( ) Polygamous ( )

9. Occupation: Farmer ( ) Civil Servant ( ) Business ( ) Student/Applicant ( ) Health worker ( ) Other ( )

10. Have you ever been transfused before? Yes ( ) No ( )

If yes, why?.....; When?.....; How many pints of blood did you received?.....

11. Have you donated blood? Yes ( ) No ( ). If yes, when?

12. Have you ever had surgery before? Yes ( ) No ( ).

If yes, when?.....Were you transfused? Yes () No (). If yes, how many pints did you received?

13. Have you been vaccinated for HBV? Yes ( ) No ( )

If yes, when?

14. Do you have multiple sexual partners? Yes ( ) No ( )

15. Do you use condom when having with any person other than your spouse? Yes ( ) No ( )

16. Have you had scarification?Yes ( ) No ( ). If yes, when?

17. Do you practice drug injection on yourself?

Never ( ) Once ( ) Twice ( ) Thrice ( ) Four times ( ) Fives times >Five times ( )

18. Do you share unsterilized sharp objects such as razor, injection needle, cuticle removal etc?

Yes ( ) No ( )

19. History of smokingNone( ) 1-5yrs( ) 6-10yrs( ) 11-15yrs( ) 16-20yrs( )>20yrs( )

20. History of alcohol consumption None( ) 1-5yrs( ) 6-10yrs( ) 11-15yrs( ) 16-20yrs( ) >