THE PREVALENCE OF HEPATITIS B AND C VIRUS INFECTIONS AMONG LIVER CIRRHOSIS PATIENTS AT THE KORLE-BU TEACHING HOSPITAL, ACCRA-GHANA

BY

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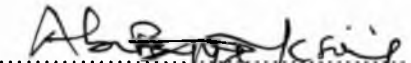
DECLARATION

THE WORK DESCRIBED IN THIS THESIS WAS DONE BY ME AT THE PATHOLOGY AND CHEMICAL PATHOLOGY DEPARTMENTS OF UNIVERSITY OF GHANA MEDICAL SCHOOL, KORLE-BU UNDER THE SUPERVISION OF PROF E. K. WIREDU (DEAN AND ASSOCIATE PROFESSOR, SCHOOL OF ALLIED HEALTH SCIENCES) AND DR R. K. GYASI (SENIOR LECTURER, DEPT. OF PATHOLOGY,UNIVERSITY OF GHANA MEDICAL SCHOOL).

ALL THE WORK RECORDED IN THIS THESIS IS ORIGINAL, UNLESS OTHERWISE ACKNOWLEDGED IN THE TEXT OR BY THE REFERENCES CITED. THIS WORK HAS ALSO NOT IN ITS PRESENT FORM OR OTHERWISE BEEN SUBMITTED TO THIS OR ANY OTHER UNIVERSITY FOR THE AWARD OF A HIGHER DEGREE.



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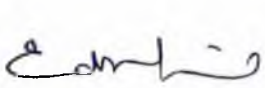

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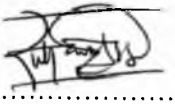

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DEDICATION

TO MY DEAR WIFE MARGARET AND OUR DAUGHTER

DEBBY.

ACKNOWLEDGEMENTS

I would first give thanks to the Lord for giving me grace and strength to complete this project. I am very grateful to my supervisors Professor Edwin Kwame Wiredu, Dean, School of Allied Health Sciences and Dr Richard Kwasi Gyasi, Senior Lecturer of the Department of Pathology. I appreciate your invaluable guidance, contributions, advice and patience. May God richly bless you.

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LIST OF ABBREVIATIONS

HBV	-	**Hepatitis B Virus**
HCV	-	**Hepatitis C Virus**
HCC	-	**Hepatocellular Carcinoma**
HBsAg	-	**Hepatitis B surface antigen**
HBcAg	-	**Hepatitis B core antigen**
HBeAg	-	**Hepatitis B e antigen**
HBX	-	**Hepatitis B X protein**
HBV-DNA	-	**Hepatitis B virus deoxyribonucleic acid**
Anti-HBs	-	**Antibody to hepatitis B surface antigen**
Anti-HBc	-	**Antibody to hepatitis B core antigen**
Anti-HBe	-	**Antibody to hepatitis B e antigen**
Anti-HCV	-	**Antibody to hepatitis C virus**
HCV-RNA	-	**Hepatitis C virus ribonucleic acid**
NANB hepatitis	-	**Non-A, Non-B hepatitis**

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ABSTRACT

Cirrhosis of the liver is an endstage chronic liver disease which is generally irreversible. About 75% of patients with posthepatitic cirrhosis have progressive disease despite supportive therapy and die within one to five years from serious complications such as variceal haemorrhage, hepatic encephalopathy or superimposed hepatocellular carcinoma. Records from the Pathology Department of the Korle-Bu Teaching Hospital, Accra-Ghana indicates that cirrhosis is the commonest liver disease leading to death through serious complications. Most studies done in other countries have indicated an association between liver cirrhosis and chronic hepatitis B and C infections, especially, where these viruses are endemic. In Ghana Hepatitis B virus infection is endemic with seroprevalence rate ranging from 6.7-15.6%. That of hepatitis C ranges between 2.8% and 5.4%. Although liver cirrhosis is the commonest liver disease causing death at the Korle-Bu Teaching Hospital, the role of hepatitis B and C virus infections have not been well established in Ghana. The study was therefore carried out to determine the seroprevalence and the roles of hepatitis B and C virus among liver cirrhosis patients at the Korle-Bu Teaching Hospital.

To achieve the above objectives, a nested case-control study design was used. Seventy consenting patients (cases) clinically diagnosed with ultrasound support as liver cirrhosis were interviewed. To test the validity of the criteria used in diagnosing cirrhosis, autopsies were done on all the cases that died during the study period. For each case interviewed four consenting patients age ($\pm$5 yrs) and sex-matched who were on admission with non-hepatic disease and not jaundiced were chosen as controls. About 10ml of blood was taken from each subject and the serum separated into well labelled micro tubes for storage at -70°C till analysis. Once thawed, samples were analysed for

hepatitis B surface antigen and anti-HCV by sensitive ELIZA test-kits. The results were analysed statistically using EPI-INFO 2000 at a 5% significance level.

Of the 70 cases 18 died and autopsy done on all confirmed the diagnosis of cirrhosis morphologically indicating that the criteria used in the diagnosis of cirrhosis were together sensitive and specific.Thirty, out of the 70 cases studied were positive for hepatitis B surface antigen giving a prevalence rate of 42.8%. The rate among controls was 7.5% (21 out of 280 controls). Hepatitis B virus infection was significantly associated with cirrhosis ($\chi^2 = 56.078$, P= 0.000). The odds ratio obtained 9.25 with 95% CI = 4.83-17.7, indicates that the risk of developing cirrhosis is about 9 fold increased in those with Hepatitis B infections than those without.

The seroprevalence of antibodies to hepatitis C virus among liver cirrhosis patients was 7.1% (5 out of 70) and 3.6% (10 out of 280) among the controls. An odds ratio of 2.07 (95% CI=0.159-1.462) obtained shows that the chances of a chronic hepatitis C virus patient developing cirrhosis of the liver is about twice that of those without hepatitis C and this was not statistically significant ($\chi^2$1.717, P=0.189).

Blood transfusion was found to be a significant means of transmitting Hepatitis B virus infection (P=0.043) but not a significant means of transmitting hepatitis C virus infection (P=0.33.).

In conclusion, it is recommended that blood screening against these viruses must continue and that other important modes of transmitting both hepatitis B and C viruses be further investigated to enable appropriate preventive measures to be applied. Immunization against hepatitis B Virus infection must be encouraged not only for children but among the general population especially groups which are at risk.

CHAPTER 1: INTRODUCTION

1.1 GENERAL INTRODUCTION

Liver cirrhosis is an end stage chronic liver disease which is generally irreversible. About 75% of patients with posthepatitic cirrhosis have progressive disease despite supportive therapy and die within one to five years from serious complications (**1**) such as variceal haemorrhage, hepatic encephalopathy or superimposed hepatocellular carcinoma (HCC).

Studies done in other countries have indicated an association between liver cirrhosis and chronic viral hepatitis due to hepatitis B virus (HBV) and hepatitis C virus (HCV) infections especially where these viruses are endemic (**2 - 6**).

Viral hepatitis refers to infection of the liver caused by a group of viruses having a particular affinity for the liver. These are hepatitis A, B, C, D and E viruses. A new viral agent bearing similarities to hepatitis C virus has been identified and cloned and designated hepatitis G virus but this virus appears to be non-pathogenic, causing neither liver disease nor exacerbation of liver disease (**7, 8**).

A number of clinical syndromes may develop after exposure to these hepatitis viruses.

These are:

1. A carrier state without clinically apparent disease or with asymptomatic chronic hepatitis,
2. Acute hepatitis which may be icteric or anicteric

3. Fulminant hepatitis with sub-massive to massive hepatic necrosis and
4. Chronic hepatitis with or without progression to cirrhosis and or HCC.

Not all of these hepatotropic viruses provoke each of the above clinical syndromes. Hepatitis A and E viruses do not generate a carrier state or cause chronic hepatitis. Hepatitis D virus is absolutely dependent on the genetic information provided by hepatitis B virus for multiplication and causes hepatitis only in the presence of hepatitis B virus as either acute coinfection or super-infection of a chronic carrier of hepatitis B virus.

Hepatitis B and C viruses are by far the most important of the hepatitis viruses causing chronic hepatitis which may progress to liver cirrhosis and or HCC (**9,10**). Chronic hepatitis is defined as symptomatic, biochemical or serologic evidence of continuing or relapsing hepatic disease for more than 6 months (**11**). Although hepatitis B and C viruses are responsible for most cases of chronic hepatitis, there are many other causes of chronic hepatitis including Wilson disease, α_1 antitrypsin deficiency, chronic alcoholism, drugs (isoniazid, methyldopa, methotrexate etc) and autoimmunity. Therefore serologic studies are important for the diagnosis of chronic hepatitis caused by the hepatitis B and C viruses.

HBV is endemic in Ghana with seroprevalence rates ranging from 6.7%-15.6% (**12-16**). Recent studies have also revealed HCV seroprevalence rates of 2.8-5.4% (**17-19**) and the Ghana National Blood Transfusion Service started screening for anti-HCV just recently in November 2002. Edington in his study on hepatic diseases in Ghana observed that the commonest liver disease leading to death at autopsy was cirrhosis of the liver (**20**) through complications such as portal hypertension with

upper gastrointestinal haemorrhage, hepatic failure with encephalopathy and hepatocellular carcinoma .

Although liver cirrhosis is the commonest liver disease causing death at the Korle-Bu Teaching Hospital, the role of HBV and HCV infections in liver cirrhosis in Ghana has not been well established.

1.2 AIM OF THE STUDY

The overall aim of the study is to investigate the prevalence of HBV and HCV infections among patients with liver cirrhosis visiting the Korle Bu Teaching Hospital.

1.3 SPECIFIC OBJECTIVES

1.3.1 To determine the seroprevalence of HBV and HCV among liver cirrhosis patients at the Korle Bu Teaching Hospital.

1.3.2 To determine the role of chronic hepatits B and C viral infections in cirrhosis of the liver.

1.4 OUTPUT OF THE STUDY

There is very little information on the role of HBV and none at all on that of HCV in cirrhosis of the liver in Ghana. The results of the study will therefore serve as a baseline data for policy formulation on prevention and control of infection by these viruses, for future comparison and monitoring of policy and interventions.

1.5 BENEFICIARIES OF THE STUDY

The results of the study will benefit the Ministry of health and the Ghana Government by serving as a basis for policy formulation on prevention and control of hepatitis B and C viral infections, which, will in the long term benefit the people of Ghana. The information provided by the study will also contribute to the world literature on hepatitis B and C viral infections in cirrhosis of the liver.

CHAPTER 2: LITERATURE REVIEW

2.1 HEPATITIS B VIRUS

Hepatitis B virus was the first human hepatitis virus from which the proteins and genome could be identified and characterized. In 1963, Blumberg, in a search for polymorphic serum proteins, discovered a previously unknown antigen in the blood of an Australian Aborigine (Australia antigen). Soon thereafter, it was recognized that the appearance of this antigen was related to type B hepatitis. Using immune electron microscopic methods, Dane eventually discovered virus-like particles that carried this antigen on their surface, in the serum of hepatitis B patients (**21**). These particles were consequently considered to be the hepatitis B virus (HBV). In 1973, the viral nature of the particles discovered by Dane was confirmed by the detection of an endogenous DNA-dependent DNA polymerase within their core (**22**).

HBV and its relatives throughout the animal kingdom now comprise an officially recognized virus family termed hepadnaviridae, the name derived from their hepatotropism and DNA genome (**23**).

2.2 HEPATITIS B VIRAL MORPHOLOGY

The mature HBV virion is a complex, double-layered spherical particle 42nm in diameter (**24**). It has an outer surface envelope (viral coat) of protein, lipid and carbohydrate enclosing a nucleocapsid which consists of HBV DNA, DNA polymerase, HB_C Ag and X protein (**11**). Using electron microscopy, the genome of HBV was shown to be partially double stranded circular DNA molecule that is 3200 nucleotides in length (**25**). The two DNA strands each have gaps, although one strand is almost complete. The DNA is associated with a DNA polymerase, the

activity of which is dependent upon the addition of all four nucleoside triphosphates and magnesium ion but not primer DNA. Endogenous DNA polymerase is capable of filing the open regions of each DNA strand.

2.3 ANTIGENIC COMPOSITION

The organization of the HBV genome is unique in that all regions of the viral genome encode protein sequences (**11**).

These are:

1. An envelope glycoprotein called hepatitis B surface antigen (HB_S Ag). Infected hepatocytes are capable of synthesizing and secreting massive quantities of non-infective surface protein (HB_S Ag).
2. A nucleocapsid core protein (HB_C Ag) and a longer polypeptide transcript with a precore and the core region designated HBeAg. Thus HBeAg consists of HB_CAg plus a precore region.
3. A region protein from the X region (HBX) which is necessary for virus replication and acts as a transcriptional transactivator of the viral genes and a wide variety of host gene promoters. HBX is also thought to play a key role in the causation of HCC (**26**).

2.4 HBV SUBTYPES AND MUTANT STRAIN

There are a number of subtypes of HBV defined by various combinations of antigenic determinants present on the HBsAg (**24**). All have the same group-specific determinant, a, but there are four major subtype-specific determinants certain pairs of which tend to behave as alleles (d and y; r and w) that is as mutually exclusive alternatives. Thus the four major subtypes of HBV are adw, ayw, adr and rarely ayr.

Rare examples of unusual specificity such as adwr or adyr may be the result of mixed infections. Recently, specific nucleotide changes have been associated with variation between the pairs of determinants. In each case, a single nucleotide change results in alternative specification of corresponding codon of arginine or lysine. Determinant d, has a lysine at position 122 and y has an arginine (**27**). Likewise determinant w has a lysine at position 160 and r has an Arginine (**28**). Recently a further subtype allele that has either isoleucin or threonine in position 126 has been identified (**29**).

Since all subtypes are able to induce cross-protection after immunization the significance of serological or other sub-typing is mainly of epidemiological and phylogenetic interest (**24**). However it cannot be excluded that some of the neutralizing antibodies are subtype specific as is the case with most other viruses. While there are clear differences in the geographical distributions of the subtype (**30**), there seems to be no correlation between virus subtype and virulence.

On occasion infectious mutant strains of HBV emerge during active replication of the wild type HBV strain and some may have ominous consequences. First, some mutants replicate successfully but are incapable of HBeAg expression despite continued HB_C Ag production. The loss of circulating HBeAg and hence of anti-HBe antibody formation is associated with fulminant hepatitis. Second, vaccine-induced escape mutants appear to replicate in the presence of vaccine-induced immunity (**11**).

2.5 PATHOGENESIS OF HBV INFECTION

HBV infections pass through two phases namely proliferative and integrative phases. Pathogenesis involves immunologically mediated hepatocyte necrosis by sensitized cytotoxic $CD8^+$ T cells attributed to cellular expression of viral antigens during the episomal phase of viral replication (proliferative phase). Cell surface expression of viral HB_SAg and HB_CAg in association with the major histocompatibility complex (MHC) class 1molecules leads to activation of cytotoxic $CD8^+$ T lymphocytes and hepatocyte destruction (**11**). The incorporation of the viral DNA into the host genome (integrative phase) may occur in hepatocytes not destroyed by the immune response. With cessation of viral replication at this point and the appearance of antiviral antibodies, infectivity ends and liver damage subsides. However, the risk of HCC persists.

2.6 EPIDEMIOLOGY AND NATURAL HISTORY OF HBV INFECTION

Liver disease due to HBV is an enormous problem globally. It can cause acute hepatitis, chronic non-progressive hepatitis, chronic progressive hepatitis ending in cirrhosis and/or HCC. HBV can also produce fulminant hepatitis with massive liver necrosis, an asymptomatic carrier state with or without progressive sub-clinical disease or act as the backdrop for hepatitis D virus infection. There is an estimated worldwide carrier rate of 350 million (**31**). In the United States there are 300, 000 new infections per year and the prevalence in the general population is about 1 % (**32**). Carrier rates of 9-20 % are reported in Sub-Saharan Africa (**33-36**) and 5-18 % in the Middle East (**37-40**). Carrier rates ranging from 5-35% are found in the Western Pacific and South East Asia except in Australia, New Zealand and Japan where the mean carrier rate is less than 2%(**31**). In Ghana studies done put the prevalence rates as follows: 6.7-10% among blood donors (**12, 13**), 6.4% among pregnant women (**41**), 15.6% among school children (**17**) and 54.1% among jaundiced patients (**42**).

HBV remains in blood during the last stages of a prolonged incubation period of 4-26 weeks (mean 6-8 weeks) and during active episodes of acute and chronic hepatitis. It is also present in all physiologic and pathologic body fluids, with the exception of stool. HBV is a hardy virus and can withstand extremes of temperature and humidity. Thus, whereas blood and body fluids are the primary vehicles of transmission, virus may also be spread by contact with body secretions such as semen, saliva, sweat, tears, breast milk and pathologic effusions (**11,43**). Transfusion of blood and blood products, dialysis, needle-stick accidents among health workers, intravenous drug abuse and homosexual activity constitute the

primary risk categories for HBV infections. In one third of patients the source of infection is unknown (**11**). In endemic regions such as Africa and South-east Asia, spread from an infected mother to a neonate during birth (vertical transmission) is common(**44,45,46**). These neonatal infections often lead to the carrier state for life (**44,47**).

Many subjects infected with HBV will develop a clinical or sub-clinical self-limiting acute hepatitis, with spontaneous clearance of hepatocytes supporting HBV replication within a few weeks of infection. However about 5-10% of individuals with HBV acute hepatitis will develop a chronic infection (**11**) and this group runs the major risk of developing cirrhosis and liver cancer. The age at which an individual is infected determines the likelihood of a chronic infection developing. Ninety-eight percent of babies born to mothers with chronic replicative HBV infection (usually, but not invariably, HBeAg positive) become infected, and 95% of these will develop a persistent infection (**48**). However, only about 10% of adults infected with HBV develop chronic infection (**49**).

2.7 FACTORS LEADING TO HBV PERSISTENCE

The reasons why certain individuals develop chronic infection, rather than a transient acute infection, may relate to specific viral factors or host factors. Viral factors include secretion of HBeAg, secretion of HBsAg, HBV integration and HBV mutants (**49**). Secretion of HBeAg is thought to reduce the cytotoxic T-cell response against infected hepatocytes, possibly by inducing immune tolerance against nucleocapsid-derived peptides displayed on the cell surface (**50**). This

reduces the degree of hepatic inflammation, lessening the risk of death of the host cell and elimination of the virus which is disadvantageous to the virus.

In this way a protracted infection is more likely; a healthy host with persistent infection is more likely to spread the virus to other individuals than a sick host with severe liver disease.

In addition to secreting soluble nucleocapsid proteins into the serum, the virus also secretes surplus surface (envelope) protein (HBsAg) into the blood (**49**). This excess HBsAg can be seen in the serum by electron microscopy as 22nm-sized particles. These particles may confer a biological advantage on the virus, as they will 'divert' antibody to HBsAg away from the intact whole virus particles and thus reduce the chances of virus neutralization by these anti-envelope antibodies (**49**).

It is now clear that HBV DNA becomes covalently integrated into the hepatocyte genome at some time during the chronic infection (**51,52**). The cells containing integrated sequences must evade the immune elimination processes and probably do so by not expressing HBc and HBe antigens (the putative targets for cytotoxic T cells). Since the preferred site for integration on the viral genome is in the promoter region of the HBV core gene, this transcription unit is destroyed during the integration process and thus no nucleocapsid antigens are produced (**53**). HBsAg, however continues to be expressed in these cells because this gene is intact in the integrated viral sequence. As long as the cell is not recognized by cytotoxic T cells as being infected, integration of the virus will result in viral persistence.

Host factors that may lead to HBV persistence include deficient response to interferon (IFN), antibody to nucleocapsid antigens, anti-idiotype response and gender and hormonal factors (**49**). In acute HBV infection, IFN-α is responsible for the initial increase in MHC class I antigen display on hepatocyte membranes and the activation of the 2,5-0ligoadenylate synthetase[2,5-OAS], endonuclease and protein kinase systems, which result in an effective anti viral state within the liver (**49**).

However, in chronic HBV-infected subjects there is evidence of abnormal activation of hepatocytes by interferon: levels of 2,5-OAS are only minimally elevated and MHC class I proteins are present only in very low density on these infected hepatocytes (**54,55**). MHC class I display is believed to be important for recognition and elimination of HBV-infected hepatocytes by cytotoxic T cells, and the decreased MHC expression, in addition to the reduced 2,5-OAS activation, may increase the likelihood of persistent HBV infection (**56**).

Antibodies to nucleocapsid antigens (anti-HBc and anti-HBe) have been shown to block cytotoxic T cell killing of HBV-infected hepatocytes (**57**). This blocking effect may be the result of simple steric hindrance if the B-and cytotoxic T-cell epitopes are displayed close to each other on the cell membrane. Thus circulating anti-HBc may modulate T-cell mediated lysis of infected cells and may be one mechanism leading to viral persistence (**58**).

One important method of immunoregulatory control is achieved by synthesis of antibodies against the variable region of antibodies. These antibodies, known as anti-idiotypes, can either enhance or, more commonly, limit the immune response

Hepatitis B Acute Infection

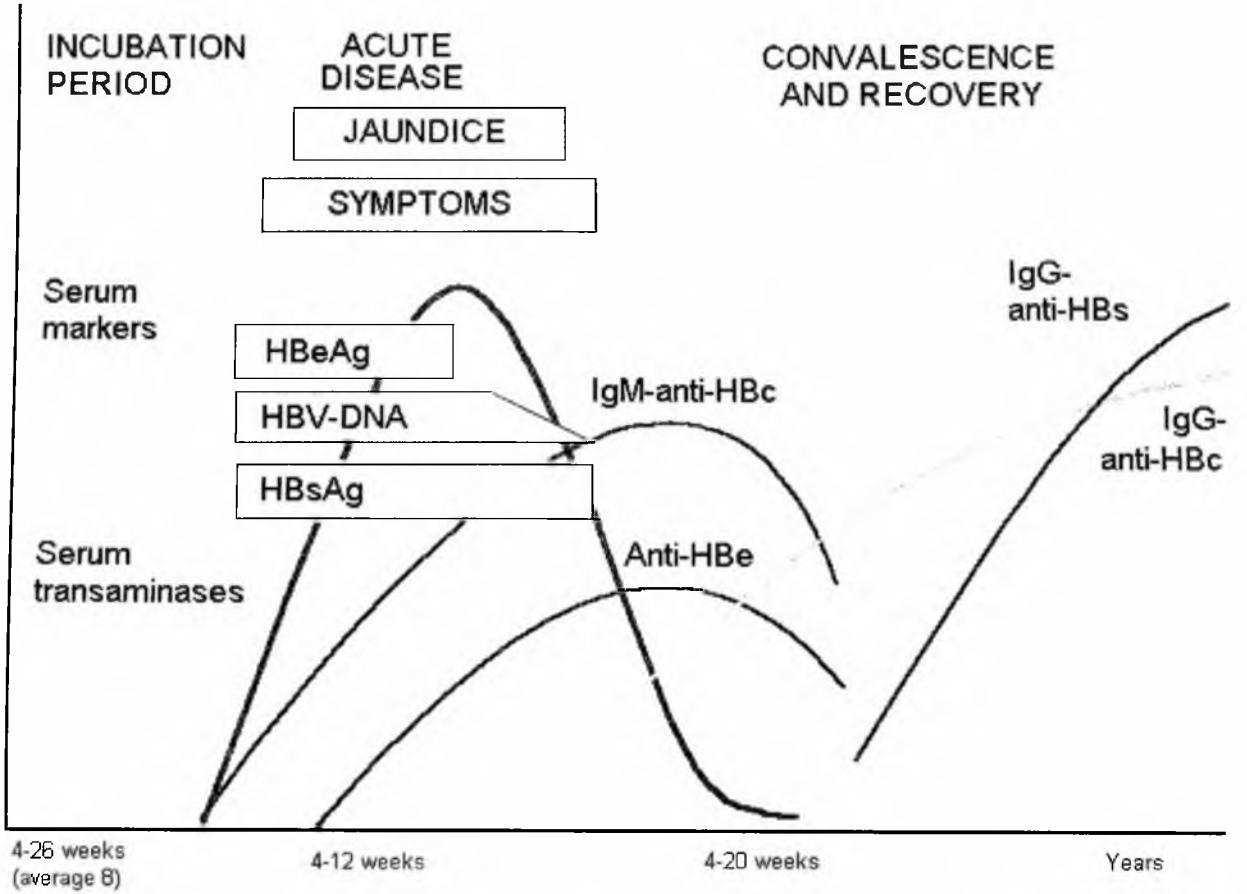


Fig. 1 Adapted from The Pathologic Basis of Disease, Robbins and Kumar, eds. 6th ed.

(**59**). One recent study looking at anti-idiotype antibodies in HBV infection showed that 70-80% of patients with persistent infection had these antibodies (**60**).

Following exposure to HBV infection in adulthood, the likelihood of remaining chronically infected is greater in males than in females (**49,61**). The reasons for this difference are not known, although it may be speculated that they are hormonal in origin.

2.8 CLINICAL FEATURES, SERUM MARKERS AND SEROLOGIC DIAGNOSIS OF HBV INFECTION

2.8.1 ACUTE HBV INFECTION

. In newly infected subjects there is a mean incubation period of 6-8 weeks from exposure to clinical symptoms and the length of time depends on the size of the inoculum and host factors (**62**). Clinical symptoms include a prodrome of anorexia, nausea, vomiting, arthralgia, urticaria and a low grade fever which precede jaundice. Tender hepatomegaly and splenomegaly may occur.

The sera of patients with acute hepatitis B may display an entire spectrum of antigens, antibodies and DNA related to the virus (fig.1) at one time or another (**63**). These markers are in dynamic change and the changes are a reflection of virus replication and the patient's immune response. HBs Ag appears before the onset of symptoms, peaks together with elevated transaminase levels during overt disease and then declines to undetectable levels in 3 to 6 months (**11**). HBsAg is a marker

of active infection and it remains the primary diagnostic and screening marker for hepatitis B (**64**). It is however now possible to use several other serological tests in a work-up of patients suspected of hepatitis B in order to avoid errors and to resolve the complexities of the diagnostic staging in chronic hepatitis B (**65**). HBeAg, HBV DNA and DNA polymerase appear in the serum soon after HBsAg before onset of symptoms and all signify active viral replication (**11**). HBeAg usually declines within weeks, persistence indicates probable progression to chronic disease. The disappearance of HBeAg, HBV DNA and DNA polymerase from serum and the seroconversion to anti-HBe precedes clearance of HBsAg and such events predict recovery (**63**).

IgM anti-HBc is usually the first antibody to appear followed shortly by anti-HBe (**11**). IgG anti-HBc slowly replaces the 1gM. IgG anti-HBs does not rise until the acute disease is over and is usually not detectable for a few weeks to several months after the disappearance of HbsAg (**11**). Anti-HBs may persist for life, conferring immunity.

2.8.2 CHRONIC HBV INFECTION

In most adult cases of acute hepatitis B, serum HBsAg disappears within 3-4 months after exposure but in about 5% of patients antigenaemia will be detected for more than 6 months (**62**). A 6-month persistence of HBsAg by convention defines the carrier state and most remain chronically infected and experience several possible outcomes (**11**).

Hepatitis B Chronic Infection

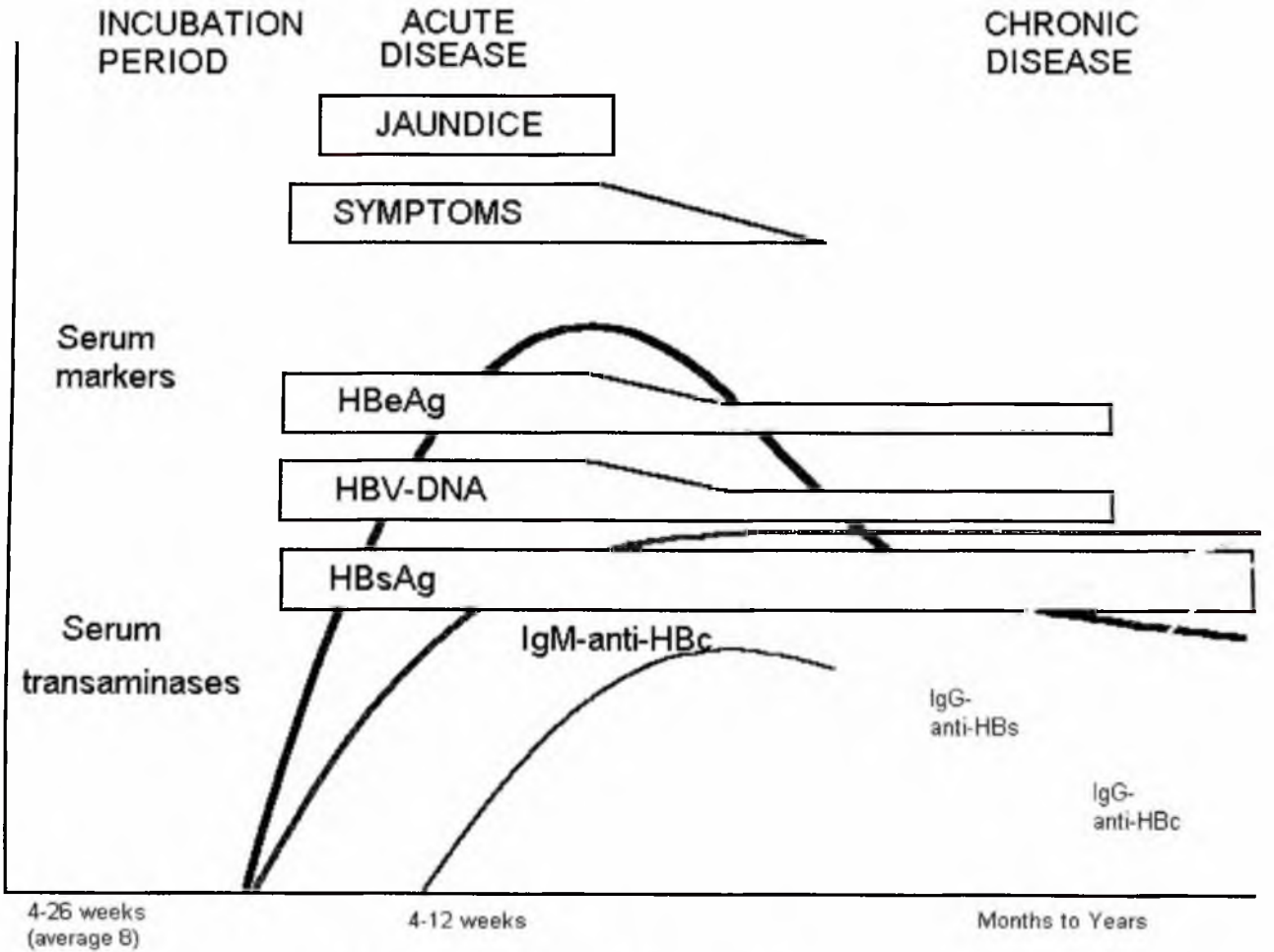


Fig. 2 Adapted from The Pathologic Basis of Disease, Robbins and Kumar, eds. 6th ed.

After the acute phase, the marker pattern becomes distinguished as a rather stable one (fig. 2); HBsAg persists whilst IgM anti-HB_C declines slowly but markers of viral replication-HBeAg and HBV DNA - remain detectable, with anti-HBe and anti-HB_S usually undetectable (**63**). Elevated Alanine-aminotransferase (ALT) values indicate ongoing active hepatitis. Some carriers will have persistently active hepatitis and will progress to cirrhosis and possibly HCC (**66**).

At unpredictable intervals after the acute phase, many patients become asymptomatic carriers. That is, while HBsAg and anti-HBc persist, ALT levels return to near normal and seroconversion from HBeAg to anti-HBe occurs (**65**). HBV DNA declines or is undetectable, but patients remain infectious. This transition from the active to asymptomatic chronic infection can occur directly after the acute phase or it may happen years later. Occasionally asymptomatic carriers may also experience a return to active hepatitis and reappearance of HBeAg and HBV DNA (**62**). A few chronic carriers have serum levels of HB_SAg below detectable limits. While asymptomatic carriers appear to be in an inactive state of hepatitis they remain at significantly increased risk of cirrhosis and hepatocellular carcinoma.

2.9 HEPATITIS C VIRUS

Hepatitis C Virus was discovered in 1989 by Choo et al(**67**).It is believed to be the most important cause of transfusion associated hepatitis, being responsible for 90-95% of all cases of post transfusion Non-A, Non-B (NANB) hepatitis (**68**). Much of our understanding of NANB hepatitis as well as the discovery of its major

causative agent, hepatitis C virus came from studies in Chimpanzees (**67**). The first proof that NANB hepatitis was indeed an infectious disease caused by a transmissible agent came from almost simultaneous independent reports of successful transmission of human NANB hepatitis to chimpanzees and the demonstration of serial transmission to other animals (**69,70,71**). The first specific serological assay for HCV infection was not developed until 1989 and this resulted from the partial cloning of the HCV genome following its recovery from plasma of an experimentally infected chimpanzee (**72**).

2.10 MORPHOLOGY AND SUBTYPES OF HCV

HCV occupies a genus in the Flaviviridae family (**73**) and is a small enveloped, single-stranded RNA Virus. It has a genome composed of about 9500 nucleotides that codes for a single polyprotein precursor 3000 amino acids long that is processed to yield a variety of structural (Virion) and non-structural (NS) proteins (**74**).

As observed for other RNA viruses there is a substantial fluidity of the HCV genome resulting from an error-prone replicase and the absence of repair mechanisms that operate during DNA replication (**75**). In addition the process of host selection and adaptation of a rapidly mutating genome has led to the evolution of many distinct (yet still fluid) HCV genotypes (**76**).Presently six major types designated by Arabic numerals (1,2,3 etc) and further divided into subtypes (more than 70 and growing) designated by letters (a,b,c etc) have been reported (**77**).

The distribution of HCV genotypes has been found to vary geographically (**78**). Genotypes 1, 2 and 3 and their subtypes are distributed worldwide. In contrast, genotype 4 appears to be a pan-African type (the principal genotype in Zaire and Egypt) and genotype 5 has been found to be the principal genotype in South Africa (**79,80**). Genotype 6 and its variants have been found mainly in Asia (**78,81**). Studies suggest that the clinical features of liver disease depend on HCV genotypes (**82,83**). It is also noteworthy that the success of interferon treatment seems to be type or subtype related (**84,85**). These observations make the identification of infecting HCV genotypes from different geographic regions of great interest.

In a study of HCV genotypes in Ghanaians living in Kumasi, the commonest genotype was type 2 and the sequences of the isolates did not exactly match any of the type 2 subtypes suggesting that these isolates are indigenous to Ghana (**19**).

2.11 EPIDEMIOLOGY AND NATURAL HISTORY OF HCV INFECTION

It is estimated that over 170 million people are infected with HCV worldwide (**86**). Studies of volunteer blood donors and general populations have shown considerable geographic variation in HCV seroprevalence rates (**87,88**): 0.5-1.5% in Western Europe, Northern Europe, North America and Australia; 1.5-2.5% in Japan and the Mediterranean region; and as high as 14% in Egypt. Prevalence in different regions of Saudi Arabia range from 1.5% to 5.7% (**89,90**). In West Africa, a study done in Nigeria indicated anti-HCV prevalence of 5% (**91**) and studies done in Ghana put the prevalence rate between 2.8 and 5.4% (**17-19**).

Hepatitis C Acute Infection

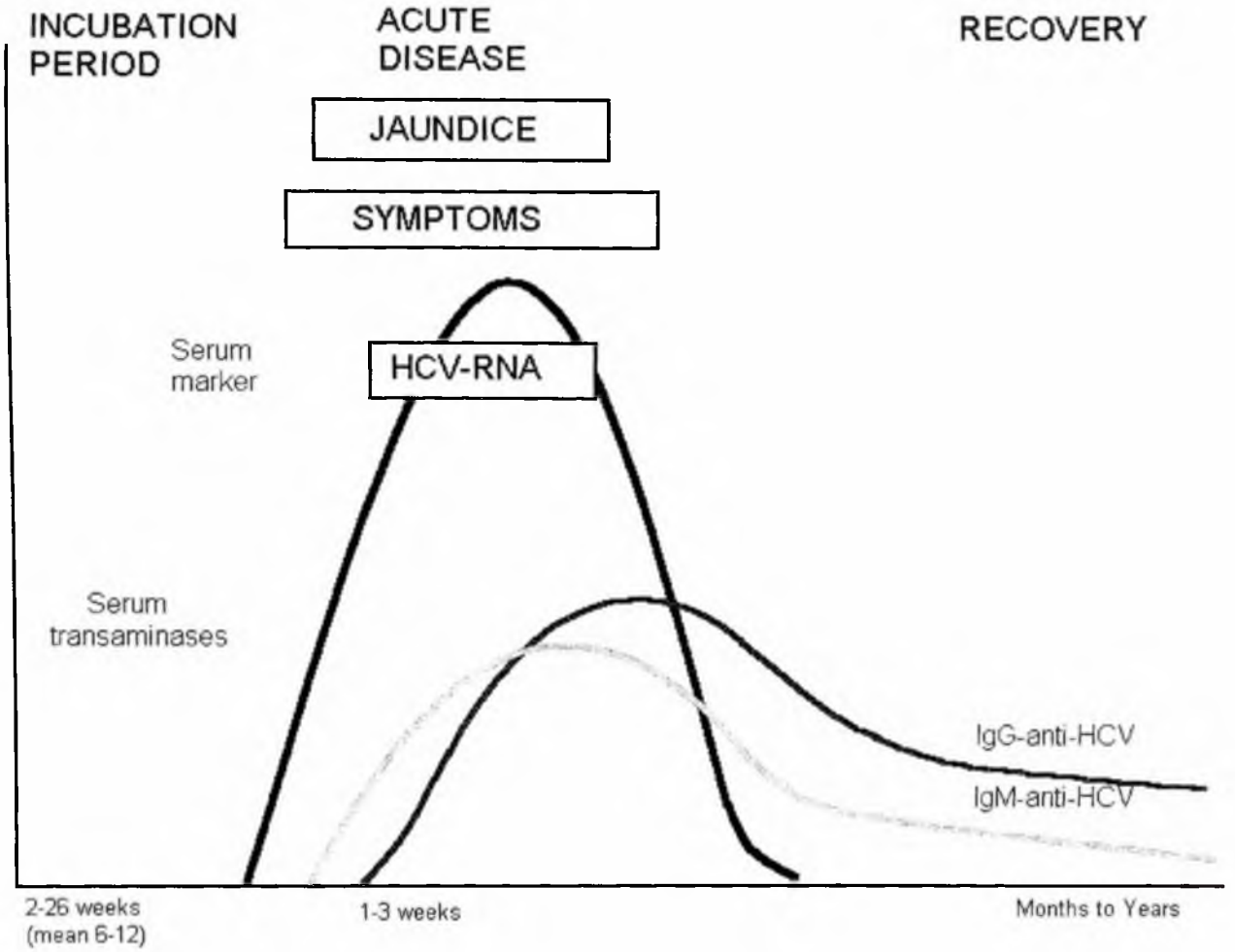


Fig. 3 Adapted from The Pathologic Basis of Disease, Robbins and Kumar, eds. 6th ed.

The known routes of transmission of HCV are inoculations and blood transfusions whilst sexual and vertical transmission are infrequent (**11,92,93**). However, up to 40% of all HCV cases cannot be traced to any obvious source (**94, 95**). Haemodialysis patients, haemophiliacs and intravenous drug abusers are high risk groups (**96**).

In more than 85% of HCV acute hepatitis the infection persists and leads to chronic hepatitis (**97**). A characteristic feature of infection is repeated bouts of hepatic damage resulting from reactivation of pre-existing infection or the emergence of an endogenous new mutant. This typically manifests as episodic elevations in serum transaminases (**11**). Once chronic hepatitis is established, it may slowly progress to worsening stages of fibrosis and cirrhosis and eventually to the development of HCC (**98,99**).

2.12 CLINICAL FEATURES, SERUM MARKERS AND DIAGNOSIS OF HCVINFECTION

HCV acute hepatitis has a mean incubation period of 6-12 weeks (fig.3) and has similar but usually milder clinical features than HBV acute hepatitis. However, individual cases may be severe and indistinguishable from HBV hepatitis (**11**).

In patients with HCV infection, an episodic pattern of aminotransferase elevation is common but a specific diagnosis of hepatitis C can be made by demonstrating the presence in serum of anti-HCV (**100**). When a second or third generation immunoassay (that detects antibodies to non-structural and nucleocapsid proteins) is

Hepatitis C Chronic Infection

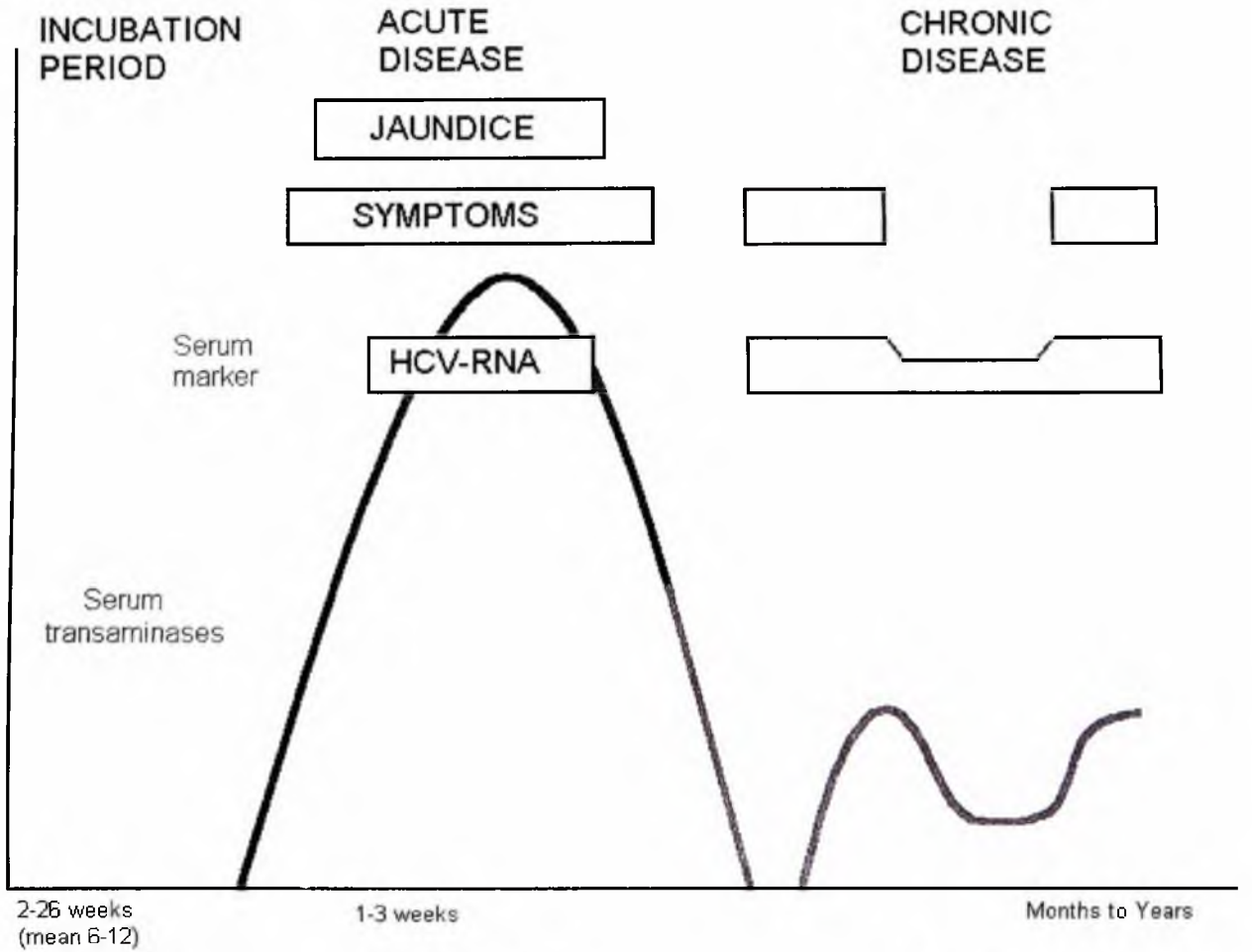


Fig. 4 Adapted from The Pathologic Basis of Disease, Robbins and Kumar, eds. 6th ed.

used, anti-HCV can be detected in acute Hepatitis C during the initial phase of elevated aminotransferase activity. This antibody may never become detectable in 5-10% of patients with acute hepatitis C and levels of anti-HCV may become undetectable after recovery from acute hepatitis C (**63**). In patients with chronic hepatitis C, anti-HCV is detectable in over 95% of cases (**63**). Assays for HCV RNA are the most sensitive tests for HCV infection and represent the " gold standard" in establishing a diagnosis of hepatitis C (**100**). HCV RNA can be detected even before acute elevation of aminotransferase activity and before the appearance of anti-HCV in patients with acute hepatitis C. In chronic hepatitis C (fig.4), HCV RNA remains detectable indefinitely, continuously in most but intermittently in some patients (**63**).

2.13 CIRRHOSIS OF THE LIVER

2.13.1 DEFINITION AND CLASSIFICATION

Cirrhosis of the liver is an end stage chronic liver disease characterized by three morphologic features.

1. Diffuse bridging fibrous septa in the form of delicate bands or broad scars replacing multiple adjacent lobules.
2. Diffuse parenchymal nodules created by regeneration of encircled hepatocytes and
3. Disruption of the architecture of the entire liver.

Cirrhosis of the liver can be classified either by morphology or by aetiology (**101**). Morphologically, there are three kinds of cirrhosis based on the size of the regenerated hepatocyte nodules. These are:

1. Micronodular(< 3mm diameter nodules)
2. Macronodular(>3mm diameter nodules) and
3. Mixed nodular (a mixture of both micronodules and macronodules).

The aetiology of cirrhosis varies both geographically and socially. The following is the approximate frequency of aetiologic categories in the western world (**11**).

Alcoholic liver disease	60-70%
Viral hepatitis	10%
Biliary disease	5-10%
Primary haemochromatosis	5%
Wilson disease	rare

α_1 -antitrypsin deficiency	rare
Cryptogenic cirrhosis	10-15%.

2.13.2 CLINICAL FEATURES AND DIAGNOSIS OF CIRRHOSIS

The early stages of cirrhosis are usually asymptomatic. The initial non-specific symptoms may include anorexia, weight loss, weakness, loss of libido and impotence. The major clinical features depend on the two main pathological processes of hepatocellular failure and portal hypertension **(1,11)**. These include hepatic encephalopathy, jaundice, abdominal distension with ascites and dependent oedema. The liver may be enlarged at the early stages but shrinks progressively and becomes hard at later stages. Patients may develop hepatocellular carcinoma, infections and renal failure as complications.

The diagnosis has to be suspected clinically from the history, a firm nodular feel of the liver, signs of portal hypertension and hepatocellular insufficiency(**1**). Liver function tests and coagulation abnormalities reflect parenchymal cell damage. Transaminase levels are elevated if active necrosis is present. Abdominal Ultrasound is a useful non-invasive procedure for supporting the diagnosis (**161**). However, where available a liver biopsy could be done for a histological diagnosis.

2.14 EPIDEMIOLOGICAL ASSOCIATION BETWEEN LIVER CIRRHOSIS, HCC AND HEPATITIS B AND C VIRUS INFECTIONS

Once cirrhosis is established, it is usually difficult to clearly distinguish an aetiologic diagnosis on morphologic grounds alone. However, by means of

serological studies, the association of viral hepatitis with liver cirrhosis has been studied in various places in the world and well documented (**2 - 6**).

In the natural history of HBV infection it is estimated that 10-33% of those who develop persistent infection end up with chronic hepatitis of which 20-50% may develop liver cirrhosis (**11**). Several studies done in various places have documented moderate to high prevalence of HBsAg among hepatic cirrhosis patients (**2,3,42,102**).

In Nigeria, a study by Baba et al (**102**) on the prevalence of HBs Ag among patients with liver disease indicated that 10 (56%) out of the 18 liver cirrhosis patients studied were positive for HBsAg. A similar study carried out in Ghana by Archampong (**42**) indicated that 7(39%) out of the 18 cases of liver cirrhosis were positive for the HBsAg. One hundred and fourteen cirrhotic livers at autopsy were studied for the presence of hepatitis B surface antigen by orcein staining by Sundaram C et al (**2**) in South India. Sixty-eight percent were positive for the antigen. There were 13 cases of macronodular, 55 mixed nodular and 46 micronodular cirrhosis and their antigen positivity was 100%, 98.7% and 21.7 respectively.

A total of 516 patients with chronic hepatitis B were followed up longitudinally to determine their outcome in Taiwan and during a mean follow up period of 5.7 $\pm$ 3.4 years (range 1-17years), cirrhosis occurred in 71 patients with a calculated annual incidence of 2.4%(**3**).

In the United States it is estimated that about 85% of acute HCV infections end up with chronic hepatitis and that about 20% of this may develop liver cirrhosis (**11**).

A prevalence rate of 22% for anti-HCV antibodies was found among 150 non-alcoholic liver cirrhosis patients in Taiwan (**4**). A long-term clinical and histopathological follow up of 65 cases of chronic post–transfusion hepatitis carried out by Di Bisceglie et al (**5**) indicated that 82% had antibody to hepatitis C and that 20% of the anti-HCV positive patients developed liver cirrhosis between 1.5 and 16 years after blood transfusion. Longitudinal studies conducted in patients who had acquired hepatitis C by blood transfusion for 15-25 years indicated that 20-30% of them developed cirrhosis (**6**).

Several HBV and HCV case-control studies have proven a possible aetiologic role for these viruses in HCC either by inducing liver cirrhosis (**103 -106**) or by being directly oncogenic (**107 - 112**). HCC is one of the most common malignant neoplasms in the world (**11,113**). Epidemiological survey from different areas has shown a relation between this neoplasm and several factors, especially infection with HBV (**113-116**). The role of HBV as the sole aetiologic agent in HCC has been reported extensively in clinical experimental studies (**117-119**) and is particularly important in countries with a high prevalence of HBV infection (**113,120**). However, in low prevalence areas, HCC is more commonly related to other factors especially cirrhosis in patients who do not have HBsAg (**120,121**). Whether cirrhosis itself is a preneoplastic state or whether onset of HCC in patients with cirrhosis is triggered by other factors, remains unsettled. It has been suggested that HCV may act as such a trigger in many patients with cirrhosis (**120,121**).

In Asia and Africa, hepatitis B surface antigen frequently occurs in patients with HCC (**122**). In Italy, there is evidence that the incidence of HCC may be increasing

(**123**) and that in addition to HBV and alcoholism, there may be other important predisposing factors (**124,125**). Foli et al investigated the association between HBsAg and HCC in Ghana (**160**) and discovered that 33% of 114 HCC cases were positive for HBsAg compared with 9.3% of 268 control cases.

In Taiwan an HBV endemic area, a close association between HBV and HCC was first reported in 1971 and 80% of the Chinese patients with HCC were found to be chronic HB_SAg carriers (**126**). This was supported by another study which showed HBV as the major cause of chronic liver disease and HCC in the country (**127**). However, HCV-related chronic liver diseases have in addition been found by other investigators (**123,128**). Colambo et al in 1989 established that HCV is an important factor associated with HCC and NANB hepatitis (**129**). Among Spanish patients, experimental results indicate that HCV infection may have a role to play in the pathogenesis of chronic liver disease, apparently related to other agents such as alcohol, and that the virus may be found in a large proportion of patients with cryptogenic cirrhosis (**130**). Other studies have indicated a possible causal role of HCV infection in HCC (**127 - 132**).

In Ghana viral hepatitis has been recognized as an endemic disease. The reported prevalence of asymptomatic HBsAg carrier is between 6-15.6%(**12-15**). Because of this high asymptomatic carrier rate of HBsAg, Foli et al (**16**) investigated the frequency in order to assess the importance of HBV infection among patients in Korle Bu Teaching Hospital. The results indicated that the majority of severe

hepatitis cases admitted to the hospital in Accra was due to HBV and the disease may, though infrequently, progress to cirrhosis. They therefore suggested that the role of HBV infection in the genesis of chronic liver diseases in the country be further investigated (**16**).

2.15 PATHOGENESIS OF LIVER CIRRHOSIS AND HEPATOCELLULAR CARCINOMA IN CHRONIC HBV/HCV INFECTIONS

The central pathogenetic process in liver cirrhosis is progressive fibrosis (**11**). Chronic inflammation and hepatocyte damage leads to release of inflammatory cytokines such as Tumour necrosis factor- alpha (TNF-α), Transforming growth factor- beta (TGF-β) and Interleukin-1 (IL-1) which stimulate hepatic stellate cells to synthesize and deposit collagen types I and III in the lobule creating delicate or broad septal tracts (**11**). Throughout this process of liver damage and fibrosis, the remaining hepatocytes are stimulated to regenerate and they proliferate as spherical nodules within the confines of the fibrous septa. The net outcome is a fibrotic nodular liver with disruption of the entire liver architecture resulting in compromised delivery of blood to hepatocytes and ability of hepatocytes to secrete substances into plasma (**11**).The causes of liver cirrhosis are many and varied but once cirrhosis is established, it is usually difficult to clearly distinguish an aetiologic diagnosis on morphologic grounds alone.

Although the molecular origins of HCC remains unclear, chronic HBV and HCV infections are believed to be associated with HCC for the following reasons:

1. Chronic hepatitis by these viruses may lead to liver cirrhosis which is a major risk factor for HCC. Repeated cycles of cell death and regeneration are important in the pathogenesis of HCC because accumulations of mutations during continuous cycles of cell division may eventually transform some hepatocytes (**133**).
2. In virtually all cases of HBV-associated liver cancer the viral DNA is integrated into the host cell genome and the tumours are clonal with respect to these insertions. This indicates that the viral integration precedes or accompanies transformation (**133,134**).
3. The HBV X protein is proposed to play a role in carcinogenesis, acting as a transactivator of cellular and viral promoters. This protein is believed to disrupt normal growth control of infected cells by transcriptional activation of several host growth-promoting genes such as insulin-like growth factor II and receptors for insulin-like growth factor I (**133,135**). HBV X protein also binds to the tumour suppressor gene p53 and may interfere with its growth-suppressing activity (**26**).
4. Cirrhosis almost invariably precedes the onset of hepatocellular carcinoma in HCV chronic liver infections (**135,136**). However, rarely, the tumour has been reported to arise in non-cirrhotic livers of HCV-infected individuals which, supports the suggestion that the virus may be directly oncogenic (**137,138**). HCV is an RNA virus and cannot integrate into the host cells genome or express reverse transcriptase activity as HBV does. So its role in carcinogenesis has generally been assumed to be that of a chronic necro-inflammatory agent causing cirrhosis (**135**). Negative (replicative) and positive (genomic) strands of the virus are present in both liver and tumour

tissue (**139,140**) and this indicates that HCV not only infects hepatocytes but replicates in them during and even after malignant transformation. One study has suggested that irregular regeneration of the liver not respecting acinar architecture is an important morphological pathway to cancer in chronic hepatitis and cirrhosis due to HCV (**141**). On the molecular level, accumulation of mutant forms of the HCV core protein in liver cell nuclei has been suggested to be the most likely oncogeneric event possibly by down-regulating the activity of p53 gene (**142,143**). Hepatocellular carcinoma has also developed in mice transgenic for the HCV core gene (**144**). Finally, the non-structural protein gene NS3 has been shown to transform NIH3T3 mouse fibroblasts (**145**).

CHAPTER 3: MATERIALS AND METHODS

A nested case-control study was used in achieving the study objectives. Seventy consenting patients (cases) clinically diagnosed as liver cirrhosis on the Medical and Surgical wards of the Korle-Bu Teaching Hospital and supported by abdominal ultrasound, were interviewed as and when they came on admission. In selecting each of the above cases it was ensured that **all** the following criteria were met:

1. deranged liver function test suggestive of chronic liver disease with or without the presence of jaundice,
2. diffuse liver nodularity detected by palpation and ultrasonographic studies,
3. presence of ascites clinically or by ultrasonographic studies,
4. signs of portal hypertension clinically or by ultrasonography and
5. the ultrasonographic studies were done by qualified Radiologist at the Radiology Department of the Korle Bu Teaching Hospital.

This was done over a period of one year and a questionnaire (refer appendix II) was administered to each one for information on name, age, sex, occupation and history of blood transfusion. For each case interviewed, four consenting patients age (±5years) and sex-matched were selected as controls based on all of the following criteria:

1. patient on admission with a non-hepatic disease,
2. no history or clinical evidence of hepatic disease and
3. a normal liver function test.

Blood was taken from all subjects and the serum separated into well labelled microtubes for storage at -70^{0}c till analysis.

Once thawed, the samples were analyzed for HBsAg, anti-HCV antibodies and liver function. For the detection of HBsAg, all samples were initially screened with a Serodia haemagglutination test kit. After that all the positive samples were confirmed using an ELISA test kit. Anti-HCV antibodies were detected using the anti-HCV ELISA third generation Kit. Absorbance values measured at 450nm which were greater than the calculated cut-off values were considered positive in the ELIZA tests (refer appendices III and IV for ELIZA test procedure details). Liver function tests (albumin, alanine and aspartate aminotransferases) were run on samples using an automatic analyzer. Controls with deranged liver function were excluded from the study. To test the validity of the criteria used in diagnosing cirrhosis, autopsies were performed on all the cases that died during the study period. The results were analysed statistically using EPI-INFO 2000 at a 5% significance level.

CHAPTER 4: RESULTS

There were a total of 70 cases of liver cirrhosis and 280 controls selected on the basis of the criteria stated in the methodology section. Liver biopsies for histological diagnosis of cirrhosis were not done because it would have necessitated the same procedure having to be done also on the controls who did not have liver disease clinically. This would have created ethical problems. Autopsy was therefore done on all the cases that died during the study period to confirm the clinical and ultrasonographic diagnosis of liver cirrhosis. Of the 70 cases, 18 died and autopsy done on all confirmed the diagnosis of cirrhosis morphologically indicating that the criteria used in the diagnosis of cirrhosis were together sensitive and specific.

4.1 AGE DISTRIBUTION OF CIRRHOSIS PATIENTS(CASES)

Fig 5 shows the age distribution of the cases. The mean age is 46 years (SD 15.8) with a range from 15 to 90 years. The mean age of male cases of 46.1years was not different from that of female cases 46.0 years. The mean age of HBV positive cases (39.1yrs) was significantly lower than that of HBV negative cases (50.7) with a P value of 0.002. However, the mean age of HCV positive cases (47.2) was not significantly different from that of HCV negative cases (45.9), the P value being 0.860.

Fig. 5 Age distribution of cirrhosis patients

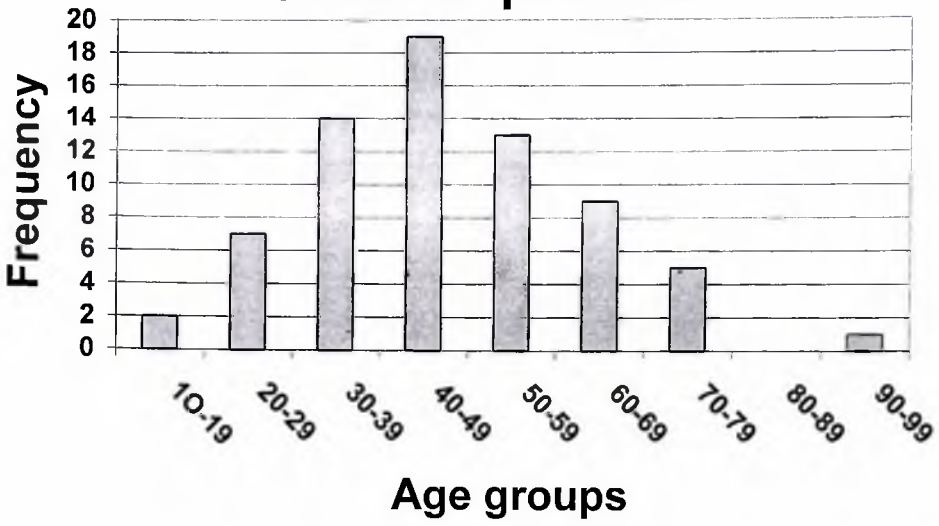
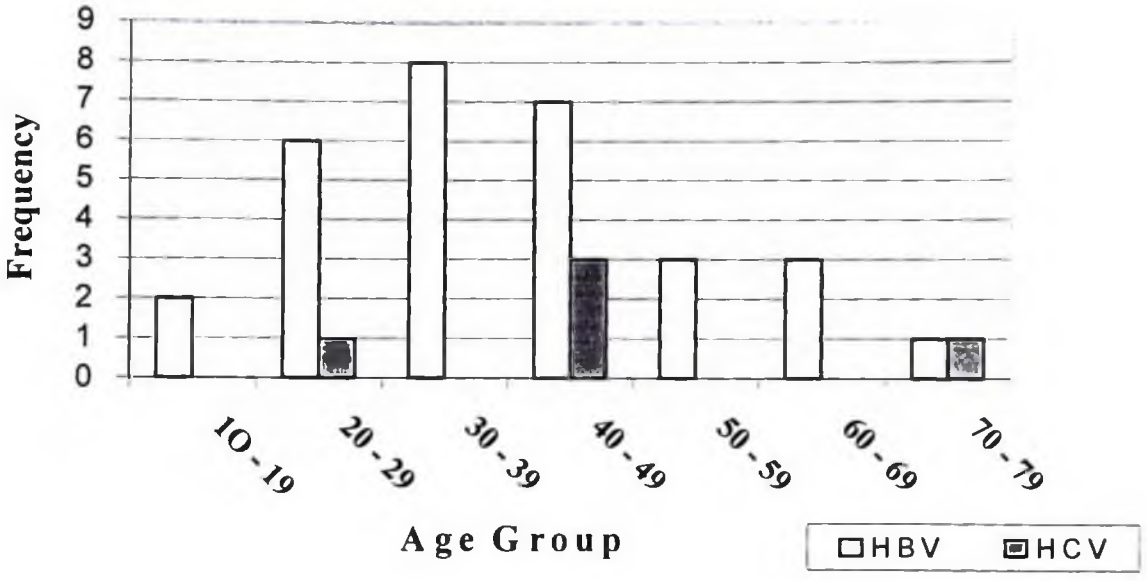


Fig. 6 shows the age distribution of HBV and HCV positive cirrhosis cases. Fifty percent of the HBV positive cases were within the age groups 30-49 years whilst three out of the only five HCV positive cases were in age group 40-49 years.

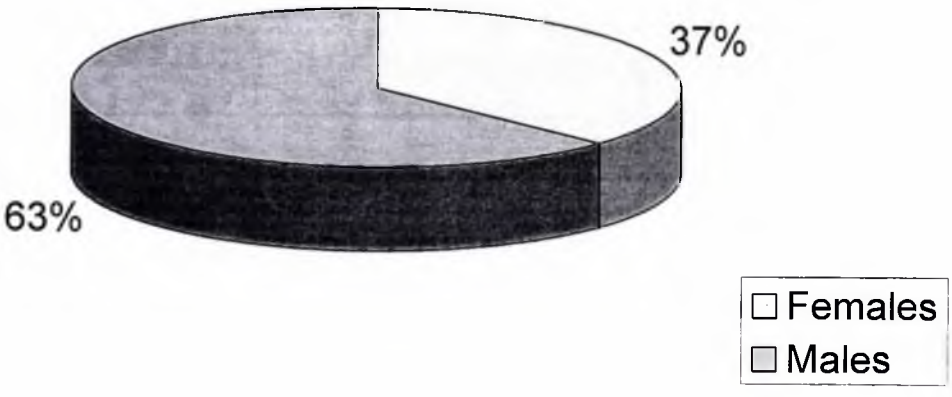
Fig. 6 Age Distribution of HBV Positive and HCV Positive cirrhosis cases



4.2 SEX DISTRIBUTION OF ALL CASES WITH CIRRHOSIS

The sex distribution of cases is as shown in the diagram below (fig 7). There were 44 (63%) males and 26 (37%) females.

Fig. 7 Sex distribution of all cases with cirrhosis



Tables 1-4 were constructed to determine whether there is any significant association between HBV/HCV status and gender. Although there were more male cases of cirrhosis than females it is clear from these tables and their corresponding P values (all > 0.05) that there was no significant association between HBV or HCV status and gender in both cases and controls.

Tab. 1 Sex distribution of HBV positive and HBV negative cases

GENDER	HBV NEGATIVE	HBV POSITIVE
FEMALE	14	12
MALE	26	18
TOTAL	40	30

χ^2=0.031 p=0.858 odds ratio 0.807 95%CI= 0.303 – 2.146

Tab. 2 Sex distribution of HBV positive and HBV negative controls

GENDER	HBV NEGATIVE	HBV POSITIVE
FEMALE	98	6
MALE	161	15
TOTAL	259	21

χ^2=0.372 P=0.541 ODDS RATIO=1.521 95%CI=0.571-4.052

Tab. 3 Sex distribution of HCV positive and HCV negative cases

GENDER	HCV NEGATIVE	HCV POSITIVE
FEMALE	24	2
MALE	41	3
TOTAL	65	5

Fisher exact χ^2=0.117 P=0.736 ODDS RATIO=0.878

95%CI=0.136-5.633

Tab. 4 Sex distribution of HCV positive and HCV negative controls

GENDER	HCV NEGATIVE	HCV POSITIVE
FEMALE	97	7
MALE	172	3
TOTAL	269	10

Fisher exact χ^2=3.409 P=0.993 ODDS RATIO=0.241

95%CI=0.061-0.956

4.3 PREVALENCE OF HBV INFECTIONS AMONG CASES AND CONTROLS

Thirty out of the seventy cases of cirrhosis studied were positive for HBsAg giving a prevalence rate of 42.8% (Tab. 5). Of the 280 controls 21 were positive for HBsAg giving a prevalence rate of 7.5% among controls. This difference in HBsAg prevalence between the cases and controls is statistically significant (P=0.000) and the risk of developing cirrhosis from HBV is about nine-fold that of the uninfected person.

Tab. 5 Prevalence of HBV among cases and controls

HBV	CASES	CONTROLS
POSITIVE	30	21
NEGATIVE	40	259
TOTAL	70	280
PREVALENCE	42.8%	7.5%

[χ^2=56.078, P=0.000, Odds Ratio=9.25 and 95% CI:].

4.4 PREVALENCE OF HCV INFECTIONS AMONG CASES AND CONTROLS

The number of cases positive for anti-HCV was 5 out of the 70 giving a prevalence rate of 7.1%. Ten out of 280 controls (3.6%) were positive for anti-HCV (Tab. 6). The risk of HCV infection resulting in cirrhosis of the liver is about twice the risk

of the event not happening. However, the difference in anti-HCV prevalence between cases and controls was not statistically significant (P=0.189).

Tab. 6 Prevalence of HCV among cases and controls

HCV	CASES	CONTROLS
POSITIVE	5	10
NEGATIVE	65	270
TOTAL	70	280
PREVALENCE	7.1%	3.6%

[Fisher exact χ^2 =1.717, P=0.189, Odds Ratio=2.07, 95% CI=0.159, 1.462].

Only three cases and one control subject were positive for both the HBsAg and anti-HCV.

4.5 ASSOCIATION BETWEEN HBV/HCV INFECTIONS AND BLOOD TRANSFUSION HISTORY

The overall association between HBV infection and blood transfusion history is shown in Tab. 7. About 8% of the study population (cases and controls) who were HBV positive had had previous blood transfusion compared to 2% of the HBV negative individuals. The statistical analysis shows that individuals who receive blood transfusion have four times the risk of those who do not and the difference is significant (P=0.043).

Tab.7 HBV infection and transfusion history among both cases and control subjects

TRANSFUSION	HBV POSITIVE	HBV NEGATIVE
YES	4	6
NO	47	293
TOTAL	51	299
% YES	7.8%	2.0%

[Fisher exact χ^2 =5.302; P=0.043; Odds Ratio = 4.141; 95% CI=1.126, 15.230]

Table 8 shows HCV infection and blood transfusion history. Statistical analysis shows that even though the risk of developing HCV infection from blood transfusion is about three times as from other sources, the difference is not significant (P=0.330)

Tab. 8 HCV infection and transfusion history among both cases and control subjects

TRANSFUSION	HCV POSITIVE	HCV NEGATIVE
YES	1	8
NO	14	327
TOTAL	15	335
% YES	0.06%	0.02%

[Fisher exact χ^2=1.033; P=0.330; Odds Ratio=2.901 95% CI= 0.339 - 24.827]

CHAPTER 5:DISCUSSION

The gold standard for the diagnosis of cirrhosis is a morphologic one using either histopathological examination of liver biopsies or gross examination of the liver. However, these methodologies were not used for the initial diagnosis of cirrhosis in all cases as this would have required that the same methods be used to select controls. To overcome this weakness in the study, stringent combined clinical and radiological criteria were used. This choice was vindicated by the fact that all 18 cases of cirrhosis who died were proved by autopsy to have cirrhosis giving a 100% sensitivity and specificity.

Cirrhosis of the liver in general can occur at any age but manifests particularly in young adults leading to premature death (**146**). In this study the mean age of the cases was 46 years (SD 15.8) with a range of 15-90 years. The age pattern shown in fig. 1 rises to a peak in the fifth decade and declines gradually after that. This shows that most people develop chronic hepatitis either in childhood or in early adulthood and progress to cirrhosis and die from about middle age onwards. This age pattern is different from that seen in cirrhosis in advanced countries where most cases of cirrhosis are within the age groups 40 – 70 years and are mostly alcohol associated (**147,148**).

In HBV endemic regions such as Africa and Southeast Asia, spread from an infected mother to a neonate during birth (vertical transmission) is common (**44,46**). About 98% of babies born to mothers with chronic replicative HBV infection become infected and about 95% of these will develop a persistent infection (**48**). It is also estimated that up to 15% of the population in HBV endemic areas acquire

the infection in early childhood and cirrhosis may ultimately develop in about 25% of these chronic carriers (**1**).

When the age distribution of HBV positive cases is examined separately, up to about 75% of the HBV positive cirrhosis cases are below 49 years. This confirms that the majority of the people with HBV associated cirrhosis in endemic areas get infected either in childhood or early adulthood and then progress through chronic hepatitis to cirrhosis. With HCV infection it is difficult to assess the importance of its prevalence among the age groups in this study since only a few cases were positive for anti-HCV.

The mean age of HBV positive cases was significantly lower than that of HBV negative cases (P=0.002) and this could be explained by the fact that HBV infection occurred early in life hence chronic hepatitis and cirrhosis develop in early adulthood. A study by Acquaye et al indicated that 6.4% of Ghanaian pregnant women are positive for HBsAg and that a significant 15% of HBV carrier rate in the population is due to perinatal transmission (**41**). HBV status was however not found to be associated with sex, which implies that both sexes are at equal risk. The study did not find any significant association between HCV status and age or gender and this is in agreement with findings of other studies (**149,150**) and probably indicates that HCV could be acquired at any age and both sexes are equally at risk.

One of the specific objectives in this study was to determine the seroprevalence of HBV infection among patients with liver cirrhosis. Thirty out of the 70 cases studied were positive for HBsAg. This gives an HBV seroprevalence rate of 42.8%

among the cirrhotic patients seen at the Korle-Bu Teaching Hospital in Ghana. The rate among controls was 7.5% (21 out of 280 controls). The prevalence of HBV infections among patients with cirrhosis of the liver has been studied in different places of the world with varying results. In areas where HBV infection is endemic (such as Southeast Asia and Sub-Saharan Africa where prevalence rates among the general population ranges from 5-35% and 9-20% respectively), it is estimated that about 25% of chronic HBV carriers develop cirrhosis (**1**).

In Ghana, a Sub-Saharan West African Country, HBV is endemic with prevalence rates of 6.7-15.6% among the population. Therefore, an HBV prevalence rate of 42.8% observed among cirrhotics supports the known association between HBV and liver cirrhosis worldwide. A recent study by Baba et al in Nigeria, a West African Country, revealed that 10 out of 18 (56%) cases of liver cirrhosis were positive for HBsAg (**102**). Soni et al in a seroprevalence study of HBV among cirrhotics in Natal, South Africa, found 16 out of 77 (21%) positive for HBsAg (**151**). As high as 75.5% prevalence of HBsAg was found by Cenac et al in Niger when they studied 49 cases of cirrhosis (**152**). In Taiwan, where HBV is endemic with general population prevalence of about 20% (**153**), as high as 74.5% prevalence of HBV among 102 cirrhosis cases has been recorded (**105**). An autopsy study by orcein staining for HBsAg among 114 cirrhotic livers at postmortem in South India revealed a 67.5% prevalence (**2**). Kato et al found 34% of 255 cirrhotics positive for HBsAg in Japan (**154**) whilst Sulaiman observed an HBV prevalence of 41.1% among 114 cirrhotics in Jakarta, Indonesia (**155**). In the developed Western World where HBV seroprevalence rates are low (less than 1% of the general population), HBV prevalence among cirrhosis of the liver is less than 10% (**11**).

The HBV prevalence rate as determined by this study is most likely to be the minimum because several studies have shown that the method used in this study (ELIZA) is not as sensitive for detecting HBV infection as polymerase chain reaction (PCR) which detects viral DNA (**156 – 158**). Attallah et al determined the prevalence of HBV infection in liver cirrhosis by detecting both HBsAg and HBV DNA. They discovered that of 48 cirrhotic cases, 10 (21%) had HBsAg and of the remaining 38 cases without HbsAg, 64.5% were HBV DNA positive by PCR. (**156**). In another study it was discovered that using PCR, serum HBV DNA could be detected in 28% of HBsAg negative cirrhotic patients (**157**). Liang et al screened for HBV DNA in 866 healthy blood donors without HBsAg and found that 1.7% were positive for HBV DNA (**158**).

The role of HBV in the development of liver cirrhosis was also determined in this study. HBsAg was significantly associated with liver cirrhosis (χ^2 =56.078, P=0.000). The odds ratio of 9.25 (95% CI=4.83-17.7) found in this study indicates that patients with liver cirrhosis are about nine times more likely to have been infected with HBV than a non-cirrhotic. In a similar study in Taiwan an odds ratio of 12 was observed (**4**).

The seroprevalence of anti-HCV among liver cirrhosis patients found in this study of 7.1% is relatively low compared with previous studies elsewhere. A prevalence of 18.4% among 49 cirrhotics cases for anti-HCV and 6.4% among 47 controls was found in Niger in 1995 (**152**) whilst Soni et al had 18 out of 77 cirrhosis cases (23%) positive for anti-HCV in South Africa (**151**). In Ghana this is the first study

of anti-HCV seroprevalence among cirrhosis patients and so there are no previous figures to compare with. Tsai et al had an anti-HCV sero-prevalence rate of 22% among 150 cirrhosis patients and 2% in 150 controls in Taiwan (**105**) whilst Kato et al recorded as high as 49% (126 out of 255 cirrhotics) in Japan (**154**). A longitudinal study by Di Bisceglie et al in Maryland, U. S. A indicated that 8 out of 39 cases of chronic HCV (20%) developed cirrhosis of the liver after a mean follow up period of 9.7 years (**120**).

The prevalence of anti-HCV among the cases of 7.1% in the study was not significantly different from that among the controls of 3.6% (χ^2=1.717, P=0.189). The odds ratio of 2.07 means that the chances of a chronic HCV patient developing cirrhosis of the liver is twice that of a non HCV infected person. However, the lack of significance of this indicates that the risk is small. This however contrasts sharply with the finding of Tsai et al in Taiwan who observed an odds ratio of 13.8 for anti-HCV in cirrhosis (**4**). Natural history studies in the U. S. A. have also indicated that approximately 20% of individuals with chronic HCV progress to fibrosis and cirrhosis (**97**).

Two possible explanations could be offered here for the low prevalence of HCV observed in this study. Firstly, it is possible that a lot more people who get infected in our environment are able to overcome the acute infection and do not progress to chronic liver disease and cirrhosis. Secondly, studies have shown that the severity of liver disease caused by HCV depends on the genotype and subtype (**82, 85**). For example, Zein et al (**82**) discovered that genotypes 1a and 1b were associated with severe hepatitis and lower rates of response to interferon therapy than genotypes 2a

and 2b. In the only study of HCV genotypes done in Ghana (**19**) the commonest genotype was type 2 and the sequences of the isolates did not exactly match any of the known type 2 subtypes. This led to the suggestion that the isolates found were probably indigenous to Ghana. It is therefore possible that the HCV genotypes that occur in Ghana probably less frequently cause severe and chronic hepatitis leading to cirrhosis. Further prevalence studies need to be carried out in other parts of Ghana to confirm the low HCV prevalence among Ghanaian cirrhotic patients and also determine the natural history of infection by the specific subtypes prevalent in the country.

Blood transfusion has been known to be one of the major means of transmission of HBV and HCV infections (**11,68**). In this study it was found that there was a significant association between transfusion history and HBV infection. Although blood transfusion was significantly associated with HBV infection compared with controls, many HBV positive cases (tab. 9) did not admit to a past history of blood transfusion. This is an important finding because it means that though transfusion is a significant means of transmitting HBV, attention must also be given to preventing HBV infections from sources other than blood transfusion. It has been found that apart from blood and its products, saliva, sweat, tears, breast milk, pathological effusions, vertical transmission and unprotected sex are means by which the HBV could be transmitted (**11,63**). The history of blood transfusion was not found to be significantly associated with HCV infections. Only one HCV positive subject had a past history of transfusion. Wansbrough-Jones et al in a study of HCV genotypes in Ghana also found that there was no association between HCV and a history of blood transfusion (**19**).

Studies done in other places have also found that a history of blood transfusion was not always significant among HCV carriers (**91,149**). It is stated in the literature that the source of infection in about 40% of HCV cases is still unknown (**94,95**). It is therefore suggested that blood transfusion may not be the only important way of transmitting HCV and that other possible means of transmission be further investigated in Ghana including sexual and vertical transmission, use of infected needles and blades, tattooing and intravenous drug use.

In order to shed more light on other possible means of transmission Wansbrough-Jones et al (**19**) in their study on HCV genotypes in Ghana investigated an association between HCV and intravenous therapy and scarification. They discovered that only one out of 168 subjects with the history of intravenous therapy was positive for anti-HCV and only 9 out of 295 subjects with history of scarification were positive for anti-HCV. No significant association was therefore established between intravenous therapy, scarification and HCV infection in Ghana. In the U. S. A. Conry-Cantilena et al studied 481 voluntary blood donors for the route of exposure and transmission of HCV. They found that history of blood transfusion ($P< 0.001$), intranasal cocaine use ($P<0.001$), intravenous drug use ($P=0.001$), ear piercing among men ($P<0.05$) and sexual promiscuity ($P=0.002$) were all significantly associated with HCV (**159**). In their study sexual promiscuity was defined as a history of sexually transmitted disease, sex with a prostitute, five or more sexual partners per year or a combination of these. The transmission of HCV infection is however found to be rare between stable and monogamous sexual partners (**63**). The findings of Conry-Cantilena et al raise the possibility of sexual transmission of HCV and this needs to be examined further especially in Ghana.

This is because HCV prevalence rates determined in Ghana among blood donors, pregnant women, and children (**17-19**) and in cirrhotic patients in this study are all low. The possibility of sexual transmission of HCV might lead to an increase in the prevalence rate through unprotected and casual sex as has occurred in HIV infection resulting in the explosive rise in prevalence. If the possibility of significant sexual transmission of both HBV and HCV is established in Ghana it would be worthwhile taking advantage of existing AIDS prevention programmes to educate people about how to avoid HIV, HBV and HCV infections through safe sex.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

In this study we have determined the prevalence of HBV and HCV infections among liver cirrhosis patients at the Korle Bu Teaching Hospital. The prevalence of HBV was found to be 42.8% among cirrhosis patients and that of HCV was 7.1%. It has also been shown in the study that HBV is significantly associated with the development of liver cirrhosis.

The study did not show any significant association between liver cirrhosis and HCV infections at the Korle Bu teaching Hospital. It has also been confirmed by this study that blood transfusion is an important means of transmitting the HBV infection in our environment. However, most of the HCV infections in this study were transmitted through means other than blood transfusion, which are not known yet. This however does not rule out completely the danger of transmission through blood transfusion since prevalence of HCV carriers among blood donors at the Korle Bu Teaching Hospital is 5.4%

It is therefore recommended that screening of blood against HBV and HCV infections must be continued. Secondly, immunization against HBV infection must be encouraged more not only for children under the EPI immunization programme but also among the general population especially groups that are at risk. Finally, it is suggested that further studies be done to investigate other possible modes of transmission of HCV and HBV infections, especially the roles of maternal –to-foetal and sexual transmissions, in our environment. This will enable appropriate preventive measures to be applied since no vaccine has as yet been produced for HCV.

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APPENDIX I

cirrhosis Consent form for the study of prevalence of HBV and HCV infections among patients at the Korle Bu Teaching Hospital

Information (to be read or translated to subjects in their own mother tongue.)

Dear Patient,

We kindly ask your permission to enter into a study, which we will proceed to describe. We would like to start by stressing that this study is strictly voluntary. Should you decide not to participate, it will have no consequences for your treatment. Your participation would be much appreciated because the information gathered will help us to know more about the above diseases which will help in their management.

The study in a few words.
Liver cirrhosis is becoming a more common disease in our environment. The aim of the study is to find out the possible role of HBV and HCV infections in the causation of the disease. Knowing the cause of a disease can help in the management and prevention of the disease.
We will do this by taking a small quantity (10ml) of blood from a participant and examine it at the laboratory to find out whether there is any of HBV or HCV infection present. All informatiom gathered will be treated in strict confidentiality.If you have any questions on this study please feel free to ask and we will be glad to answer.
Thank you.
Yours sincerely

Prof E. K. Wiredu
Pathology dept
Korle-bu.

Dr. R. K. Gyasi
Pathology dept
Korle-bu

Dr Blankson Aboagye
Pathology dept
Korle-bu.

I hereby agree to enter into this study:

.....
Name and signature/thumbprint of subject.

APPENDIX II

QUESTIONNNAIRE

DATE:
DEPARTMENT: OPD/WARD
NAME:
SEX:
AGE:
OCCUPATION:
CLINICAL HISTORY

JAUNDICE:PRESENT/ABSENT

BLOOD TRANSFUSION: YES/NO

CLINICAL DIAGNOSIS ENTERTAINED:

RELEVANT CLINICAL INVESTIGATIONS:

1. LIVER FUNCTION:
ALBUMIN:
ALT:
AST:

2. SEROLOGY:
HBsAg: +VE / -VE

anti-HCV: +VE / -VE

ULTRASOUND REPORT: