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DEPARTMENT OF CLINICAL MICROBIOLOGY

HEPATITIS B AND C INFECTION IN PREGNANT WOMEN ATTENDING ANTENATAL CLINIC AT THE CATHOLIC HOSPITAL, BATTOR IN THE VOLTA REGION, GHANA.

By

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DECLARATION

I hereby declare that this submission is my own work towards the MSc and that, to the best of my knowledge; it contains no materials previously published by another person or material which has been accepted for the award of any other degree of the university. All references used in work have fully been acknowledged.

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DEDICATION

I dedicate this work to the Almighty God for his Grace, guidance and protection for seeing me through a successful completion of this work. I am forever grateful.

I also dedicate this work to my children Elaine Yayra Attipoe and Keren Elinam Attipoe, you are the best.

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ABSTRACT

Viral hepatitis is caused by hepatotrophic viruses with Hepatitis B and Hepatitis C being the frequent viruses affecting humans. Infections with HBV and HCV in pregnancy results in complications to the neonate and mother. Determination of the infection in pregnant women as well as the associated risk factors helps to identify neonates at risk of mother to child transmission and hence appropriate measures taken to help prevent the infection. A cross sectional study was carried out at the Catholic Hospital, Battor, to investigate the seroprevalence of HBV and HCV virus infections and associated risk factors among pregnant women attending the antenatal clinic. Structured questionnaire were administered to obtain the socio demographic data and Enzyme Linked Immunosorbent Assay, ELISA from Human diagnostic worldwide, Germany) was used to investigate the presence HBsAg, anti-HBc and anti HCV. One hundred and thirty five (135) pregnant women were enrolled in the study. HBsAg was detected in 37 of these women, giving an overall prevalence of 27.4%. Among these women 5 (13.5%) tested positive for HBeAg indicating that this proportion of patients was highly infectious and therefore likely to transmit the virus to their offspring. The prevalence of hepatitis C in the study population was 8.8% and 60.7% tested positive for anti HBc. Parity, educational background and the use of protection during sex were factors that did not have any statistically significant association in the acquisition of these infection but the age of the subjects had a significant association with the acquisition of both HBV and HCV. Among the associated risk factors analyzed, having multiple sexual partners was the only significant factor in the acquisition of HBV infection whiles history of previous blood transfusion was associated to the acquisition of HCV infection. The results from this study reveals a high prevalence of HBV and HCV among pregnant women in the area.

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 LIST OF ACRONYMS OR ABBREVIATIONS

ALF	Acute liver failure
ANC	Antenatal clinic
Anti-HBc	Hepatitis B core antibody
CDC	Centre for disease control
DNA	Deoxyribonucleic acid
EIA	Enzyme immunoassay
ELISA	Enzyme linked immunosorbent assay
EM	Electron microscopy
HBeAb	Hepatitis B envelope antibody
HBeAg	Hepatitis B envelope antigen
HBIG	Hepatitis B immune globulin
HBsAb	Hepatitis B surface antibody
HBsAg	Hepatitis B surface antigen

HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HDV	Hepatitis D virus
IFN	Pegylated interferon
IgG	Immunoglobulin G antibodies
IgM	Immunoglobulin M antibodies
MNC	Mean negative control value
MPC	Mean positive control value
NASBA	Nucleic acid sequence based amplification
NAT	Nucleic acid test
NK cells	Natural killer cells
PCR	Polymerase chain reaction
RIBA	Recombinant immunoblot assay
RNA	Ribonucleic acid
RT-PCR	Real time polymerase chain reaction
STD	Sexually transmitted disease
TBM	3, 3', 5, 5', tetramethylbenzidin
TMA	Transcription mediated amplification
WHO	World health organization

CHAPTHER ONE

INTRODUCTION

1.0 Background to the study

Hepatitis is inflammation of the liver characterized by the existence of inflammatory cells in the tissue of the organ principally caused by viral infections. There are five hepatotrophic viruses Hepatitis A B C D and E that are recognized to cause hepatitis and of these, Hepatitis B virus and Hepatitis C virus are amongst the most regular viral infections in human beings (Eke *et al.*, 2011; El-Serag, 2012). Hepatitis can also be caused by toxins (some drugs, plants and alcohol), other infections and certain autoimmune diseases (Ahmedin *et al.*, 2004).The major public health problem, particularly in developing countries among the liver diseases are the one caused by hepatitis B and C (Haider *et al.*, 1994; Santiago-Munoz *et al.*, 2005) and are extremely prevalent in the sub-Saharan Africa (Kwan *et al.*, 1997; Kramvis and Kew, 2007). Hepatitis is called acute when infection lasts for less than six months and chronic when infection continues longer. Most infection occur with limited or no symptoms, but often leads to vomiting, jaundice, malaise, fatigue anorexia (low appetite) and abdominal pain. (Ryder and Beckingham, 2001).

The mode of Hepatitis B virus transmission is through infected blood, by sexual means and mother to child (vertically) in the perinatal duration. Perinatal transmission is the principal mode of hepatitis B virus (HBV) transmission globally (Tran, 2009). Most people are infected by vertical transmission, or in the early-childhood in endemic areas, (Wright, 2006). Without immunization of the pregnant women, up to 90% of newborns born to mothers will become chronic carriers of the infection (McMahon *et al.*, 1985; Chang, 2000; Sandesh *et al.*, 2005). Parenteral routes such as intravenous drug use or blood product transfusion, sexually and vertically during delivery is mainly the mode in which hepatitis C is transmitted (Dienstag, 1983; Melbye *et al.*, 1990; Wejstål *et al.*, 1992). Mother to child transmission of hepatitis C

virus occurs in 3%-10% of pregnancies complicated by hepatitis C virus infections (Berkley *et al.*, 2008). The World Health Organization (WHO) approximated that 3.0% of the world's populaces are infected with hepatitis C virus chronically where most of these cases are coming from Africa which is reported to have the highest prevalence rate of hepatitis C virus infection (Lavanchy, 1999; Madhava *et al.*, 2002).

The prevalence of hepatitis B virus amongst women who are pregnant globally is about 5.0% ranging from 0.6% in low endemic regions to greater than 20.0% in high endemic areas in the Far East and Africa whiles the occurrence of hepatitis C virus amongst women who are pregnant globally is between 1.0% and 8.0% (Petrova and Kamburov, 2010; Arshad *et al.*, 2011). In Southern African countries, the occurrence of hepatitis B virus among pregnant women is 2.0% to 2.9% except South Africa which has a prevalence of 4.6% (Alter, 2007; Sinha and Kumar, 2010), whiles the prevalence of HCV in these same countries is reported to be 0.1% (Njouom *et al.*, 2011). In Central Africa, the prevalence of hepatitis B virus among women who are pregnant ranges from 6% to 9.5% whiles the prevalence of HCV is 4.3% (Ugbebor *et al.*, 2011; Kfutwah *et al.*, 2012). HBV prevalence in Western Africa is high varying between 6.2% and 16% whereas the prevalence of HCV ranges from 2.2% to 3% (MacLean *et al.*, 2012; Okusanya *et al.*, 2013).

Chronic infection with hepatitis B virus and hepatitis C virus are frequently asymptomatic but there is a high vertical transmission rate which can proceed to cirrhosis of the liver and hepatocellular carcinoma. During pregnancy, infections with viral hepatitis are related with high maternal risk, neonatal and foetal problems (Ali and Adam, 2011). Foetal and neonatal hepatitis may lead to chronic virus carriage, which might result to impaired physical and mental health in the future. Chronic virus carriage is usually caused by neonatal hepatitis, which in turn may lead to liver cirrhosis and hepatocellular carcinoma among young adults (Sookoian, 2006; Wright, 2006; Shukla *et al.*, 2011). In addition, inducement of premature labor, poor

outcomes of infants such as still births, neonatal deaths (NND) and high maternal mortality have been reported to be caused by acute hepatitis in pregnancy (Bohidar, 2004; Gambarin-Gelwan, 2007). The mother is also at risk of postpartum hemorrhage and high incidence of hypertensive disorders. If the pregnant woman has had acute hepatitis B infection during late pregnancy perinatal transmission of this disease happens, in the first post-partum or if the pregnant woman is a long-lasting HBsAg carrier (Levy and Gagnadoux, 1996).

However, early diagnosis of hepatitis B and hepatitis C infections in women who are pregnant can aid in treatment and management of the disease much more efficiently. A preventive measure includes immunization with hepatitis B virus (HBV) vaccine which is the most effective mode of hepatitis B virus infection prevention. Hepatitis B immune globulin (HBIG) also safeguards the infant by passive immunization if given just before or soon after exposure to hepatitis B virus. In addition, screening of pregnant women for HBV infection and routine screening of blood donors for HBsAg, strict surveillance, good personal hygiene and proper measures to control the environmental factors should be practiced to reduce transmission.

Treatment regimen following a positive diagnosis of the infection involves the use of interferon alpha 2b, P EG-interferon alpha 2a, , lamivudine, adefovir, entecavir, telbivudine and tenofovir (Tran, 2009).

There is no vaccination for HCV infections on the other hand but the infection can be prevented avoiding contact with infected blood, avoid sharing of razor blades, toothbrushes, shavers and needles and avoidance of alcohol intake (CDC, 1998a; Wiley *et al.*, 1998). Treatment regimen is the use of pegylated interferon (I FN) alpha and ribavirin (Fried *et al.*, 2002; Hadziyannis *et al.*, 2004). Early detection of HCV infection ensures early administration of antiviral treatment which is the most effective than beginning at a later stage (Alter *et al.*, 1990). Moreover, early identification together with counselling and life style modification reduces the transmission of the infection to other people.

In Ghana, particularly in Battor which is a rural area where the level of education is low and also the level of teenage pregnancy being high, it is expected that some of the sexually transmitted diseases like hepatitis B and hepatitis C will be prevalent among the youth. Despite the inclusion of Hepatitis B in the routine antenatal care screening, some newborns are still at risk of vertical transmission of the disease. The study therefore, was to determine the prevalence of these viruses among healthy pregnant women.

1.2 Problem Statement

Hepatitis B virus and hepatitis C virus infections are a foremost worldwide public health burdens spreading quickly in the developing countries including Ghana. Perinatal hepatitis B virus transmission results in a projected 21.0% of hepatitis B virus associated mortality whiles regionally it ranges from 13.0% in the Eastern Mediterranean region to 26.0% in the Western Pacific region. Irrespective of parity, age, gestational age, history of blood transfusion and tattooing, researches conducted in Africa has established a moderately high HBsAg sero-prevalence in women who are pregnant (Elsheikh *et al.*, 2007). In Libya, ElMagrahe *et al*. found HBsAg positivity to be 1.5% and transmission to be 60.9% whiles in Ghana, Candotti *et al.* reported of HBsAg prevalence of 15% with materno-fetal transmission of 8.4% in neonates. Limited research has been conducted on HBV and HCV in Ghana and even the few conducted are in urban settings of the country (Candotti *et al.*, 2007; Ephraim *et al.*, 2015). In the Volta Region, no study has been conducted on pregnant women to assess the occurrence of hepatitis B and hepatitis C of its inhabitants and thus this study will be the first in the region. Studies conducted by Ephraim *et al.* (2015) and Candotti *et al.* (2007) revealed that HBV and HCV infections in pregnant women has a significantly high prevalence.

From studies that has been carried out in various parts of the world, it has been established that chronic hepatitis B virus infection happens amongst 90.0% of newborns infected at birth (because of their weaker immune system), 30.0% of children infected at 1 - 5 years of age and about 1%-5.0% of individuals infected as older children and adults (Mast *et al.*, 2005; Chang *et al.*, 2009; Tran, 2009). These children become chronic carriers of HBV and serves as the main pool for continuous transmission of the disease. They are at risk of end stage renal disease, glomerulonephritis, cirrhosis and hepatic carcinoma (Levy and Gagnadoux, 1996; Wasmuth, 2009) which are major threats to the community at large, thus an awareness of the infection have to be created.

1.3 Justification and relevance of study

Comprehensive studies, research and information on the occurrence of hepatitis B virus and hepatitis C virus among women who are pregnant and their neonates has been limited in Ghana, though a lot of research has been carried out in highly endemic areas like Cameroon, Nigeria, China and Senegal. In these areas, transmission during child birth and childhood is the most common method as confirmed by results from the studies that had been carried out (Eke *et al.*, 2011; Gupta *et al.*, 2014).

In Ghana however, few studies has been carried on pregnant women to determine the occurrence of hepatitis B virus and hepatitis C virus infections in pregnant women and their effect on neonates. Nevertheless, the few that has been done was mostly in the urban areas. Considering the problems associated with infection of hepatitis B and C during pregnancy and the effect on the neonate, coupled with the fact that not many studies has been carried out in the rural communities like Battor, it is worth studying the prevalence of Hepatitis B and C infection in pregnant women. This study will provide additional information on the risk of

materno-fetal transmission, the carriage and the infection status of pregnant women in North Tongu of the Volta Region, Ghana. It will also report reliable occurrence of this infectious disease among the inhabitants and offer an opportunity for vertical transmission prevention of the disease.

1.4 Main Aim

The aim of the study is to determine the prevalence and possible risk factors-associated with hepatitis B and C virus infection in healthy pregnant women visiting antenatal clinic at Catholic hospital, Battor.

1.5 Specific objectives

1. To determine the prevalence of hepatitis B and C infection in pregnant women attending antenatal clinic at Catholic hospital, Battor
2. To assess the risk of vertical (mother-to child) transmission
3. To inform decision on prevention of (mother to child) vertical transmission of the viruses

CHAPTHER TWO

LITERATURE REVIEW

2.1 Hepatitis B

2.1.1 Historical background

Around 400 BC (Hippocrates), viral hepatitis, primarily labeled as “epidermal jaundice”, was described. Interest in the disease increased when Rudolf Virchow termed a patient with sign and symptoms of epidermal jaundice having the lower end of the common bile duct blocked with a plug of mucus in 1865. The disease was termed “catarrhal-jaundice” since it was alleged to be caused by phlegm obstructing the bile duct (Gruber and Virchow, 1865). In the early 1960s, the unearthing of Australian antigen by Baruch Blumberg and Harvey Alter was made.

Blumberg was working on blood samples he collected throughout the world to study the inherited diversity in human beings with a focus on discovering the origin behind variability of disease susceptibility and outcomes. Alter was testing serum from patients who had received multiple transfusion of blood and had developed febrile transfusion reactions by means of agar gel double diffusion (Ouchterlony). Alter begun testing the serum that
Blumberg had collected (Gerlich, 2013) focusing on detecting novel serum lipoproteins since Blumberg had proven that lipoproteins were polymorphic amongst people (reviewed by Blumberg and Alter, 1965; Alter, 2014).

In 1963, a crude detection of hepatitis B surface antigen (HBsAg) was made precisely through an Ouctherlony reaction amid serum from Australian aborigine and hemophiliac patient (reviewed by Blumberg and Alter, 1965). This was an unanticipated discovery since this preliminary precipitate didn't take up a lipid stain but rather a protein counterstain efficiently noticed the precipitate (Alter, 2014). The detected protein was termed the Australian antigen (AuAg). It was found mostly in patient diagnosed with leukemia and children with Down's syndrome (Blumberg *et al.*, 1967). By this time accruing proof proved an association between the existence of AuAg and viral hepatitis. Separate studies by Prince, Murakami and Okochi confirmed AuAg to be specifically found in patients with serum hepatitis(Okochi and Murakami, 1968; Prince, 1968).

David S Dane examined AuAg immune complexes against purified AuAg using EM in 1970. He observed bigger particles comparable in size to other viruses with an evidently noticeable inner core. Upon biochemical studies on the "Dane" particle, an inner core nucleocapsid (Hepatitis B virus core antigen) and an outer surface protein AuAg which was later called hepatitis B virus surface antigen (HBsAg) was identified (Dane *et al.*, 1970). DNA genome of hepatitis B virus was also elucidated. The existence of endogenous DNA established that the Dane particle contained complete hepatitis B virus with its nucleic acid genome

(Hirschman *et al.*, 1971; Kaplan *et al.*, 1973). Further studies by Robinson *et al.* (1974) and Will *et al.*, (1982) provided evidence that the Dane particle contained infectious HBV.

2.1.2 Structure of HBV

Hepatitis B virus (HBV) is a hepatotrophic circular genome of partially double stranded DNA virus belonging to the Hepadnaviridae family with a core antigen enclosed by a shell enclosing hepatitis B surface antigen (HBsAg) (Seeger and Mason, 2000). The virus have preference for liver cells and infects only humans and some other non-human primates.

The intact Hepatitis B virus is called a Dane particle and looks like a sphere under the electron microscope. It is a 42.0nm partly double stranded DNA virus that consist of a 7nm thick outer lipoprotein coat or envelope containing the surface antigen (HBsAg) and a 27nm nucleocapsid core (HBcAg and HBeAg) (Robinson, 1995; Hollinger and Liang, 2001). It has a vigorous polymerase enzyme-linked to single molecule of double stranded hepatitis B virus DNA. The hepatitis B viron consists of a surface and a core.

The core comprises the viral genome, a relaxed circular, partially duplex DNA of 3200 nucleotides, and a polymerase which is responsible for the synthesis of viral DNA in cells which are affected. The genome can encode four (4) sets of proteins and their regulatory components by shifting the frames over the same genetic matter. The four polypeptide reading frames (genes) are the S (surface), the C (core), the P (polymerase) and the X (transcriptional trans activating). The S genes consist of three regions, the pre-S1, pre-S2 and encodes the surface proteins (HBsAg), which is the serological hallmark of HBV infection (Chisari *et al.*, 1989).

The C gene is categorized into two compartments, the pre core and the core, and codes for two different proteins, the Core antigen (HBcAg) and the e antigen (HBeAg) which is a cleavage

product of the viral core structural polypeptide. The HBeAg is released during vigorous infection and growth of virus, when the soluble components of the core is released (Ganem and Schneider, 2001).

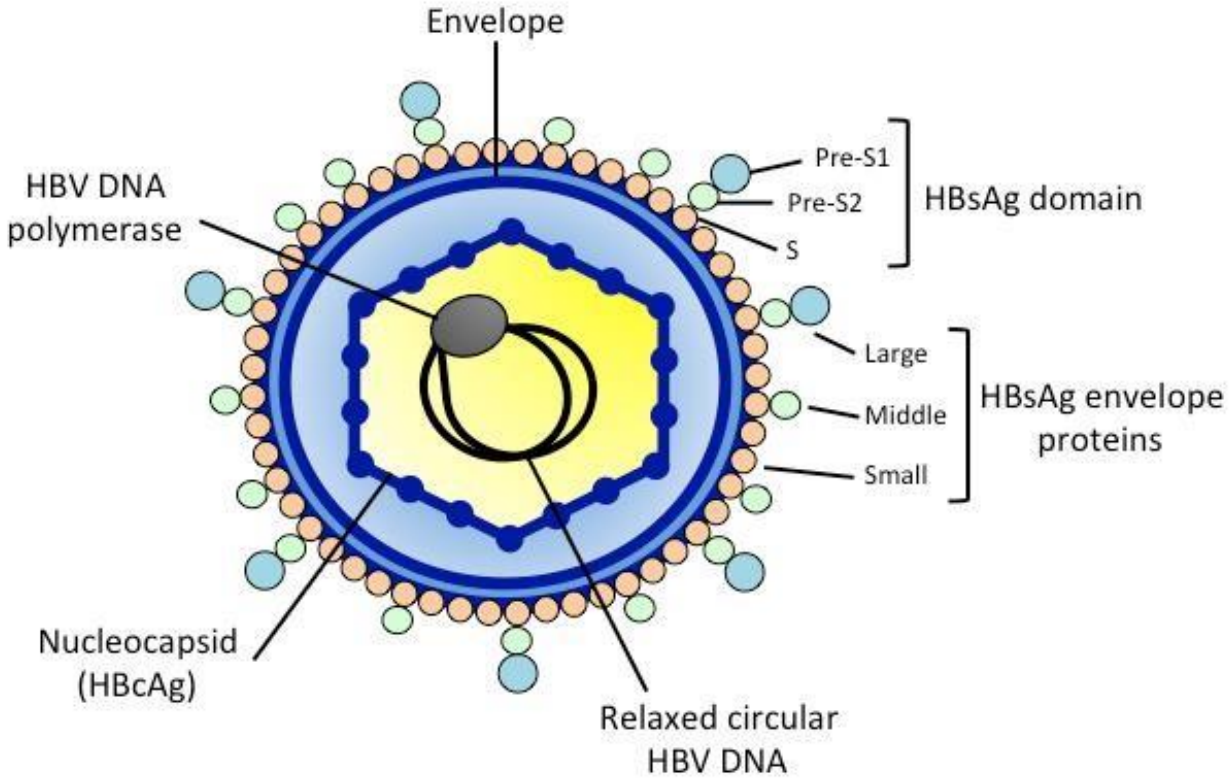


Fig.1: Structure of Hepatitis B virus. Source: www.antimicrobe.org/images-monographs/v22_fl.png

2.1.3 The immune system and HBV

In an acute HBV infection, HBsAg is used as a general marker of infection and it is the first to be detected in the bloodstream before the appearance of symptoms (about 2-8 weeks). Subsequent to show are the virion markers including soluble antigen (HBeAg) and virus specific DNA polymerase. The presence of HBcAg is not detected due to initial presence of anti-HBc (2 to 4weeks) after the surface antigen has showed. Antibodies to HBcAg (antiHBc) are suggestive of infection: IgM anti-HBc indicates an acute or current infection and vanishes

within six (6) months usually, whereas IgG anti-HBc indicates a chronic or past infection and persist for life. Hepatitis B surface antigen persevering for a duration beyond six(6) months is termed as chronic hepatitis B virus infection (Kwon and Lee, 2011). The manifestation of HBeAg and hepatitis B virus DNA shows active replication of the virus and therefore highly infective but the latter is more accurate and used mainly for monitoring response to therapy. Anti HBe in the blood indicate that the virus is not replicating anymore but the individual may still be HBsAg positive when tested and it is normally detected about 4 months after infection.

Following initial exposure to HBV, the host innate immunity plays an important role in which NK cell action may concur with ultimate viremia (Webster *et al.*, 2000). Subsequently, a multi-specific CD 8+ T cell mediated response focused in the direction of hepatitis B virus core, envelope and polymerase epitopes is essential to establish immune control of hepatitis B virus, leading to spontaneous recovery (Ferrari *et al.*, 1990; Maini *et al.*, 2000; Rehermann, 2000).

Injury of the liver is immunologically mediated in chronic hepatitis B and thus, the sternness and course of the disease does not correlate with the level of virus in the serum or amount of antigen expressed in the liver. Antigen specific cytotoxic T cells are alleged to play part in injury of the cell and account for viral clearance. However, HBV infected cells are made up of aggregated an HbS antigen which combines with and block anti-HbS antibodies, and hence limit the humoral response. Arthritis, as well as skin and kidney damage is caused by immune complexes due to the deposition of antibody and HBsAg in tissues which will activate the immune system. Development of chronic infection may be due to deficiency of an energetic and specific C D8+ cytotoxic T cell and C D4+ helper T cell reaction whereas, the use of non-specific Tcells results in low level chronic inflammation and damage of the liver (Maini *et al.*, 2000; Webster *et al.*, 2000). Similarly, spontaneous sero-conversion from HBeAg to anti HBeAb is also immunologically mediated (Ganem *et al*, 2004). Constant cellular stimulation and C D8+ T cell cyto-toxicity may happen within the liver of chronically infected patients and

is unsuccessful in attaining control of HBV, ensuing in a tenacious state of infection, hepatocyte injury, cirrhosis, advanced hepatic fibrosis and high hepatocellular carcinoma risk (Rehermann, 2000).

Individuals who have immuno-suppressed states, such as AIDS, chronic illness and malnutrition are more likely to be asymptomatic carriers because of their weak immune system. Immunity against hepatitis B virus infection is through a response to HBcAg and HBsAg.

HBsAg is detected in the blood from one to six months after infection but antibodies against the HBsAg is detectable after eight months and thus a 'window' where neither HBsAg nor anti-HBsAg can be detected. Due to the immune response, the disease resolves in most patients.

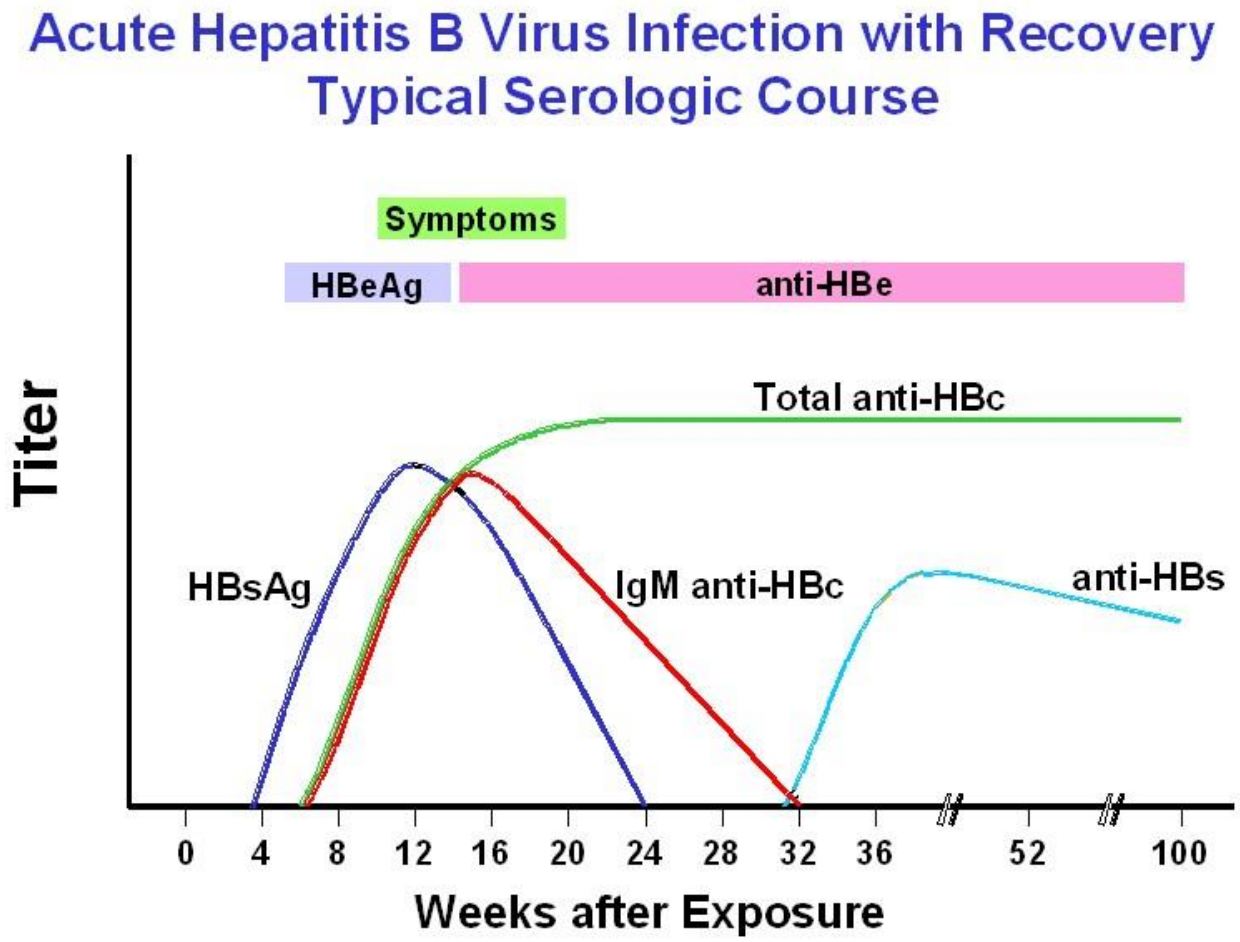


Fig. 2 Acute HBV infection with recovery typical serological course. (Source retrieved from Wikipedia March 2016)

In chronic infection, HBsAg and HBeAg, anti-HBeAg and anti-HBcAg (IgM) are present in the blood throughout the infection.

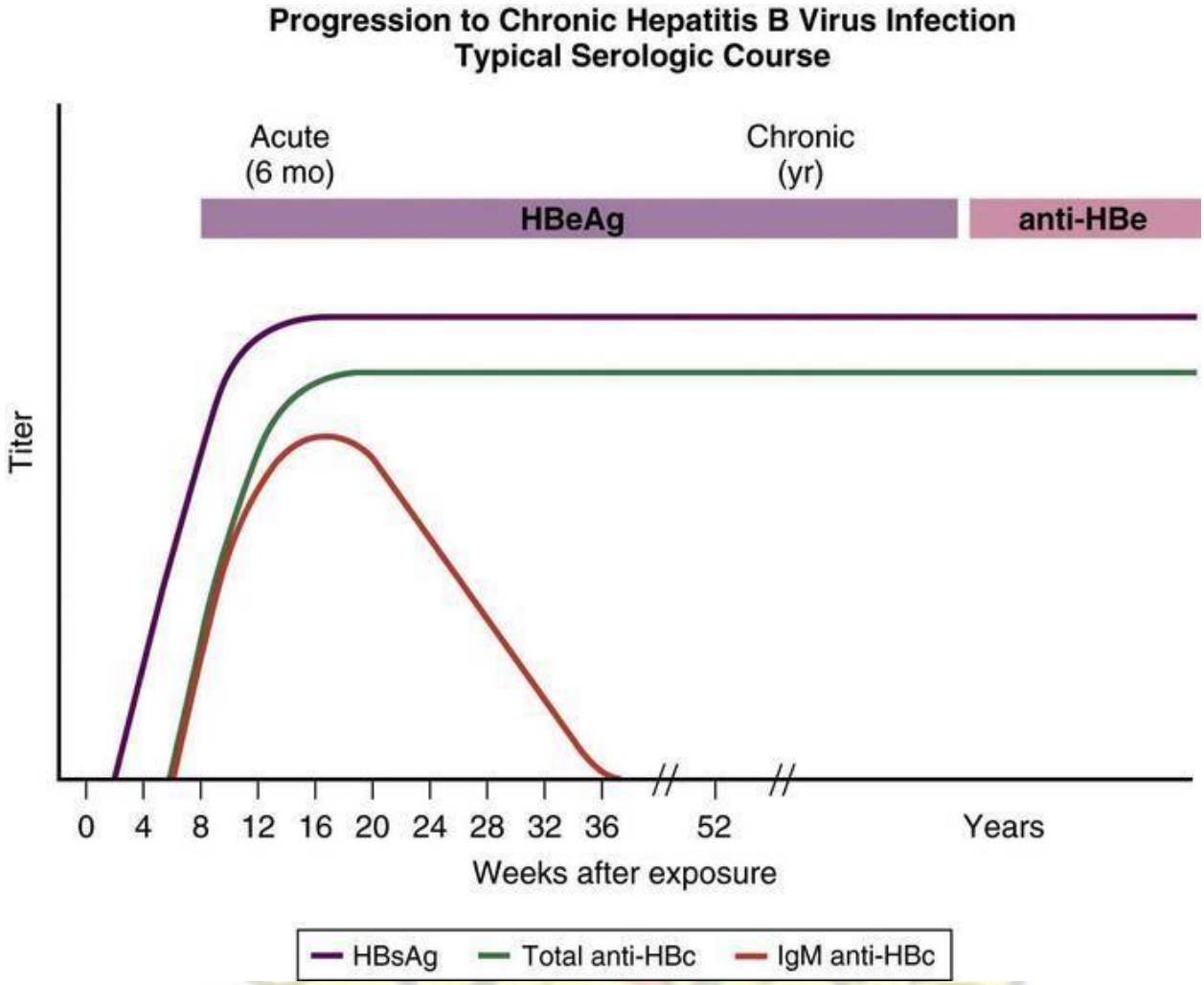


Fig 3. Progression to chronic HBV infection. (Source retrieved from Wikipedia March 2016)

2.1.4 Epidemiology of HBV infection

The hepatitis B virus is transmitted through exchange of body fluids between healthy and infected individuals. The path of transmission is through either parenteral (infected serum, blood products, blood transfusions) or non-parenteral (saliva, tears, sweat, urine, semen, skin wounds) route. It is also transmitted during child birth, unprotected sex and by sharing of needles (Ahmad *et al.*, 2004).

Studies conducted worldwide has shown that, HBV is transmitted predominantly perinatally in highly endemic areas with the age of being infected and chronicity risk being contrariwise

related (Ganem and Schneider, 2001; Tran, 2009). Perinatally acquired chronic hepatitis B infection is related to cirrhosis, glomerulonephritis, hepatic carcinoma and end stage renal disease(ESRD) in children (Levy and Gagnadoux, 1996; Wasmuth, 2009). The virus can also be transmitted horizontally to children in their first year of life (less than 5 years of age) if the mothers are HBsAg positive and Hepatitis e antigen positive (Elinav *et al.*, 2006), however, the HBV is not transmitted through the placenta but the infection occurs during child birth. Studies have shown that if the pregnant women have acute hepatitis B in the second or third trimester of gravidity or within two (2) months of giving birth there is a high perinatal infection risk. This mode of transmission occurs mainly in the Sub-Saharan Africa and Asia. Babies who are infected with hepatitis B virus perinatally has a 70-90% chance of developing chronic hepatitis B virus infection and one(1) in four(4) adult who were infected at birth develop hepatitis B virus related liver disease and mostly will die impulsively (Chang, 2000). The children become chronic carriers of HBV and remain the key pool for continuous transmission of the disease. Several of them grow into mothers and persistent carriers and thus repeating the cycle (Mast *et al.*, 2005).

HBV infection is a major public health problem with a high incidence of mortality and morbidity. The World Health Organization (WHO) has estimated the problem of hepatitis B virus infection to be roughly two (2) billion with greater than 350 million people infected chronically globally (Hollinger and Liang, 2001; WHO, 2001). The world is categorised into three (3) zones where the occurrence of chronic hepatitis B virus infection is high (>8.0%), inter mediate (2%-8%) and low (< 2%). About 45.0% of the world's populace are located in in the high endemic areas with HBsAg seropositivity above 8%. Most of these people live on the Africa continent, southeast Asia, the Pacific Basin, Middle East and some countries in Eastern Europe (Hollinger and Liang, 2001; Lavanchy, 2004). In such areas, around 80% of the inhabitants develop hepatitis B virus infection before 40 years, and 8-20% of the inhabitants

are carriers' hepatitis B virus. Sub-Saharan Africa have the higher endemicity where about fifty (50) million people are HBV chronic carriers (Burnett *et al.*, 2005). The low endemic areas includes North America, Western and Northern Europe, some parts of South America and Australia where few (20%) of the inhabitants is infected with hepatitis B virus (WHO, 2001; Shepard *et al.*, 2005; Ezechi *et al.*, 2014). Nations in the Middle East have inter mediate to high endemicity of hepatitis B infection.

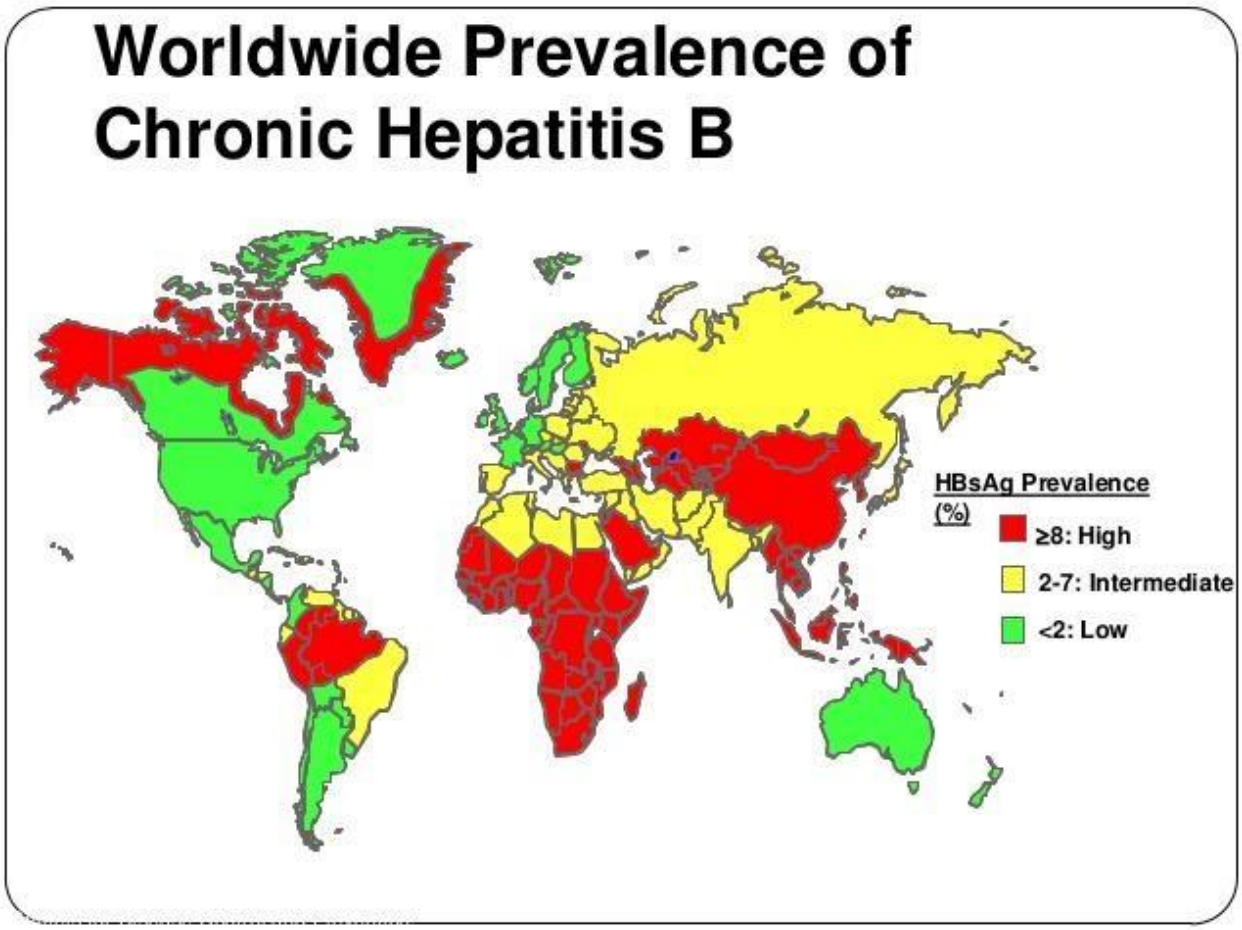


Fig. 4 Prevalence of HBV. (Source retrieved from Wikipedia March 2016)

The high rate of perinatal infection and high carrier rate is the main mode for sustaining the higher rate of prevalence in certain nations. In high endemic areas, most infections occur in early stages, particularly attained from the carrier mother at delivery. This result in the adult population having a carrier rate of 10%-20% with the rest of the population being immuned. In intermediate endemic

areas, infection is common in childhood as a result of horizontal transmission between children, particularly siblings and most infections occurs within the first 5 years of life (Elsheikh *et al.,* 2007; El-Magrahe *et al.,* 2010). The carrier rate in adult is 2%-10% with at least half or a quarter of the population being immuned. In low endemic regions, childhood infection is occasional with a rate of carriers at 0.1%-0.5% and thus most of the infection occurs in adolescent and young adults and the common mode of transmission being sexual followed by percutaneous transmission (Edmunds *et al.,* 1996).

In Africa, several transmission of hepatitis B virus happens before the five(5) years of age and it is mainly through medical procedures, close contact within households, traditional scarification and other unknown contrivances (Alter and Seeff, 2000). Africa has a lesser vertical transmission rate than Asia moderately as of lower of hepatitis B e antigen (HBeAg) prevalence in Africa, which is a foremost contributing factor of peri natal transmission (RoinGeard *et al.*, 1993).

2.1.5 Pathogenesis and Clinical presentation of HBV infection

HBV enters the bloodstream and targets the hepatocytes. The hepatocytes which are infected are distended and the cytoplasm assumes a ground appearance which is glassy. HBV is not cytopathic, and injury of the liver in HBV chronic infection is due to immunological response. Although the virus has a long incubation period (45-180 days), virus replication starts a few days after infection. The replicative process is through a reverse transcription, where the virus uncoats after absorption and the single stranded region of the genome is restored by DNA polymerase.

Hepatitis caused by virus can be acute; an unexpected sickness with a mild-to-severe course followed by comprehensive resolve. On the other hand, if the cell-mediated immune reaction

is feeble, the infection does not resolve and chronic hepatitis arises with an extended course of active disease or silent asymptomatic infection. The disease has diverse clinical sign and symptoms reliant on the individual's age infection, immune status and the phase at which the infection is detected.

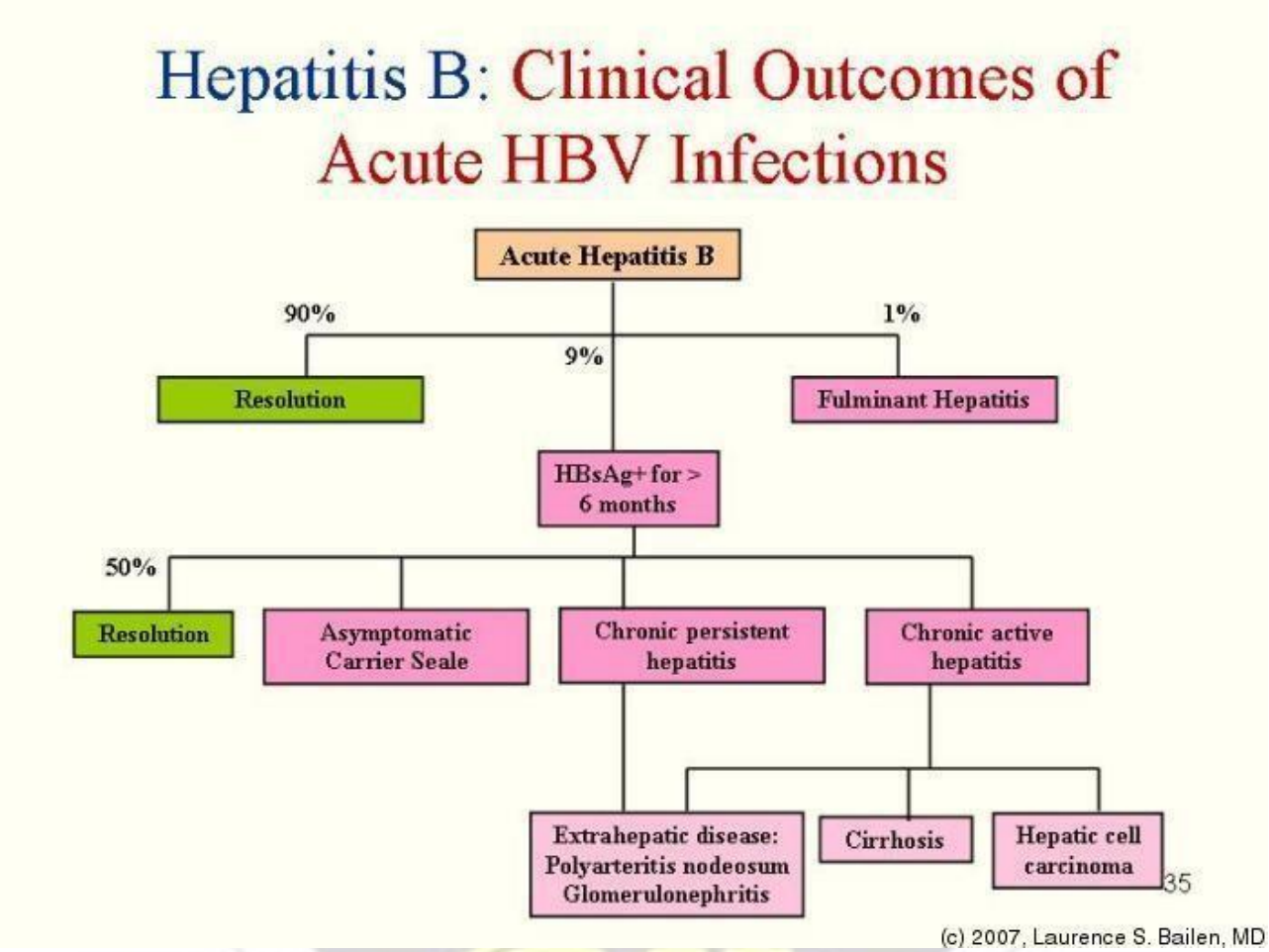


Fig 5 Clinical outcome of HBV infection. (source Laurence, 2007)

In acute infection of HBV there is a variable period of incubation, liable to the type of virus, amount of virus in the inoculum, the manner of transmission and host factors. It normally ranges between forty-five (45) and one hundred and twenty (120) days with a mean of 60-90 days (Robinson, 1995). The growth of the virus first results in systemic symptoms much like the low grade joint, runny nose, pharyngitis fatigue flu, muscle/joint aches and cough,(Ryder and Beckingham, 2001). One to two weeks later, the icteric phase of the disease begins with

the presence of urine which is dark followed by stools which are pale and the colour of mucous membranes, skin conjunctivae, and sclera become yellowish. The infected person might develop jaundice as the bilirubin level rises, which the liver normally clears it. There is a high level of HBV DNA. As there is growth of the virus in the cells of the liver, these hepatocytes die (necrose). During cell death enzymes are released by the hepatocytes. These are the liver function enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), and alkaline phosphatase (ALP). Raised levels of these liver enzymes in the blood will aid in the establishment of hepatitis (McMahon *et al.*, 1985; Hoofnagle *et al.*, 2007; Chu and Lee, 2008)

In about 2-3 weeks into the sickness, the infected person is frequently jaundiced, has an enlarged liver which is painful and raised blood concentration of liver function enzymes, AST and ALT whiles ALP , GGT and serum bilirubin is slightly elevated. There is a fluctuating level of HBV DNA and a pronounced hepatic necro-inflammation (Hadziyannis, 2006). About 4 - 12 weeks thereafter, the jaundice fades and the disease resolutions with the growth of natural protective anti bodies in around 95.0% of adults (Hollinger et al, 2001, Hollinger and Liang, 2001).

Although most adults recover completely from an acute hepatitis B infection, about 5%-10% of the virus perseveres in the human body. The figure is greater in children, about 70%-90% of newborns infected in their first few years in life and become chronic hepatitis B virus carriers (Robinson, 1995). Chronic hepatitis B is a persistent (> six (6) months) infection with tenacious levels of HBsAg and IgG anti HBcAg and the nonappearance of an antibody reaction. It is more problematic to diagnose because the infected individual is regularly asymptomatic with only an enlarged tender liver and mildly raised liver function enzyme levels whiles other may show signs of anxiety, anorexia, malaise and easy fatigability.

The clinical characteristic of chronic viral hepatitis varies broadly reflecting the interaction between numerous viral pathogens ant the patient's adaptive and innate antiviral immune reaction. Chronic hepatitis B virus infection can results to chronic hepatitis which leads to cirrhosis of the liver and finally HCC or liver failure. Some patients are asymptomatic carrier (antegenaemia) where the carrier individual on no occasion develops antibodies against HBsAg and keep the virus without injury of the liver. It is estimated that, there are about 200 million hepatitis B virus carriers globally whereas others are chronic-persistent patients with a low-grade symptoms of hepatitis. Other patients experience chronic active /aggressive hepatitis where the infected individual has an acute infection state that lingers without the normal resolution (last longer than 6 - 12 months). This leads to liver cirrhosis, hepatocellular carcinoma and finally death. Others experience severe acute hepatitis with speedy devastation of the liver which leads to sudden death (fulminant hepatitis) (Hoofnagle *et al.*, 2007; Lee *et al.*, 2008).

Hepatitis B virus infection in pregnant women is comparable to the overall adult populace and doesn't upsurge death rate nor does it produce teratogenic effect. Women infected with hepatitis B virus have an increased odds for complication during gravidity (Reddick *et al.*, 2011) and a high mother-to-child transmission causing foetal and neonatal hepatitis which can lead to serious effects on the neonate, leading to impaired physical and mental and physical health in future. Acute HBV infection in pregnancy leads to increased occurrence of low birth weight and infants prematurity, whereas chronic maternal HBV infection results in gestational diabetes mellitus, antepartum hemorrhage and preterm birth (Jonas, 2009). Further studies have shown that up to 90.0% of infected newborns in their first year of life and 30.0% - 50.0 % of infected children between one (1) to four (4) years advanced to chronic infections and approximately 25.0% of adults who were infected during child hood develop to chronic infection and die from hepatitis B virus –associated cirrhosis and cancer of the liver (WHO, 2002).

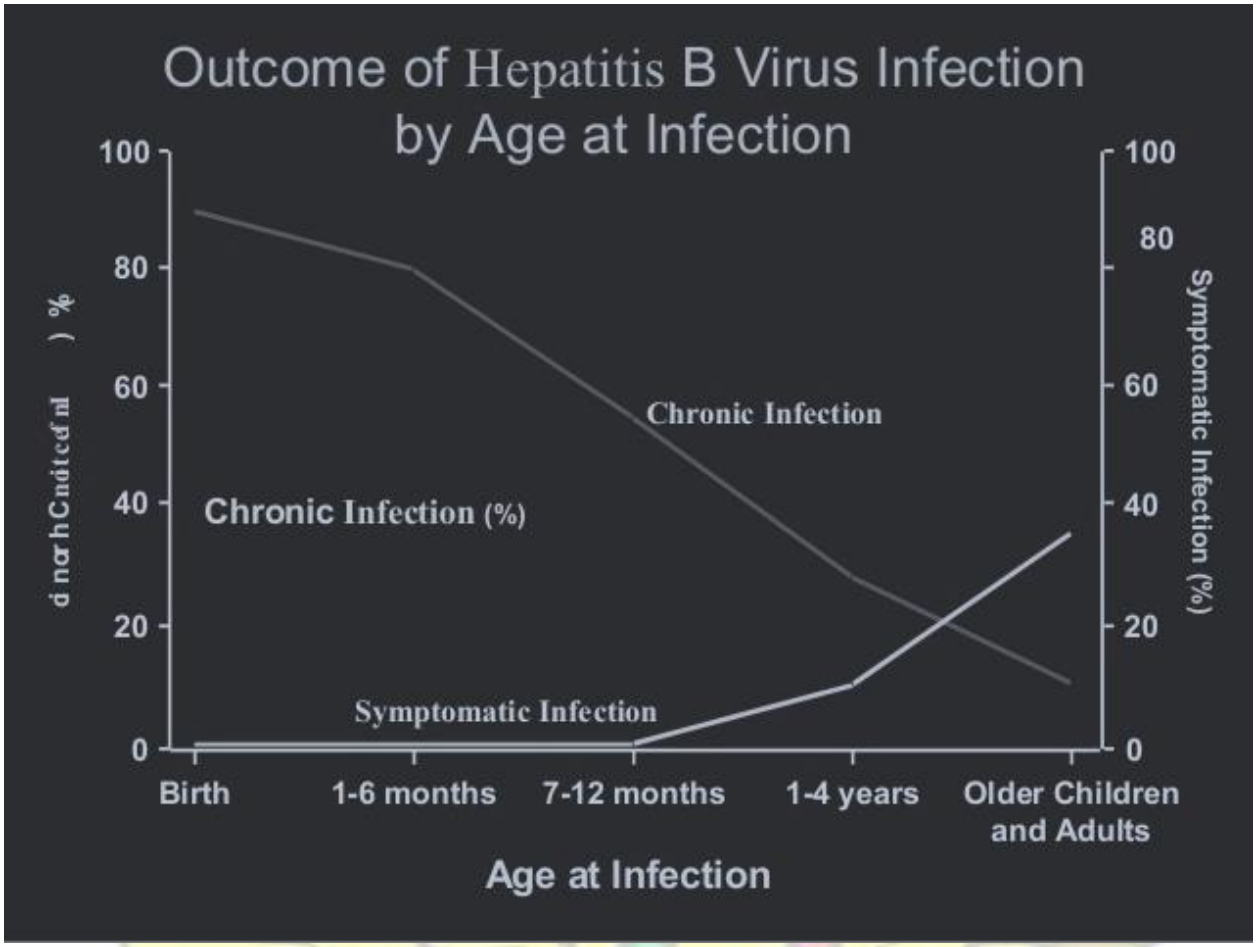


Fig. 6 Outcome of HBV infection by age at infection. (Source retrieved from Wikipedia March 2016)

Individuals who contract the disease at an older age, patients who misuse alcohol and individuals co-infected with HDV and HCV progress to cirrhosis more rapidly and have an accelerated liver disease progression and an increased risk of HCC (Chu and Lee, 2008; Jamma *et al.*, 2010).

2.1.6 Treatment and Prevention of HBV infection

About 90%-95% of grown-ups with acute hepatitis B virus infection resolves naturally and sero-convert to anti-HBs without antiviral therapy. However, there are anti-viral agents for the treatment of acute, chronic and persistent hepatitis B virus infection.

The antiviral medications for treating and managing chronic HBV infections includes PEG interferon alpha2a, interferon alpha 2b, three nucleoside analogues (lamivudine, adefovir and entecavir), telbivudine and tenofovir ((Tran, 2009).

Lamivudine suppresses HBV DNA to undetectable levels whiles interferon alpha suppresses hepatitis B virus DNA and HBeAg in about half of the managed individuals and prevents cirrhosis (Dienstag, 1983; Niederau *et al.*, 1996). The nucleoside or nucleotide analogues inhibit viral nucleocapsid formation and blocks viral DNA synthesis by premature chain termination (Perrillo, 2005; Wieland *et al.*, 2005) and are used for longer durations. Nevertheless, they inhibit with mitochondrial D NA replication and hence results in potential toxicity; effects which are poorly term in the growing fetus PEG-IFN is contraindicated in pregnancy hence, adefovir, lamivudine, entacavir, tenofovir and telbivudine are the drug of choice in pregnancy (Bzowej, 2010). Telbivudine, lamivudine or tenofovir might be used for the avoidance of perinatal and intra uterine HBV transmission in the last trimester of gravidness in HBsAg positive women with raised concentration of viremia (Xu *et al.*, 2009; Shi *et al.*, 2010; Han *et al.*, 2011).

Effective methods for preventing perinatal transmission includes

1. Screening pregnant women for HBV
2. Administering HBV vaccines to babies beginning at birth. The vaccine is a recombinant vaccine. The vaccine is given to infants at birth, 2, 4, and 15 months; it is also given as 3 injections to adolescents and high-risk adults (health care workers, IV drug users, etc.) (WHO, 2001; Jonas, 2009; Tran, 2009).
3. Administration of hepatitis B immune globulin (HBIG) at birth to babies born to infected mothers and administration of antivirals in late pregnancy to mothers with high viral load (Patton and Tran, 2014). It protects by passive immunization but it lasts only for 6 months though the protection is immediate.

2.1.7 Laboratory diagnosis of HBV infection

2.1.7.1 Serological detection

Diagnosis of HBV is mainly by serological means. It is established by rally of specific antigen and/or antibody in the sera. The diagnosis is founded principally on the identification of hepatitis B virus antigens (HBsAg), hepatitis B e antigens (HBeAg), anti-bodies counter to these antigens (HBsAb, HBcAb) and the manifestation of viral nucleic acid (HBV DNA) in the liver, blood , and additional sites of the hepatocytes (Datta *et al.*, 2014). Based on the existence or nonexistence of a mixture of antigens and antibodies, acute or chronic, recent or past infection can be diagnosed (Lok and McMahon, 2007).

The presence of serum HBsAg or detection of HBV DNA indicates an HBV infection. In acute infection, both HBsAg and IgM anti bodies to HBcAg maybe positive; however determination of hepatitis B virus DNA is the utmost dependable test if acute liver failure (ALF) is existing. The titers of HBsAg, HBeAg and HBV DNA are raised in the period of incubation but HBeAg and HBV DNA concentration start to reduce at the beginning of disease and disappears at the time of peak clinical disease (Ganem and Schneider, 2001). IgG antibodies to HBcAg develops after an acute infection. Those who clears the disease or achieve spontaneous resolution develops antibodies against the HBsAg which is associated with a long term immunity. The presence of anti HBsAb and anti HBcAb (IgG) shows immunity and recovery in a previous infection whereas, antibodies to only HBsAg will be produced in a successful vaccination. In chronic infection, HBsAg positivity will persist, in the serum for more than six months. The presence of HBeAg is the marker for high infectivity since there are large amount of HBsAg associated with infectious virus.

The diagnosis of hepatitis B virus infection is greatly dependable on the immune identification of HBsAg (Blumberg, 1997), however, there is the issue of non-identification because of low levels of antigen or diagnostic-escape mutations in the epitopes. In this instances, molecular based assays are of great importance.

2.1.7.2 Molecular Based technologies

A molecular method for the detection of HBV infections includes thermal cycling-based techniques for the amplification of HBV DNA. Nucleic acid based HBV DNA assays are more accurate in the quantification of HBV DNA levels and allow for assessment of replicative stage (Lok and McMahon, 2007). Assays for HBV DNA detection are categorized into two groups: Direct identification assays that uses probes to hybridize directly to the HBV DNA. They are simple but lacks sensitivity which can be increased by using another method of signal amplification. The other method is indirect detection and it involves *in vitro* amplification step to raise the amount of the target sequence, followed by identification of the amplified target. The techniques used includes Polymerase chain reaction (PCR), isothermal amplification-based methods eg nucleic acid sequence based-amplification (NASBA), transcription mediated amplification (TMA) etc. these procedures are more sensitive and reliable for the quantification of serum HBV DNA. The main problem with these techniques includes adulteration or false positive results which can be circumvented by the proper usage controls in the assay and adhering to specific precautions (Victor *et al.*, 1993).

2.2 Hepatitis C virus

2.2.1 Historical background

In the 1970'S, several cases of post transfusion hepatitis were associated to either Hepattis A virus or HBV infection and a novel infection entity named "non A, non B hepatitis (NANBH) was for the first period designated (Feinstone *et al.*, 1975). Blood-borne NANBH is a major

post transfusion hepatitis which regularly developed into liver cancer diseases such as cirrhosis HCC. Several years were devoted to detect the etiological agent of NANBH since the virus was unable to grow proficiently in cultured cells (Gupta *et al.*, 2014) and the agent was suggested to be a virus based on physiochemical properties (Bradley *et al.*, 1983; Bradley *et al.*, 1985). A breakthrough was made when workers at Chiron cloned cDNAs agreeing to portion of the virus genome isolated from chimpanzee that have been infected with the blood of an infected individual with post transfusion NANBH and which subsequently developed acute NANBH (Choo et al, 1989). The virus was identified as the causative agent of NANBH and named hepatitis C virus (Kuo *et al.*, 1989). The only reservoirs are human and chimpanzees.

2.2.2 Structure of Hepatitis C virus

The hepatitis C virus viron is made up of a single stranded positive RNA genome belonging to the Flaviviridae family, genus Hepacivirus (Simmonds *et al.*, 2005). It is confined in an icosahedral capsid, enclosed by a lipid bilayer within which two diverse glycoproteins are attached (Penin *et al.*, 2004).

The HCV viron is 55-65 nm in diameter and the genome contains a 9.6 kb positive strand RNA with three discrete zones. A short 5′ noncoding region (NCR) that contains two (2) domains, an internal ribosome entry site (IRES) and a stem loop structure involved in positive strand priming during hepatitis C copying. A long, unique open reading frame (ORF) that encodes the structural and non-structural proteins, and a short 3′ NCR principally involved in minus-strand priming during replication of hepatitis C virus (Penin *et al.*, 2004). The predecessor is sliced into at least ten (10) diverse proteins: the structural proteins, which form the viral article, include the core protein and the envelope glycoproteins E 1, E 2 and E7. The non-structural proteins include the p7 viro-porin, the NS2 protease, the NS3, NS4A complex harboring protease and NTPase/RNA helicase activities, the

NS4B and NS5A proteins, and the NS5B (Gupta *et al.*, 2014). HCV has six genotypes (Ferrari *et al.*, 1990) (Feinstone *et al.*, 1975; Alter, 2007) with multiple sub-types. Genotyping is the main instrument for evaluating the infection course and assessing duration of treatment and response (Amarapurkar *et al.*, 2000).

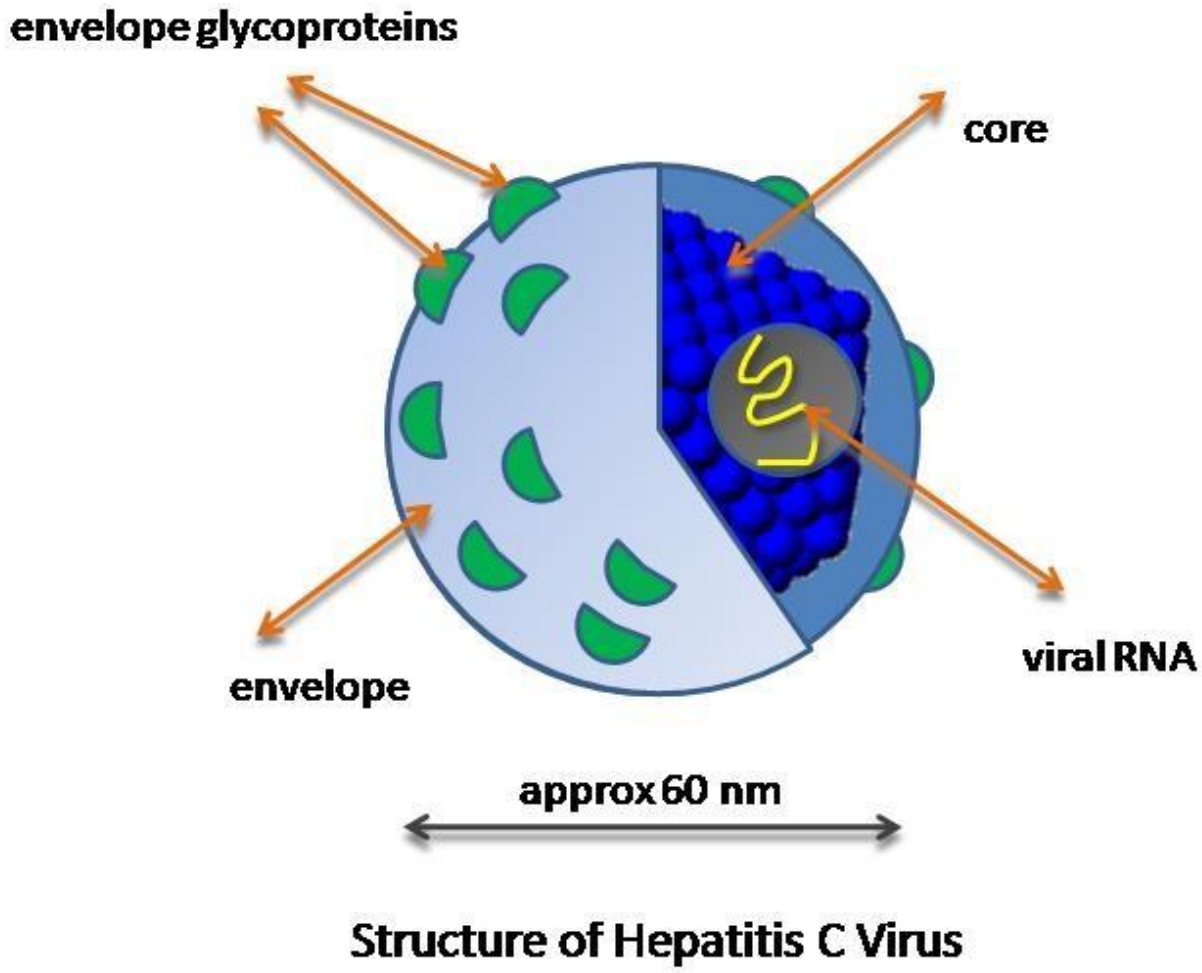


Fig 7 Structure of Hepatitis C virus. (https://upload.wikipedia.org)

2.2.3 The immune system and HCV

During HCV infection, the virus replicates within the host with an ongoing genetic mutation. The mutants are different from each other and thus when the immune system eradicates one form the other mutants continue to cause ongoing disease. As a result, humoral immune response have little effect on viral clearance (Bartosch *et al.*, 2003; Netski *et al.*, 2005). In the second and third trimester, there is an increase in HCV RNA due to production of oestrogen which suppress the intrathymic T cell differentiation and whiles activating the extrathymic pathways.

Cellular immune responses have a significant part in determining the result of acute HCV infection. CD4 and CD8 cellular response mediated by a type 1 T helper cell (Th 1)

lymphocytic response is associated with cytokine profile leading to viral clearance (Thimme *et al.*, 2001; Bowen and Walker, 2005). Spontaneous virus clearance is higher in symptomatic acute HCV infection (Meigs *et al.*, 2001).

2.2.4 Epidemiology of HCV infection

Drug injection usage is the main route of transmitting hepatitis C virus accounting for about 2 /3 of infections in the United States and Western Europe and about 80.0% in Australia (Dore *et al.*, 2003; Shepard *et al.*, 2005). HCV infection is also transmitted less commonly by accidental injuries with needles or sharps (with an incidence of 1.8% after a needle stick injury), unsafe medical or surgical procedures, and perinatally (from mother to child at birth which results in an incidence of 6% of new borns to infected mothers) (Shalmani *et al.*, 2014). Transfusions with infected blood products or transplant from infected donors also transmit the infection (Dhingra, 2002). Transmission through sexual intercourse with an infected person also occurs but the risk increases when one has multiple sex partners. Male to female transmission is more efficient than male to male. Parenteral transmission through tattooing or acupuncture with unsafe materials, body piercing and promiscuous male homosexual activity is associated with hepatitis C virus infection (van de Laar *et al.*, 2010).

The World Health Organization (WHO) has estimated that about 150-300 million people (3.0%) of the world populace have had hepatitis C virus infections (Schiff, 2002) and about 50.0% of all the cases develops to be chronic carriers and are at liver cirrhosis and liver cancer risk (WHO, 2000). High prevalence of > 2.9% are recorded in African and Asian countries, whiles developed countries like Western and northern Europe, North America and Australia have a low occurrence rate (<1.0%) (Alter, 2007; Esteban *et al.*, 2008). Egypt has the highest

worldwide prevalence of 22% countrywide (Kamal et al, 2008) whiles the bottommost occurrence (0.01% - 0.1%) is reported in the United Kingdom and Scandinavia.

More than one million new cases are recorded yearly , the higher proportion happening in Africa (Shepard *et al.*, 2005).

The prevalence of HCV infections varies from one geographical area to another. Different countries belonging to different parts of the world has comparable prevalence of hepatitis C virus infection but there are diverse outlines of age-specific rate of prevalence. In a country like United States, the occurrence is higher amongst the age group of 30-49 years whiles it is lower in the ages lesser than 20 years and higher than 50 years of age (Armstrong *et al*., 2006). A similar pattern is observed in Australia where HCV infection occurs mostly among young adults with variations occurring amongst individuals with dissimilar infection risk factors (Law *et al*., 2003). In contrast, the age-specific prevalence of HCV infections increases with age in countries like Spain, China and Italy. In these countries, most infections occur in persons 50 years and above suggestive of a risk of HCV infection in the past ie some years previously (Domínguez *et al.*, 2001; Sun *et al.*, 2001; Sagnelli *et al.*, 2005).

In Africa, central Africa has the highest prevalence of HCV with rates impending greater than 13.8% of the whole populace whereas, the prevalence among pregnant women is 4.3% (Madhava *et al*., 2002; Ugbebor *et al.*, 2011). The risk factors which were identified includes infected blood and products, intravenous drug abuse, unsterile medical and dental procedures and the use of procedures where blood exposure is involved (Madhava *et al*., 2002; Alter, 2007). Alter (2007) classified eastern region of Africa as having high to intermediate prevalence of HCV with rates ranging from 2-2.9% whiles (Modi and Feld, 2006) classified it as a low endemic area with rates 0-2% though studies in the area have been scarce.

2.2.5 Pathogenesis and clinical manifestation

Hepatitis C virus has a lengthy incubation period between the beginning of infection and clinical expression of liver disease but the virus is detected 1-3 weeks after infection. The virus enters the bloodstream and infects the hepatocytes (liver cells), undergoes replication simultaneously but does not kill the host cells and thus set up a persistent infection leading to chronic disease. It can present as an acute or a chronic hepatitis.

Studies have shown that acute HCV infections are usually asymptomatic in about 70%-80% of patients and hence infrequently diagnosed (McCaughan *et al.*, 1992). About 20-30% of adults who develop acute hepatitis C infections develop clinical sign and symptoms with the start ranging from 3-12 weeks after introduction of the virus after a prodromal phase of 6-7 weeks (Alter and Seeff, 2000; Thimme *et al.*, 2001). Symptoms may include weakness, anorexia, malaise, fatigue, weight loss, joint pains, flu-like symptoms and jaundice. At about 2-8 weeks of exposure, serum alanine aminotransferase (ALT) concentration rise indicating cells of the liver are dying (necrosis). The rise normally reaches levels more than 10 times the upper limit of normal values (Thimme *et al.*, 2001). Within 1-2 weeks after contact, HCV RNA can be identified in the serum. The HCV RNA level increases quickly in the first few weeks and then mounts between 1000000 to 100000000 IU/ml, shortly before the peak of ALT levels and onset of symptoms. Acute HCV infection can be severe, but fulminant liver failure is rare. In self-limited acute hepatitis C, signs and symptoms can persist for numerous weeks and diminish as ALT and HCV RNA levels wane (Farci *et al.*, 1996).

Antibody to HCV test positive near the start of signs roughly 1-3 months after contact and up to 30.0% of individuals will test negative for anti HCV at the start of their signs (Farci *et al.*, 1991).

Chronic hepatitis C is noticeable by the perseverance of HCV RNA in the blood for at least 6 months after the start of acute infection. HCV is self-limiting in about 15%-25% of patients, whereas about 75%-85% of patients who are infected do not get rid of the virus by six (6) months, leading to persistent liver infection and then chronic active hepatitis develops. During the chronic infection period, HCV can give rise to extra-hepatic manifestations including lethargy, muscle and joint pain, dark urine, jaundice (yellowing colour of the eyes and skin) (appears only after other symptoms have started to go away) and clay-coloured bowel movement.

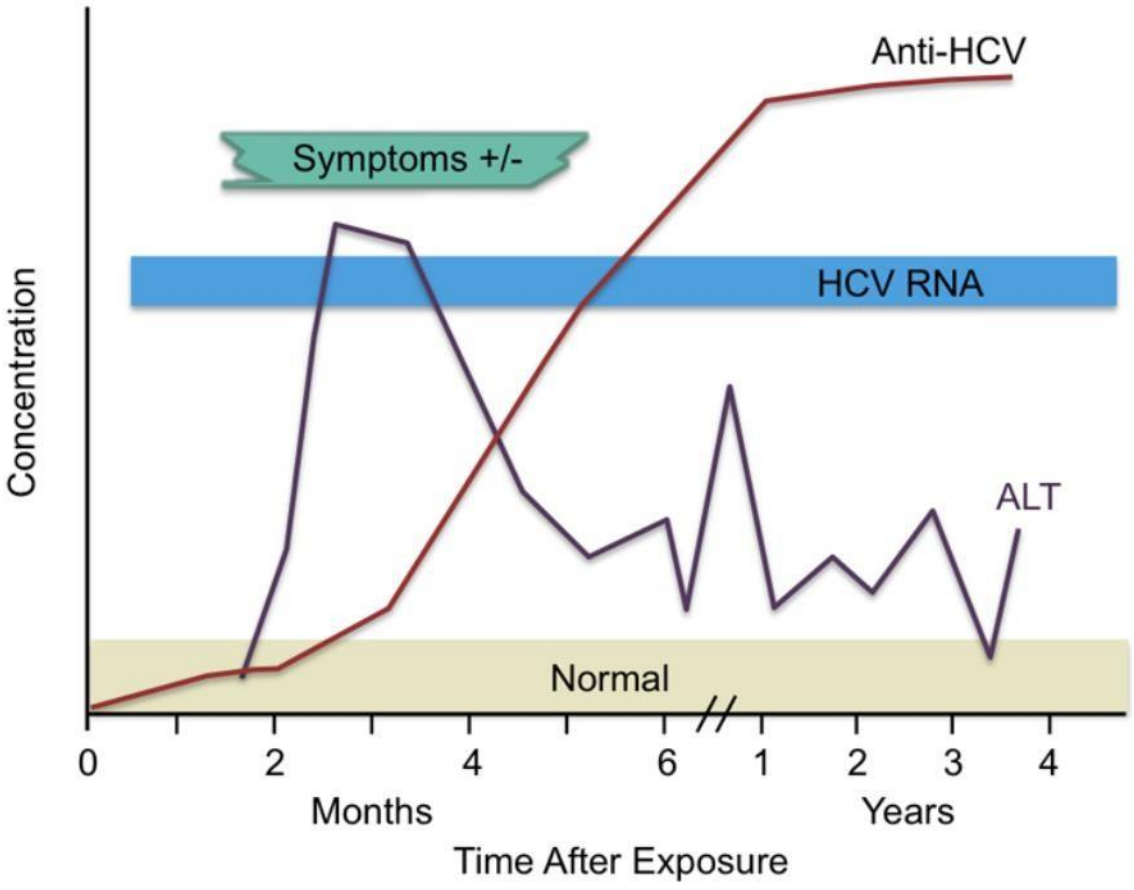


Fig. 8 Laboratory marker with acute Hepatitis C infection. (source CDC, 2012)

In pregnant women, chronic active hepatitis C infection is associated with preterm delivery and intra uterine growth hindrance (Zanetti *et al.*, 1999) and vertical transmission occurs in about 3%-10% of gravidities complicated by HCV infection (Berkley *et al.*, 2008).

2.2.6 Treatment and Prevention of HCV infection

Studies have shown that about 50%-80% of people who develop acute hepatitis C remains HCV infected and there is spontaneous resolution among infected infants and young females than among individuals who are older when they develop acute hepatitis (Seeff and Hoofnagle, 2002; Thomas and Seeff, 2005).

The present normal management for chronic hepatitis C is the mixture of pegylated interferon (IFN) alpha and ribavirin for a period of 12 months before the development of cirrhosis.

Only patients with visible HCV RNA are well-thought-out for pegylated IFN alpha and ribavirin mixture therapy. This choice was based on superiority of this combination treatment over standard interferon alpha and ribavirin. The HCV genotype is assessed before treatment, as it determines the sign, the treatment duration, the ribavirin dose and the virological monitoring procedure (Fried *et al.*, 2002; Hadziyannis, 2006).

To reduce the risk of infection, depends on measures including education, screening of blood products, condom provision, alcohol abstinence and programs to reduce vertical transmission (Zeba *et al.*, 2011). There is no vaccination for the prevention of HCV infection. The infection can be prevented by avoiding contact with infected blood, avoid sharing of razor blades, toothbrushes, shavers and needles (CDC, 1998b; Wiley *et al.*, 1998). Avoid the intake of alcohol because the combination with HCV infection accelerates the progression to cirrhosis and increased risk of liver cancer (Wiley *et al.*, 1998).

2.2.7 Laboratory diagnosis of HCV infection

There are two main methods of diagnosing and managing HCV infections: serological assay (indirect test) that detects specific antibodies to hepatitis C virus (anti HCV) and molecular assay (direct test) that can identify, measure or describe the components of HCV particles such

as HCV RNA and core antigen. Both tests play an important part in infection diagnosis, therapeutic decision making and assessment of virological response to therapy.

2.2.7.1 Serological assay

This is an indirect test which detects anti-HCV and it is used both to screen for and diagnose HCV infections. Three generations of ELISA have been developed for the serological detection of anti-HCV but the third generation assays are mainly used. The detection is built on the usage of a third generation enzyme immuno-assays (EIAs), detecting antibodies directed against various HCV epitopes. The specificity of third-generation EIAs for anti HCV is higher than 99% but their sensitivity is more problematic to assess, due to nonexistence of gold standard method (Colin *et al.*, 2001). It is associated with a 30%-50% rate false positive if used in a lower occurrence populace (CDC, 2003). However, all these assays had the disadvantage of giving a high false positive results and lack of sensitivity to detect antibodies during the window period. Moreover, they cannot distinguish between acute, past and chronic infections (Colin *et al.*, 2001).

A fourth generation has been developed but literature backing up the addition of these assays as fourth-generation on the basis of better sensitivity and specificity is inadequate (Colin *et al.*, 2001). Recombinant-immunoblot assay (RIBA) was developed as a auxiliary test to confirm serological reactivity by ELISA but no longer in use because of molecular test (Dow *et al.*, 1996).

2.2.7.2 Molecular Assay

Molecular techniques are important in the diagnosis and monitoring of treatment for HCV. It is among the principal pathogens to be recognized by purely molecular techniques since it was difficult to cultivate the virus in cell culture (Gupta *et al.*, 2014). Nucleic acid test (NAT) is regarded the ‘gold standard’ for the detection of active HCV replication and for diagnosing

acute HCV infection, since RNA is noticeable as early as one week after exposure and 4-6 weeks before sero-conversion (Glynn *et al.*, 2005; Kamal and Nasser, 2008). NATs used for the detection of HCV RNA are based on polymerase chain reaction (PCR), branched DNA signal amplification and transcription mediated-amplification. The molecular assays are direct test and includes qualitative assay (for detection) and quantitative assay (for quantification) of HCV RNA.

The qualitative NAT are normally considered as confirmatory test for HCV diagnosis and they use conventional RT-PCR or transcription-mediated amplification (TMA). It is used to confirm lower concentration viremia in individuals with reactive anti HCV outcomes and to screen blood donation for proof of HCV infections (Fontana *et al.*, 2000; Scott and Gretch, 2007). Qualitative tests are of limited importance now due to the availability of more sensitive quantitative PCR methods.

Quantitative assays has replaced qualitative assays because of its good specificity (98-99%) and sensitivity (99%) (Ghany *et al.*, 2009). Due to the high sensitivity, this method is used effectively for checking response to therapy during antiviral management of HCV infected individuals.

There is also an assay for HCV genotyping which is based on direct sequencing, reversehybridization to genotype-specific oligonucleotide probes and restriction fragment length polymorphism analysis (Pawlotsky, 2003). The HCV genotyping is of clinical importance since infection by certain genotypes is related with disparity results of treatment (Pawlotsky, 2003).

CHAPTHER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY SITES AND SAMPLING STRATEGY

3.1.1 Study Site

The study was conducted in the Antenatal clinic of the Catholic Hospital, Battor in North

Tongu district of the Volta region, Ghana and the Department of Clinical Microbiology, KNUST, Kumasi. The samples were collected at the clinic, frozen at -20°C and transported to the Department of Clinical Microbiology Virus Research Laboratory, KNUST for analysis.

3.1.2 Sampling Period

The study took place from December 2015 to February 2016. Ethical approval was obtained from the Committee on Human Research, Publications and Ethics (C HRPE), School of Medical Sciences, KNUST and Catholic Hospital, Battor.

3.1.3 Study Population

The study population was made up of women who are pregnant and visiting the antenatal clinic for their routine laboratory screening and examination between the ages of 18 years to 50 years.

3.2 Eligibility Criteria

3.2.1 Inclusion criteria

All pregnant women visiting the antenatal clinic were educated on the risk factors associated with HBV and HCV infections. Those who gave their consent were included in the study. For those who were not literate, consent was obtained by interpretation in their local dialect.

3.2.2 Exclusion Criteria

1. Pregnant women with known HBV and HCV status or previous liver conditions were excluded

2. Antenatal respondents reporting for repeat visit during the study period

3.3 Sample size

One hundred and thirty five (135) blood samples were collected during the sampling period for the cross-sectional study. This number was generated based on the previous prevalence rate of 9.5% reported by Ephraim et al, 2015 using the formula:

$N= [Z^2 (P) (1-P)] / (Error)^2$

Where N= Sample size,

Z= A constant of 1.96,

Error= 5% (0.05),

P= Previous prevalence rate

$N= [1.96^2(0.095) (1-0.095)]/ (0.05)^2$

N= [3.8416(0.095) (0.905)]/0.0025

N= [3.8416(0.085975)]/0.0025

N=0.33028/0.0025

N=132.11

3.4 Contact Process

Pregnant women attending antenatal clinic at Catholic Hospital, Battor were approached and the rational of the study explained to them. Signed informed consent was obtained from willing participants. Their socio-demographic data, knowledge about HBV and HCV infections and factors associated to it were obtained through structured questionnaire (Appendix 1).

3.5 Sampling procedure

Four milliliters (4mls) of the participant's venous blood sample were collected into a BD Vacutainer with SST II Advance Semi-separator gel (BD, Belliver Industrial Estate, Plymouth, PL6 7BP, United Kingdom) using a 5mls sterile hypodermic syringe. The blood samples were allowed to clot for 10 to 15 minutes and then centrifuged at 3500 rpm for 5 to 10 minutes. The sera were then aliquot into cryo tubes and stored at -20°C at the Catholic Hospital, Battor laboratory. The sera were then transported to the Virus Research laboratory of the department of Clinical Microbiology, School of Medical Sciences, KNUST where the sera were screened for HBsAg and anti HCV using commercially obtained ELISA kit from Human Diagnostics Worldwide, Germany.

3.6.0 Reagent preparation and laboratory testing of patients serum samples

3.6.1 Preparation of reagents and washing solution

All reagents were brought to room temperature (25°C) before use according to the manufacturer's instruction. The washing solution was prepared by diluting 1 part of the washing solution (eg 50 mls) to 19 parts of deionized water (eg 950 mls) for Hepatitis C and 1 part of washing solution (eg 50 mls) to 20 parts of deionized water (eg 1000 mls) for Hepatitis B and anti-HBc.

3.7 ELISA analyses of samples (HCV)

3.7.1 Sample Preparation

The subjects' sera, positive control and negative control were diluted in a ratio of 1 in 20 with sample diluent. 1 part (10µl) of serum was diluted with 20 parts (200µl) of the sample diluent and shook for 15 seconds prior to further processing.

The microtitre plates were arranged according to the number of samples. The ninety-six well microtitre plates were labeled, one blank (A1), two negative control (B1, C1) and three positive control (D1, E1, F1). 100µl of Positive control, negative control and diluted samples were added into their respective wells except the blank and mixed gently by tapping the plate for 15 seconds. The plate was covered and incubated for 60 minutes at 37°C. At the end of incubation, each well was washed 8 times with 350µl of diluted washing buffer allowing the microtitre to soak for 30-60 seconds. After the final washing cycle, the plate was turned onto a blotting paper to remove any remainders. 100µl of working-conjugate solution was added into each well except the blank and mixed gently by tapping the plate for 15 seconds. The plate was covered and incubated for 30 minutes at 37°C. At the end of incubation each well was washed 8 times with diluted washing buffer allowing each well to soak for 30-60 seconds, blotting the plate on a paper after the final wash. 50µl of substrate reagent A and 50µl of substrate reagent B solution was added into each of the wells including the Blank and plate incubated at 17-25°C for 30 minutes avoiding light. 100µl stop solution was added into each well and mixed gently. The plate reader was calibrated with the blank well and the absorbance read at 450nm as soon as possible or within 15 minutes after terminating the reaction using a reference wavelength of 630-690nm. The cut-off value was calculated and the results evaluated.

The principle underlining the ELISA test can be found in Appendix 3.

Calculation of Cut-off Value

The mean absorbance value for the two negative control were calculated as MNC=

(B1+C1)/2

The mean absorbance value for the three positive control were calculated as

MPC= (D1+E1+F1)/3

The cut-off Value was calculated as

Cut-off Value= (0.25*MPC) +MNC

Cut-off index S/Co = Absorbance of specimen/COV

3.8 ELISA analyses of samples (HBsAg)

The microtiter plates were arranged according to the number of samples. The ninety-six well microliter plates were labeled, one blank (A1), three negative control (B1, C1, D1) and two positive control (E1, F1). 50µl of enzyme-conjugate reagent was added to each well except the Blank. 50µl of Positive control, negative control and specimen were added into their respective wells except the blank and mixed gently by tapping the plate for 20 seconds. The plate was covered with an adhesive film or foil and incubated for 60 minutes at 37°C. At the end of incubation, each well was washed six (6) times with diluted washing buffer allowing the microtitre to soak for 30 seconds. After the final washing cycle, the plate was turned onto a blotting paper to remove any remainders. 100µl of substrate working solution (50µl substrate reagent A and 50µl substrate reagent B) was added into each well including the blank and mixed gently by tapping the plate. The plate was covered and incubated for 30 minutes at room temperature (15-25°C) in the dark. 100µl stop solution was added into each well and mixed gently. The plate reader was calibrated with the blank well and the absorbance read at 450nm. The absorbance was read within 30 minutes after stopping the reaction using a reference wavelength of 630-690 nm. The cut-off value was calculated and the results evaluated.

The principle underlining the ELISA test can be found in Appendix 3.

Calculation of Cut-off Value

The mean absorbance value for the three negative control were calculated as

MNC= (B1+C1+D1)/3

The mean absorbance value for the two positive control were calculated as

MPC= (E1+F1)/2

The cut-off Value was calculated as

Cut-off Value=MNC+0.025

3.9 ELISA analyses of samples (anti-HBc)

The microtiter plates were arranged according to the number of samples. The ninety-six well microliter plates were labeled, one blank (A1), three negative control (B1, C1, D1) and two positive control (E1, F1). 50µl of enzyme-conjugate reagent was added to each well except the Blank. 50µl of Positive control, negative control and specimen were added into their respective wells except the blank and mixed gently by tapping the plate for 20 seconds. The plate was covered with an adhesive film or foil and incubated for 60 minutes at 37°C. At the end of incubation, each well was washed six (6) times with diluted washing buffer allowing the microtitre to soak for 30 seconds. After the final washing cycle, the plate was turned onto a blotting paper to remove any remainders. 100µl of substrate working solution (50µl substrate reagent A and 50µl substrate reagent B) was added into each well including the blank and mixed gently by tapping the plate. The plate was covered and incubated for 30 minutes at room temperature (15-25°C). 100µl stop solution was added into each well and mixed gently. The plate reader was calibrated with the blank well and the absorbance read at 450nm. The absorbance was read within 30 minutes after stopping the reaction using a reference wavelength of 630-690nm. The cut-off value was calculated and the results evaluated.

The principle underlining the ELISA test can be found in Appendix 3.

Calculation of Cut-off Value

The mean absorbance value for the three negative control were calculated as

MNC= (B1+C1+D1)/3

The mean absorbance value for the two positive control were calculated as

MPC= (E1+F1)/2

The cut-off Value was calculated as

Cut-off Value= (0.4*MNC) + (0.6*MPC)

Cot-off index S/Co=absorbance of specimen/COV

3.10 Validation of assay and interpretation of results

Samples of patients that gave absorbance less than the cut-off value were considered negative for the assay whiles patients with results equal to or greater than the cut-off value were considered reactive.

3.11 Measure of infectivity using One Step HBV (5 in 1) Test

The level of infectivity was measured using a One Step HBV (5 in 1) test cassette from Blue Cross Bio-Medical (Beijing) Co., Ltd. It is a quantitative immunoassay for the determination of hepatitis B markers (HBsAg, HBsAb, HBeAg, HBeAb, HBcAb) in human serum to aid in the assessment of hepatitis B infection. The test strip was removed from the pouch and 2-3 drops of the sample was added to each of the sample well on the cassette. The results were read after 15 minutes. A pink colored control (C) band and a distinct pink

colored band in the test (T) region is positive for HBsAg, HBsAb, HBeAg whilst negative for HBeAb and HBcAb. Only one colored band in the control (C) region with no band in the test (T) region is Negative for HBsAg, HBsAb, HBeAg whilst positive for HBeAb and HBcAb. The results is invalid when no band appears in either regions or only one color band appears in the test region.

3.12 Analysis of results

The results were statistically analysed using the Stata Statistical package. Significant differences between the proportions and the groups or variables were determined using binary regression. P-values of 0.05 were considered significant.

CHAPTHER FOUR

RESULTS

4.1 Socio-demographic characteristic of pregnant women

A total of 135 pregnant women were recruited in the study. They were orally interviewed and blood samples taken from them at the Catholic Hospital, Battor to determine the seroprevalence of Hepatitis B and Hepatitis C infection. The ages of the participants enrolled were between 18 years to 45 years and the mean age was 28.63 years with majority of the women 46.7% falling in the age category 21-30 years. Thirty seven (37) out of the 135 participating pregnant women

representing 27.4% had no formal education while 98(72.6%) had at least primary education. Majority of the women were married 97 (71.9%) and 18(13.3%) were cohabiting whiles 20 (14.8%) were single. Among the 135 participants, 49 (36.2%) were traders, 8 (5.9%) were students, 26 (19.3%) were involved in different kinds of vocational jobs and 11 (8.1%) were public servants. Sixty two (46%) of the subjects were primigravida with 73 (54%) being multigravida. A total of 53 (39.2%) have had 2 or more sexual partners before the current partner, 47(34.8%) have had one previous sexual partner and 35 (25.9%) never had any previous sexual partner. Those in their second trimester of pregnancy recorded the highest number 75(55.5%) followed by those in the first trimester with 43(31.9%) with the third trimester recording the least number 17 (12.6%). This is represented in Table 1.

Table 1: Socio-Demographic Characteristics of Pregnant Women

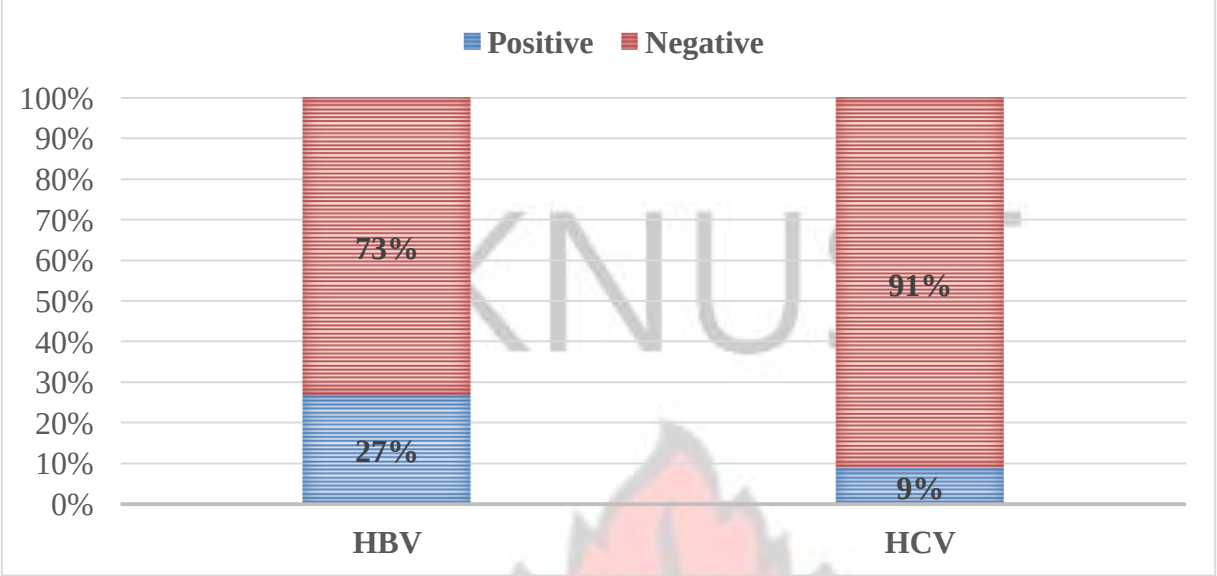
1. Age group	**Frequency**	**(%)**
< 20	19	14.1 46.7
21 -30	63	38.5
31 – 40	52	0.7
41 – 50	1	**100**
Total	**135**	
2. Marital Status		
Single	20	14.8 13.3
Co-habiting	18	71.9
Married	97	**100**
Total	**135**	
3. Education background		

None	37	27.4
Basic	71	52.6 8.9
Secondary	12	11.1
Tertiary	15	**100**
Total	**135**	
4. Occupation		
Unemployed	19	14.1 19.3
Vocational Jobs	26	16.3
Farming	22	8.1
Public Servants	11 8	5.9
Students	49	36.3
Trading	**135**	**100**
Total		
5. Parity		
Nullparity	30	22.2 23.7
Primiparity	32	54.1
Multiparity	73	**100**
Total	**135**	
6. Gestational age		
1st Trimester	43	31.9 55.5
2nd Trimester	75	12.6
3rd Trimester	17	**100**
Total	**135**	
7. Previous sexual partners		
None	35	25.9 34.8
Single	47	39.3
Multiple (2 or more)	53	**100**
Total	**135**	

4.2 Overall seroprevalence of HBV and HCV

In this study, 49 pregnant women (36.2%) out of the 135 examined had serological evidence of infection with viral hepatitis. Of these, 37/135(27.4%) tested positive for HBV and 12/135(8.8%) tested positive for HCV antibody however, none of the pregnant women was co-infected with both HBV and HCV as illustrated in Fig. 1.

Fig. 1 Prevalence of HBV and HCV



4.3 Prevalence and age distribution of respondents

The prevalence of HBV infection showed that women between the ages of 41-50 years had less infection (5.4%) with P=0.361 whilst HCV infection was low in women in the ages 1820 years and 41-45 years (8.33%) with P=0.152 and 0.859 respectively. Respondents in the age groups 21-30 years, had the highest prevalence of Hepatitis B infection, 62.2% and Hepatitis C infection 50%. The prevalence among these age groups was significant having a P-value of (P=0.015 for HBV and P=0.014 for HCV) (Table 2).

Table 2: Prevalence of Hepatitis B & C in relation to the age groups of respondents using binary logistic regression

	HCV infection among pregnant women (N=135) (n=12)				HBV infection among pregnant Women (N=135) (n=37)			
Age groups	POS(+)	Odds ratio	CI 95%	P-value	POS(+)	Odds ratio	CI 95%	P-value
18 – 20	1(8.33%)	1.045	0.99-1.145	**0.152**	5(13.5%)	0.982	0.785-1.253	**0.183**
21 -30	6(50%)	1.025	0.98-1.521	**0.014**	24(64.8%)	1.012	0.981-1.325	**0.015**
31 – 40	4(33.33%)	1.036	0.98-1.47	**0.142**	7(18.9%)	1.240	0.841-1.532	**0.215**
41 – 45	1(8.33%)	1.014	0.94-1.531	**0.859**	1(2.7%)	2.154	1.560-3.152	**0.361**

4.4 Educational background against HBV and HCV prevalence

None of the respondents in this study with secondary or tertiary educational level had HCV infection whiles pregnant women with tertiary education had the lowest infection with HBV (10.8%) and P=0.08. Pregnant women with basic educational level had the highest infection with both HBV and HCV (48.7% and 66.7%) though the values were insignificant (P=0.687 and 0.824). This was followed by those without any formal education, having HBV prevalence of 21.6% and P=0.896 and HCV prevalence of 33.3% and P=0.991 (Table 3).

Table 3: Educational background against prevalence of Hepatitis B&C

Educational background	HCV Positive among pregnant women				HBV Positive among pregnant Women			
	(N=135) (n=12)				(N=135) (n=37)			
	POS(+)	Odds ratio	CI 95%	P-value	POS(+)	Odds ratio	CI 95%	P-value
None	4(33.3%)	1.420	0.984-2.153	**0.991**	8(21.6%)	1.091	0.891-1.520	**0.896**
Basic	8(66.7%)	1.315	0.534-1.789	**0.987**	18(48.7%)	1.418	0.851-2.251	**0.687**
SHS	0%	1.00		**1.00**	7(18.9%)	2.750	1.425-3.152	**0.170**
Tertiary	0%	1.00		**1.00**	4(10.8%)	1.254	0.984-2.542	**0.08**

4.5 Gestational age and occupation against the prevalence of HBV and HCV

Pregnant women in their second trimester had the highest percentage (43.2% and 92.7%) for HBV and HCV infections respectively. This was followed by pregnant women in the first trimester who had 40.5% and 8.3% for HBV and HCV infection respectively. However, there was no significant difference (>0.05) between the various trimesters. None of those in the third trimester was infected with HCV but 16.2% had HBV infection (Table 4).

With regards to occupation, the highest frequency (51.4% and 33.3%) of HBV and HCV infection was observed among women in the trading occupation whiles students and public servants had the least infection (5.4%) for HBV and no HCV infections. However, no significant association was observed between seropositivity and occupation of the study subjects (p=0.318 and 0.719) for HBV and HCV (Table 4).

Table 4: Gestational age and occupation against the prevalence of HBV and HCV

	HCV Positive among pregnant women (N=135) (n=12)				HBV Positive among pregnant Women (N=135) (n=37)			
	POS(+)	Odds ratio	CI 95%	P-value	POS(+)	Odds ratio	CI 95%	P-value
Gestational Age 1st Trimester	1(8.3%)	0.739	0.286-1.892	**0.528**	15(40.5%)	1.196	0.651-2.197	**0.564**
2nd Trimester	11(92.7%)				16(43.2%)			
3rd Trimester	0				6(16.2%)			
Occupation								
Unemployed	3(25%)				5(13.5%)			
Vocational	3(25%) 0				6(16.2%)			
Public Servant		0.719	0.675-2.554	**0.876**	2(5.4%)	1.108	1.214-2.432	**0.318**
Farming	2(16.7%)				3(8.1%)			
Student	0				2(5.4%)			
Trading	4(33.3%)				19(51.4%)			

4.6 Associated risk factors of respondents against prevalence of HBV and HCV Table 5 illustrates the prevalence of HBV and HCV according to some associated risk factors. According to the table, pregnant women with multiple sexual partners' showed a significant association with HBV seropositive, 28 (75.5%) out of 37 with a P=0.003). The other risk factors such as marital status 24(64.9%), sexual protection 31(83.8%), blood transfusion 28(75.7%), tattoo 3(8.3%), alcohol intake 6(16.6%) and parity 8(21.6%) did not show any association with HBV infection. However, pregnant women who had blood transfusion 6(50%) showed a significant association with HCV seropositive (P=0.005) whiles risk factors such as marital status 9(75%), sexual protection 11(91.7%), multiple sexual partners 4(33.4%), tattoo 2(16.7%), alcohol intake 3(25%) and parity 3(25%) did not show any association with HCV infection.

Table 5: Prevalence of HBV& HCV according to associated risk factors

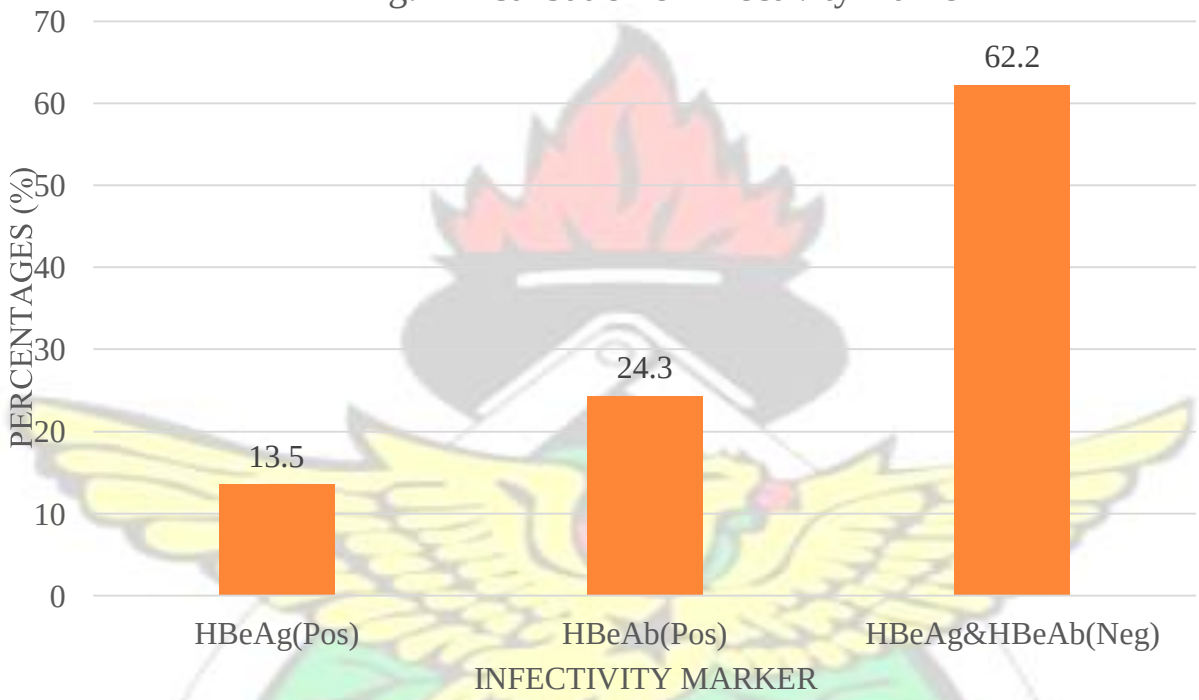
Variable(s)	HCV Positive among pregnant women (N=135) (n=12)				HBV Positive among pregnant Women (N=135) (n=37)			
	POS(+)	Odds ratio	CI 95%	P-value	POS(+)	Odds ratio	CI 95%	P-value
1. Marital status a. Single b. Co-habiting c. Married	0% 3(25%) 9(75%)	0.249	0.181-1.556	**0.531**	7(18.9%) 6(16.2%) 24(64.9%)	1.321	0.756-2.307	**0.328**
2.Sexual protection a. Yes b. No	1(8.3%) 11(91.7%)	0.945	0.094-9.482	**0.961**	6(16.2%) 31(83.8%)	0.712	0.206-2.454	**0.59**
3.Blood transfusion a. Yes b. No	6(50%) 6(50%)	6.627	6.177-24.72	**0.005**	9(24.3%) 28(75.7%)	1.293	0.453-3.680	**0.631**
4. No. of Sexual partner(s) a. One b. Two c. Three or more	1(8.3%) 7(58.3%) 4(33.4%)	0.933	0.405-2.147	**0.871**	4(10.8%) 5(13.5%) 28(75.7%)	0.251	0.129-0.490	**0.003**
5. Parity a. Nultiparity b.Primiparity c. Multiparity d. Grand parity	2(16.7%) 3(25%) 4(33.3%) 3(25%)	1.007	0.649-1.562	**0.975**	9(24.3%) 13(35.1%) 7(18.9%) 8(21.6%)	1.175	0.865-1.597	**0.302**
6. Tattoo a. Yes b. No	2 (16.7%) 10(83.3%)	0.370	0.254-3.215	**0.541**	3(8.3%) 34(91.7%)	1.124	0.954-2.152	**0.570**

7. Alcohol a. Yes b. No	 3(25%) 9 (75%)	 1.254	 0.821-2.152	 **0.086**	 6(16.6%) 31(83.4%)	 1.245	 0.865-1.892	 **0.276**

4.6 Measure of infectivity among f the pregnant women

Of the HBsAg positive women, 5(13.5%) were positive for HBeAg, 9(24.3%) were positive for HBeAb and 23(62.2%) were negative for both HBeAg and HBeAb. Fig. 2 Distribution of infectivity marker

Fig. 2 Distribution of infectivity marker



CHAPTHER FIVE

DISCUSSION

5.1 General prevalence of Hepatitis B and Hepatitis C

Screening asymptomatic people is important in disease detection, prompt diagnosis and intervention in relation to a typically asymptomatic infections such as chronic HBV and HCV infections. HBV and HCV infections are contagious diseases that can be transmitted vertically from mothers to their neonates or horizontally through blood products and body secretions (SeyedReza *et al.*, 2011). HBV infection during pregnancy results in a severe disease to the mother and chronic infection to the baby (Wright, 2006) whiles neonates born to HCV infected women are at risk of low birth weight, congenital anomaly and also preterm birth (Connell *et al.*, 2011). Viral hepatitis is the commonest cause of hepatic dysfunction in pregnancy.

The current study was a hospital-based cross sectional study to investigate the seroprevalence of HBV and HCV infection and associated risk factors among pregnant women attending antenatal clinic at the Catholic Hospital, Battor in the Volta Region of Ghana. The study revealed that out of 135 pregnant women that were included in the study, 37 women tested positive for HBV giving a prevalence of 27.4% and 12 women tested positive for HCV giving a prevalence of 8.9 % respectively among the participants. None of the participants was a carrier of both viruses. The prevalence in this study supports WHOs report, which classify Africa among the highly endemic areas with prevalence greater than 8% for HBV (Hwang and Cheung, 2011).

The prevalence observed in this study was higher than 9.5% and 7.7% for HBV and HCV and 0.6% for HBV-HCV co infection reported by Ephraim et al, 2015 in Ashanti Region Ghana. Likewise, Murad *et al.* (2013) also reported a 10.8%, 8.5% and 0% of HBV, HCV and HBVHCV co-infection among pregnant women in Saana Yemen and Ugbebor *et al.* (2011) reported of 12.5%, 3.6% and 0.57% for HBV, HCV and HBV-HCV co-infection in Nigeria.

Esan *et al*. (2014) also, in a study among pregnant women in Ido-Ekiti, Nigeria reported of a 6.78%, 1.39% and 0.15% of HBV, HCV and HBV-HCV co-infection respectively. The differences between the prevalence maybe explained by the fact that Battor is a rural area where most household items are shared and thus there can be acquisition of the infection by horizontal means. Most of the attendants at the antenatal clinic are from different rural areas where different social and cultural practices are observed which might also increase the prevalence. The differences can also be attributed to different socio-economic status, difference in geographical areas, varying sample size and different test procedures.

From this study, a high proportion of the subjects showed evidence of previous exposure to HBV. Out of 135 pregnant women that were screened, 82(60.7%) were positive for total antiHBc and hence has serological evidence of previous HBV exposure. This proportion is higher than the anti-HBc seroprevalence among pregnant women in developed world with reports ranging from 7.1% in Switzerland, 13.4% in France, 29.65% in China and 40.8% in Cameroon (Ding *et al*., 2013).

The presence of HBeAg was evaluated to assess the level of infectivity among the subjects since HBeAg is a marker of high infectivity and a measure for the risk of vertical transmission of HBV. HBeAg positive mothers are known to have a high viral load and that is the main route of transmission of HBV to newborns (WHO., 2009; Kfutwah *et al.*, 2012). From the study 5(13.5%) of HBV positive subjects were HBeAg positive. This results is not consistent with Kfuwah *et al.* (2012) who did not find any HBeAg positive samples among patients who tested positive for HBsAg. Wang *et al*, found out in a research that, the risk of vertical transmission and resulting chronic carrier infection from HBsAg positive mother to her baby is higher in HBeAg pregnant women with high HBV DNA titres. Hence the findings in this research suggest that about 13.5% of neonates will be at risk of vertical transmission of HBV in Battor, Volta Region of Ghana.

5.2 Socio-demographic characteristic and prevalence of HBV and HCV

The infection status of the study respondents whether HBV or HCV followed no particular pattern in relation to educational background, occupation, gestational age and marital status with the exception of the age of the participants where a statistically significance was observed. From Table 2, the highest proportion of HBV and HCV infection was recorded among pregnant women in the age groups 21-30 years. The prevalence of HBV amongst the group was 23(62.2%) with a P-value of P=0.015 which is significant and the prevalence of HCV was 6(50.0%) with a P-value of P= 0.014 which is also significant. Other studies reported of a higher prevalence of HBV among pregnant women between the ages of 25-35 years by Habiba and Memon (2007) in Pakistan and between 26-35 years by Esan *et al.* (2014) in Nigeria. Eke *et al.* (2011) also reported a highest prevalence of HBV among pregnant women between 20-24 years which he attributed to early marriage of the women in South-Eastern Nigeria. The highest age specific prevalence observed among the group in this study can be attributed to the fact that most of these women are of the reproductive age and they are most sexually active and fertile in addition they readily access antenatal care in the area. These further suggest that there is a risk of mother-to-infant transmission among this group.

Other socio-demographic characteristics evaluated included educational background, marital status, occupation, gestational age and parity. Reports from other studies may link some of these factors as associated risk in HBV acquisition but this study did not find any statistically significant association between any of these factors and the risk of HBV and HCV infection. However, it was noted from this study that, the few pregnant women (10.8%) who had HBV infection were all within the tertiary category and none of the women within the senior high and tertiary category had HCV infection. Nevertheless, pregnant women with basic educational background had the highest (41.7% and 35.14%) for HCV and HBV infections respectively but no statistically significant association was made between education and HBV HCV sero-

prevalence (p>0.05). However, Anaedobe *et al*. (2015) in a research on pregnant women in Southwestern Nigeria found a significant association between education and HBsAg. There is a possibility that regardless of their educational background, they had multiple sexual partners with only few using protection (condom) during sexual intercourse. In agreement to this, a research carried out by Murad *et al.* (2013) found out that women with less than secondary school level of education are at a higher risk whiles Doa'a *et al.* (2010) also suggested that low level of maternal education is a risk factor for HCV infection in children.

With regards to occupation, most of the respondents were traders. It was realized that the prevalence among this group was 51.4% and 33.3% for HBV and HCV infections respectively with a large number of them being market women. Students and public servants had the lowest prevalence of 5.4% and 0% for HBV and HCV infections respectively. This can be attributed to their knowledge about the infection and associated risk factors since most of the public servants are nurses and teachers and this also indicates the positive influence of education and public awareness on the carrier rate of HBV infection. Despite the high prevalence in the various category, there was no significant association between HBsAg positivity and occupation of the study subjects (p=0.318 and 0.876) for HBV and HCV respectively. The finding is supported by a research in Iran that found no significant association between occupation of the study participants and HBV seropositivity (SeyedReza *et al.*, 2011).

Most of the pregnant women in their second trimester (92.7% and 43.2%) were infected with HCV and HBV respectively. None of the study subjects in the third trimester tested positive for HCV but 16.2% tested positive for HBV whiles 40.5% and 8.3% of the first trimester subjects tested positive for HBV and HCV. This is in consonance with results from Ethiopia and Nigeria where the highest prevalence of HBsAg was detected in pregnant women in their second trimester (Ndams *et al.*, 2008; Zenebe *et al.*, 2014). No statistically significant association was observed between gestational age and HBV HCV infection (p>0.05). The

results is consistent with reports from Benin where no statistically significance association was found between gestational and the acquisition of HBV and HCV (Ugbebor *et al.*, 2011).

More than half (71.9%) of the study participants were married and 14.8% were single. The prevalence of HBV and HCV was highest (64.9% and 75%) among the women who were married. The women who were co-habiting followed with a prevalence of 16.2% and 25% for HBV and HCV infections respectively whiles no single woman had any infection with HCV but 18.9% had HBV infection. The high prevalence could be due to the fact that this married women were exposed to the infection earlier before marriage and could not clear it before marriage.

5.3 Associated risk factors and the prevalence of HBV and HCV

Despite the high prevalence of HBV infection among pregnant women in this study, just one predisposing factor showed statistical significant association. This finding was in accordance with earlier studies which showed that most of those testing positive for HBV had just one or two predisposing factors (Fomulu *et al*, 2013).

Sexual transmission has been recognized as a major source of HBV transmission and from this study, having multiple sexual partners 28(75.7% with p=0.003) was a statistically significant predisposing factor for HBV infection and thus confirms a strong association between having multiple partners and the prevalence of HBV. This was in agreement with
Molla *et al*. (2015) who also found a significant association between history of multiple sexual exposure and HBV infection. In addition, (Rabiu *et al.*, 2010) and Obi *et al.* (2006) also demonstrated that having a history of multiple sexual partners is a significant risk factor for HBV infection during pregnancy.

Previous history of blood transfusion did not have any significant association (p=0.631) with

HBV infection in this study which was in agreement with studies conducted in Ghana, Cameroon and Nigeria (Eke *et al.*, 2011; Fomulu *et a*l., 2015; Ephraim *et al.*, 2015). In other research carried out in Ethiopia, Nigeria and Egypt, history of previous blood transfusion was significantly associated with HBV infection (Kamal and Nasser, 2008; Olokoba *et al.*, 2011; Zenebe *et al.*, 2014).

More than half of the women in this were multigravida (54%) and out of these 7 (58.3%) tested positive for HCV and 15 (40.5%) tested positive for HBV which is in accordance to a study by Azhar *et al*. (2012) who reported of a higher frequency of HBV infection among women who are multigravida. Khattak *et al.* (2008) in a research carried out suggested that increased risk of HBV and HCV infection among this group can be due to their past pregnancies, any surgical procedure in the past, hospital admission and blood transfusion, with each pregnancy and childbirth increasing the chance of exposure to HBV and HCV. A research in Pakistan by Khan *et al.* also associated high parity with HCV infections. However, no statistically significant relation was made between the parity status of the study subjects and HBV and HCV positivity ($p=0.302$ and $p=1.007$) which is in accordance with results from Ethiopia and Benin (Ugbebor *et al.*, 2011; Zenebe *et al.*, 2014).

Though tattooing is an important risk factor in the acquisition of and HCV infections, in this study fewer participants (16.7%) had tattoo with majority (83.3%) not having any form of tattoo. No significant association was made with HCV infection and tattoo on the body ($p>0.05$). This finding is consistent with results from a research carried out in Iran and Sudan where no significant association was made between tattoo and HCV and HBV seropositivity (Elsheikh *et al.*, 2007; SeyedReza *et al.*, 2011). However, this findings is not in agreement reports from Ghana and Ethiopia where tattooing was identified as a risk factor in the acquisition of HBV and HCV (Zenebe *et al.*, 2014; Ephraim *et al.*, 2015).

The prevalence of HBV and HCV infection among the pregnant women who ever consumed alcohol was lower (8.3% and 25%) than those who never consumed alcohol. Despite the fact that this finding was not significant ($p>0.05$), other studies have linked alcohol intake to the prevalence of HCV and the progression of chronic hepatitis C to cirrhosis and HCC.

Condom usage was low among the partners of the women. From the study the prevalence of HBV infection among the subjects who use condom as a form of protection were 16.2% whiles those not using any condom for protection were 83.8%. With regards to HCV infection, 8.3% use a form of protection whiles 91.7% does not use any form of protection.

Although, this finding was not significant ($p>0.05$) for both HBV and HCV infection, it is consistent with Frambo *et al*. who also reported of no significant association in a research on pregnant women in Cameroun.

From this study, a history of previous blood transfusion showed a significant association ($p=0.005$) with HCV infection. This is in consonance with earlier studies by (Ward *et al.,* 2000; Candotti *et al.,* 2007; Ephraim *et al.*, 2015) who observed a significant association between blood transfusion and HCV infection. Although the risk for HCV transmission have declined since the start of screening, the risk still exist in some developing countries due to financial constraint and failure to adequately screen donors (Kamal and Nasser, 2008; Zahran *et al.*, 2010).

CHAPTHER SIX

CONCLUSION, LIMITATIONS AND RECOMMEMDATIONS

6.1 CONCLUSION

The seroprevalence of HBsAg in pregnant women obtained from this study was 27.4% which is of high endemicity according to WHO classification and HCV was 8.9% which is also of high endemicity. Five of the HBsAg positive pregnant women representing 13.5% were HBeAg positive suggesting that they are highly infectious and can transmit the infection to their neonates. Due to the high prevalence of HBV and HCV infection among pregnant women in Battor, North Tongu district of Volta region, it is important to give public education which is aimed at routine screening, modification of risky social lifestyle and vaccination of those who test negative and are at risk. This is to reduce the future risk of mother to child transmission of the infection.

Having multiple sexual partners was associated with the acquisition of HBV infection whiles previous history of blood transfusion was associated with acquisition of HCV infection and they showed a statistically significant association P=0.003 and P=0.005 respectively.

6.2 RECOMMENDATIONS

Based on the findings and conclusion of the study, the following recommendations are made:

Hepatitis C screening should be included in the routine antenatal tests to identify those at risk. The pregnant women should be educated on the risk factors and preventive measures of these viral infections since there is no vaccine for the infection.

It is important to vaccinate all pregnant women who test negative for hepatitis B to reduce mother to child transmission of the disease. Hepatitis B immunoglobulin (HBIG) must be given as post exposure prophylaxis to due to the high risk of developing chronic HBV infection among infants born to HBsAg positive mothers.

6.3 LIMITATIONS

The level of infectivity is more accurate when the HBV DNA is measured which indicates active virus replication and hence more accurate than HBeAg and also used to monitor response to therapy. But due to financial constraints, HBeAg was used to evaluate the infectivity in this study.

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APPENDIX

APPENDIX 1: Copy of Questionnaire

KWAME NKRUMAH UNIVERSITY OF SCIENCE & TECHNOLOGY

SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF CLINICAL MICROBIOLGY

A questionnaire on "Hepatitis B and C virus co-infection among a cohort healthy pregnant women attending Antenatal Clinic at Catholic Hospital, Battor".

I am a final year student of the above named department carrying out a project on the topic above as part of the requirement for the award of a master's degree.

Viral hepatitis is a life-threatening liver disease, caused by hepatitis B and C virus, and is a major public health problem, particularly in developing countries. Viral hepatitis during pregnancy is associated with high risk of maternal complications. Among pregnant women, chronic infection with HBV and HCV are often asymptomatic, and can lead to coagulation defects, postpartum hemorrhage, organ failure and high maternal mortality and poor outcomes of their newborns such as still births, neonatal deaths (NND), jaundice, anorexia (poor appetite), malaise, acute and chronic liver disease (liver cirrhosis) and hepatocellular carcinoma. Maternal mortality has been shown to increase in pregnant women with liver cirrhosis.

The purpose of the study is to determine the prevalence rate of hepatitis B and C co-infection among pregnant women and associated risk factors. It will also help to know neonates at risk of being infected and the necessary measures taken to prevent them from getting infected. The study involves answering questions about your demographic characteristics, medical, sexual and gynecological history and your response will help to achieve the aim of the study.

I will be glad if you can read this consent form or have it explained to you so as to make a decision to participate.

Participants consent

I have read or someone has explained to me all the necessary details I need to know concerning this study. I have therefore decided without any coercion to participate in this study.

Signature / Thumb print

Participants are required to answer all the questions and they are to be sincere in answering these questions. All information collected is solely for the purposes of research and shall not be used for other purposes, all participants information shall be kept safe. No name will be recorded.

ID of Respondent…….

Tel no……..

Please tick

1. Age……..

2. Educational Background

None [ ] Primary [ ] JHS [ ] Secondary [ ] Tertiary [ ]

3. Occupation:

Housewife/Self Employed [ ] Farmer [ ] Unemployed [ ] Other

4. Where have you lived most of your life? Urban [ ] Rural [ ] Urban slum [ ]

5. Religion:

Christian [ ] Muslim [ ] Others [ ]

Sexual History

6. What is you marital Status?

Single [ ] Married/Cohabited [ ] Divorced/Widowed/Separated [ ]

7. Before your present relationship have you had any other sexual relationship before?

Yes [ ] No [ ]

8. IF YES how many people? ……

9. During sexual relations (intercourse) with your partner(s) former or present do you use any protective measures?

Yes [ ] No [ ]

10. If yes what protective measures did you use……..

11. How often? Rarely [ ] Sometimes [ ] Most times [ ]

Gynecological History

12. How many pregnancies have you had?

13. How many children do you have?

14. Did you lose any pregnancy through still birth, miscarriage or abortion?

Yes [ ] No [ ] If yes specify…….

Medical History

15. Do you smoke or have you ever smoked?

Yes [ ] No [ ]

16. Does your partner smoke or has your partner ever smoked?

Yes [ ] No [ ]

17. Any history of alcohol consumption?

Yes [ ] No [ ]

18. Any history of blood transfusion?

Yes [ ] No [ ]

19. Any history of intravenous drug use?

Yes [ ] No [ ]

20. Have you ever been diagnosed of the following?

(1) Hypertension [ ] (2) Cancer [ ] (3) HIV [ ] (4) Sexual transmitted infections [ ] (5) None of the above [ ]

21. Do you have any form of tattoo?

Yes [ ] No [ ]

Knowledge of the Causes of hepatitis B and C viral infection

23. Have you ever heard of hepatitis B and C?

Yes [ ] No [ ]

24. Do you know about the causes and mode of transmission?
Yes [ ] No [ ]

25. If yes specify…….

26. Have you ever had a screening before?

Yes [ ] No [ ]

27. Have you had any form of hepatitis B vaccination?

Yes [ ] No [ ]

28. How old is the pregnancy?

APPENDIX 2: **ASSAY VALIDATION**

HBsAg: The test run was considered valid provided the following criteria were met:

1. mean negative control value :MNC<0.200
2. Mean positive control value :MPC>0.500
3. MPC-MNC≥0.300
4. Individual NC values should lie within 0.5*MNC and 2.0*MNC

Anti-HBc: The test run was considered valid provided the following criteria were met:

1. Colour in A1 (blank): colourless or light yellow
2. MNC≥0.400
3. MPC≤0.100
4. MPC-MNC≥0.300

HCV: The test run was considered valid provided the following criteria were met:

1. Colour in A1 (blank): colourless or light yellow
2. MNC≤0.200

3. MPC≥0.600

4. MPC-MNC≥0.400

APPENDIX 3: **The principle underlining ELISA test**

HCV

The Human anti-HCV ELISA is a 3rd generation indirect ELISA detecting anti-HCV antibodies. It uses a specific HCV recombinant antigens (core, NS 3, NS 4, NS 5) coated on microtitre wells. Specimen's antibodies, if present, or control bind to the HCV antigen. At the solid phase.

After the incubation unbound specimen components are removed by washing. For the second incubation step anti human IgG HRP conjugate is added, which binds specifically to the immobilsed human anti-HCV IgG antibodies, and forms a sandwich immunocomplex. After a second washing step to remove excess conjugate, 3,3',5,5'-tetramethylbenzidin/substrate is added. A blue colour develops changing to yellow after stopping the reaction. The intensity of the colours is directly proportional to the HCV-IgG antibody concentration in the specimen.

HBsAg

The anti-HBs test is a solid phase enzyme immunoassay system that utilizes a sandwich method to detect anti-HBs antibodies in serum or plasma. The test sample and peroxidase conjugated HBsAg (human) are added to a microwell coated with purified HBsAg (human).

The amount of peroxidase-HBsAg conjugate bound to the well is proportional to the concentration of anti-HBs in the specimen which acts as a link between the fixed and HRP conjugated HBsAg.

After incubation unbound enzyme conjugate is washed off. 3,3',5,5'-tetramethylbenzidin /Substrate solution is added and during further incubation a blue colour develops. The intensity of the colour which changes to yellow after the reaction with sulpheric acid solution is proportional to the amount of anti-HBs present in the specimen.

Anti- HBc

The human anti-HBc ELISA is based on the competitive antibody ELISA technique for detection of total anti HBc antibodies in patient specimen. Anti-HBc-ab in a test specimen compete with anti-HBc peroxidase conjugate(human) for a limited number of binding sites on the HBcAg coated well. Thus the amount of anti-HBc peroxidase conjugate bound to the well in inversely proportional to the concentration of anti-HBc-ab in the specimen.

After incubation of specimen the anti-HBc peroxidase unbound enzyme conjugate is washed off. 3,3',5,5'-tetramethylbenzidin/substrate solution is added and during further incubation a blue colour develops. The intensity of this colour which changes to yellow after stoping the reaction with sulpheric acid solution is inversely proportional to the amount of anti-HBc-ab in the specimen.