SUPERVISORS:

1. DR KAGIMU MAGID
MBChB (MUK) M.Med (KHARTOUM) MSc (London)

2. DR OCAMA PONSIANO
MBChB, M.Med (MUK)

3. DR OPIO KENNETH C,
MBChB, M.Med (MUK)
DECLARATION

I declare that the work presented in this dissertation is the result of my own study. It has not been and will not be presented anywhere else for any other degree(s)

Signed ........................................
Dr Nazziwa Esther Rosette

This dissertation has been submitted for examination with the approval of the following supervisors;

Signed ........................................
Dr Kagimu Magid (supervisor)

Signed ........................................
Dr Ocama Ponsiano (supervisor)

Signed ........................................
Dr Opio Kenneth C (supervisor)
DEDICATION

This dissertation is specially dedicated to my mother Resty Muziribi for her constant support and encouragement.

I also dedicate this work to Dr Ojwang Joseph Conrad for his support and encouragement and to my family for their support.
ACKNOWLEDGEMENTS

I would like to thank my supervisors, Dr Kagimu Majid, Dr Ocama Ponsiano, and Dr Opio Kenneth for accepting to supervise this research and for their constant guidance and input as well as for the time they have spent reading the various drafts of this study. I also extend my thanks to Dr Namulema Teddy for helping me with data collection and for the support she has given me during this study.

I am grateful to Mr Andama Alfred for his assistance with the laboratory investigations.

I am indebted to Kulika for sponsoring my postgraduate education.

I am grateful to GILEAD and IDI for their financial assistance toward this research.

I would like to thank my fellow postgraduates for their support throughout this study.

I am indebted to all the patients who agreed to participate in this study. Their cooperation has made this work possible.
# TABLE OF CONTENTS

Abbreviations and acronyms .................................................................................. ix
Operational definitions ......................................................................................... x
Abstract .................................................................................................................. xii

CHAPTER ONE .......................................................................................................1
1.0 Literature review .............................................................................................1
1.1 Epidemiology of Hepatitis B and Hepatitis C .......................................................1
1.2 Transmission of HCV and HBV ..........................................................................3
1.3 Clinical features of HBV infection .......................................................................5
1.4 Clinical features of HCV infection ....................................................................9
1.5 Laboratory diagnosis of HBV ...........................................................................11
1.6 Laboratory diagnosis of HCV ...........................................................................12
1.7 Treatment of HBV ............................................................................................13
1.8 Treatment of HCV ............................................................................................14

CHAPTER TWO .....................................................................................................15
2.0 Problem statement and justification .................................................................15
2.1 Statement of the problem ..................................................................................15
2.2 Justification of the study ..................................................................................16
2.3 Research question ............................................................................................17
2.4 Study objectives ...............................................................................................17
2.41 General objectives .........................................................................................17
2.42 Specific objectives ..........................................................................................17

CHAPTER THREE ..................................................................................................18
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0 Methodology</td>
<td>18</td>
</tr>
<tr>
<td>3.1 Study design</td>
<td>18</td>
</tr>
<tr>
<td>3.2 Study setting</td>
<td>18</td>
</tr>
<tr>
<td>3.3 Study population</td>
<td>18</td>
</tr>
<tr>
<td>3.4 Accessible population</td>
<td>18</td>
</tr>
<tr>
<td>3.5 Selection criteria</td>
<td>19</td>
</tr>
<tr>
<td>3.5.1 Inclusion criteria</td>
<td>19</td>
</tr>
<tr>
<td>3.5.2 Exclusion criteria</td>
<td>19</td>
</tr>
<tr>
<td>3.6 Study measurements</td>
<td>19</td>
</tr>
<tr>
<td>3.7 Sampling method</td>
<td>19</td>
</tr>
<tr>
<td>3.8 Sample size estimation</td>
<td>19</td>
</tr>
<tr>
<td>3.9 Recruitment</td>
<td>20</td>
</tr>
<tr>
<td>3.11 Data collection</td>
<td>22</td>
</tr>
<tr>
<td>3.12 Data management and analysis</td>
<td>22</td>
</tr>
<tr>
<td>3.14 Quality assurance</td>
<td>23</td>
</tr>
<tr>
<td>3.15 Ethical considerations</td>
<td>23</td>
</tr>
<tr>
<td>3.16 Procedure</td>
<td>24</td>
</tr>
<tr>
<td>Fig. 1 Patient flow chart</td>
<td>25</td>
</tr>
<tr>
<td>CHAPTER FOUR</td>
<td>26</td>
</tr>
<tr>
<td>4.0 Results</td>
<td>26</td>
</tr>
<tr>
<td>4.1 Baseline characteristics</td>
<td>26</td>
</tr>
<tr>
<td>4.2 Prevalence of HBV and HCV</td>
<td>27</td>
</tr>
<tr>
<td>4.3 Factors associated with HBV and HCV</td>
<td>30</td>
</tr>
</tbody>
</table>
4.3.1 Sociodemographic and behavioral factors associated with HBV and HCV……30
4.3.2 Clinical features of liver disease associated with HBV and HCV………………33
4.3.3 Liver function tests and HBV and HCV………………………………………34
5 CHAPTER FIVE……………………………………………………………………35
5.0 Discussion…………………………………………………………………………35
5.1 Prevalence of HBV and HCV……………………………………………………35
5.2 Factors associated with HBV and HCV…………………………………………36
5.21 Sociodemographic and behavioral factors associated with HBV and HCV……36
5.22 Clinical features of liver disease and HBV and HCV…………………………37
5.23 HBV, HCV and liver function tests……………………………………………37
5.2.4 Limitations of the study………………………………………………………37
6 CHAPTER SIX……………………………………………………………………39
6.0 Conclusions and recommendations……………………………………………39
6.1 Conclusions………………………………………………………………………39
6.2 Recommendations………………………………………………………………40
7.0 References………………………………………………………………………42
APPENDICES
I: Patient information and screening consent form ………………………………50
II: Consent for the parent or guardian ……………………………………………54
III: Data collection tool………………………………………………………………57
V: Laboratory procedures……………………………………………………………63
VI: Liver biopsy protocol……………………………………………………………66
List of tables:

Table 1: Sociodemographic characteristics .........................................................26
Table 2: Prevalence of Hepatitis B .................................................................28
Table 3: Prevalence of Hepatitis C .................................................................29
Table 4: Sociodemographic and behavioral factors ........................................31
Table 5: Clinical features ...........................................................................33
Table 6: Liver function tests ......................................................................34

Figures:

Figure 1: Patient flow chart ......................................................................25
Figure 2: Prevalence of HBV and HCV .....................................................27
Figure 3: Prevalence of HBV infection ......................................................28
Figure 4: Prevalence of antibodies to Hepatitis C ...................................29
Figure 5: Region of origin and HBsAg positivity .......................................32
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>Alanine amino transferase</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate amino transferase</td>
</tr>
<tr>
<td>BCM</td>
<td>Below the costal margin</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EIA</td>
<td>Enzyme immunoassay</td>
</tr>
<tr>
<td>GE</td>
<td>Gastroenterology</td>
</tr>
<tr>
<td>GGT</td>
<td>Gamma glutamyl transpeptidase</td>
</tr>
<tr>
<td>HBeAg</td>
<td>Hepatitis B e antigen</td>
</tr>
<tr>
<td>HBsAg</td>
<td>Hepatitis B surface antigen</td>
</tr>
<tr>
<td>HBV</td>
<td>Hepatitis B virus</td>
</tr>
<tr>
<td>HCC</td>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td>HCV</td>
<td>Hepatitis C virus</td>
</tr>
<tr>
<td>IFA</td>
<td>Immunofluorescent assay</td>
</tr>
<tr>
<td>LFT</td>
<td>Liver Function Tests</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PI</td>
<td>Principal investigator</td>
</tr>
<tr>
<td>RIBA</td>
<td>Recombinant immunoblot assay</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribose nucleic acid</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
OPERATIONAL DEFINITIONS

For the purpose of this study, HBV infection, HCV infection, liver disease, acute hepatitis, chronic hepatitis, liver cirrhosis, and HCC were defined as follows:

Liver disease

Liver disease included only acute hepatitis, chronic hepatitis, liver cirrhosis and hepatocellular carcinoma.

HBV infection

Positive HBsAg test in a patient.

HCV infection

Positive HCV antibody test in a patient, indicative of current or past infection.

Acute Hepatitis

History of right upper quadrant pain ± jaundice for less than six months with ALT above 40 U/L

Chronic Hepatitis

History of right upper quadrant pain ± jaundice for more than six months with ALT above 40 U/L

Liver Cirrhosis

Abdominal Ultrasound showing a reduced liver span (< 12 cm) with ascites, splenomegaly, with the following features;

- Diffuse liver surface irregularity or nodularity

- Increased echogenicity of the liver parenchyma

Or

Liver biopsy and histopathology showing liver cirrhosis
Hepatocellular carcinoma

Possible HCC:
Clinical features of a palpable, irregular, hard liver with ultra sound findings of a liver mass and alpha fetoprotein < 200 U/L.

Probable HCC
Clinical features of a palpable, hard and irregular liver with ultra sound findings of a liver mass and alpha fetoprotein > 200U/L.

Definite HCC
Liver biopsy and histopathology showing HCC.
ABSTRACT

Introduction.
Both hepatitis B and hepatitis C viral infections are endemic in Uganda and are a known cause of liver disease, including acute hepatitis, chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. Liver disease is one of the leading causes of morbidity and mortality on the gastroenterology ward in Mulago Hospital. Patients with liver disease may benefit from identification and treatment of both HBV and HCV because the treatment of these viruses may halt or slow the progression of the liver disease and avert mortality.

It is possible that hepatitis B and C contribute to the etiology of liver disease among patients in Mulago hospital but we do not know by how much.

This study was conducted with the aim of determining the possible contribution of HBV and HCV to the aetiology of liver disease in Mulago hospital.

Objectives

The main objective of this study was to determine the prevalence of HBV and HCV among patients with liver disease in the gastroenterology ward and clinic of Mulago hospital.

The other objective was to determine the factors associated with hepatitis B and hepatitis C among patients with liver disease in Mulago hospital.

Study design.

This was a cross sectional study.

Study setting

Gastroenterology ward and Clinic, Mulago Hospital Kampala.
Methods.

All patients with a provisional diagnosis of liver disease on the GE ward or clinic were informed about this study and requested to participate in it. Those patients who agreed to participate in the study were screened for liver disease using a brief history, liver function tests, abdominal ultra sound and for those with liver masses, serum alpha feto protein or liver biopsy was also done.

Those patients that fulfilled the study definitions for acute hepatitis, chronic hepatitis, liver cirrhosis or HCC and met the inclusion criteria were asked to give a written and informed consent and recruited into the study.

For each patient recruited, history was taken, a physical examination done, blood taken off for HBsAg and Hepatitis C antibodies testing.

A questionnaire was administered to each patient to assess for selected sociodemographic and behavioral factors and record patient data.

Results:

One hundred and twenty patients were recruited into the study. The prevalence of HBV among patients with liver disease was 45% while that of possible HCV infection was 4.2%. There was no HBV/HCV co-infection. Younger patients and patients from the northern part of Uganda were more likely to have HBV (p=0.01 and 0.001 respectively). History of blood transfusion and therapeutic cuts were significantly associated with HBV (p=0.047 and 0.009 respectively). Elevated AST was significantly associated with HBV infection (p=0.037).
Conclusions:

There is a high prevalence of HBV among patients with liver disease especially in the younger patients and in patients from the northern part of Uganda. HCV is a much less common infection.

Blood transfusion and therapeutic cuts are the risk factors that are significantly associated with HBV among patients with liver disease in Mulago Hospital.

Recommendations:

All patients with liver disease should be routinely tested for HBV infection and drugs for HBV should be availed to the gastroenterology ward and clinic to treat this infection.

All medical staff working on the gastroenterology ward should be immunized against HBV.

In view of the limited resources, it is not cost effective for now to routinely screen patients with liver disease for HCV infection. However, facilities for testing for markers of active HCV and HBV infections should be made available to improve patient diagnosis and treatment in the national referral hospital.
CHAPTER ONE

1.0: LITERATURE REVIEW

1.1: Epidemiology of Hepatitis B and Hepatitis C

1.1.1: Hepatitis B

Hepatitis B is a global health problem with more than 350 million chronic carriers worldwide.\(^1\)

Four million acute infections occur every year and Hepatitis B is one of the leading causes of chronic hepatitis, liver cirrhosis and HCC, accounting for one million deaths annually.\(^2,8\)

The distribution of HBV infection differs greatly throughout the world. In areas, where the prevalence is high, such as Africa, South East Asia and China, more than half of the population is infected at some time in their lives, while more than 8% are chronic carriers of the virus.\(^3\)

The prevalence of hepatitis B surface antigen among patients with suspected liver diseases in a Nigerian hospital was 38%\(^4\), while in Niger 73% of patients with chronic hepatitis, cirrhosis, and hepatocellular carcinoma were positive for HBsAg\(^5\).

HBV prevalence was 42.9% among patients with liver cirrhosis in Ghana\(^6\) and 14% among blood donors in Kenya.\(^7\) In Gambia, HBV is endemic with 15-20% of the population being chronic carriers.\(^9\)

In Uganda, the prevalence of HBV was 11% among Makerere University medical students,\(^10\) 8.1% among health workers in Mulago hospital,\(^11\) 17.8% among the HIV positive patients in Mulago hospital,\(^13\) and 9% among health workers in Uganda.\(^12\)

In the emergency ward the prevalence of hepatitis B was 8.8%\(^15\).
According to the 2005 national sero survey, 10% of Ugandans have HBV infection.\textsuperscript{16}

In Uganda still, 38.5% of patients with HCC had HBV.\textsuperscript{14}

1.1.2: Hepatitis C

According to WHO, about 170 million people are infected with hepatitis C and 3-4 million persons are infected with HCV each year.\textsuperscript{17}

The prevalence of HCV infection in sub-Saharan Africa is estimated at 3% with modest regional variation.\textsuperscript{18} The highest infection rates have been noted in Egypt with a prevalence of 22%\textsuperscript{19}, Cameroon with 12.5% and Burundi with 11%.\textsuperscript{20}

In Niger 19.1% of the patients with chronic active hepatitis, liver cirrhosis and hepatocellular carcinoma were positive for HCV.\textsuperscript{5} In Ghana 7.1% of patients with liver cirrhosis had HCV\textsuperscript{6}.

In Uganda, 4% of children and 12% of mothers were found to have HCV antibodies.\textsuperscript{21} While 4.1% of blood donors in central Uganda were repeatedly reactive for the HCV enzyme immuno assay.\textsuperscript{22} In Pakistan, 40.8% of patients with chronic liver disease had hepatitis C.\textsuperscript{72}

The prevalence of HCV infection in HIV patients in Uganda was 2.9% \textsuperscript{24} while Walusansa found a prevalence of HCV of 3.3% among HIV positive patients and 7% among HIV negative patients in Mulago hospital.\textsuperscript{25} Seremba found HCV prevalence of 5.3% in the emergency ward.\textsuperscript{15}
1.2: Transmission of HBV and HCV

1.2.1: Hepatitis B

HBV is transmitted by contact with blood or body fluids of an infected person.
HBV is 50-100 times more infectious than HIV.²

World wide, most infections occur from infected mother to child, from child to child contact in household settings and from reuse of unsterilised needles and syringes.²

In many industrialized countries, the pattern of transmission is different with most HBV infections resulting from sexual activity, injection drug use or occupational exposure.⁴⁰

The modes of transmission of hepatitis B in order of importance are outlined below

Child to child transmission

In Africa, infection in children is most often acquired horizontally in the preschool years.¹, ³, ³⁷ The exact mode of transmission of HBV among African children is uncertain but probably involves percutaneous infection through saliva or traces of blood as well as through unsterile needles during cultural scarification and other vehicles³⁸

Parenteral transmission

Sixteen million HBV infections are spread annually through unsafe injection practices.²⁶

Following a needle stick injury, the risk of transmitting HBV is 30%.²⁷

Sexual transmission

This accounts for the majority of infections in industrialized countries.², ⁴

Perinatal transmission

Perinatal transmission accounts for 50% of all new HBV infections. The risk of transmission depends on the mother's antigen status. The highest transmission rates are seen in mothers who are positive for both HBsAg and HBeAg.⁴²
The risk of perinatal transmission of HBV is 10-20% but about 95% of these infections occur at delivery.\textsuperscript{44}

About, 85% of the babies born to mothers who are HBeAg positive become infected with HBV.

In industrialized countries, mother to infant and child to child transmission of HBV accounted for up to one third of chronic infections before childhood hepatitis B vaccination programmes were implemented.\textsuperscript{2}

In areas where prevalence is high such as South East Asia infection is the result of either neonatal transmission or transmission from child to child.\textsuperscript{40}

\textit{Health care worker to patient transmission}

Transmission of HBV from health care worker to patients has been documented.\textsuperscript{41}

\textit{Transmission through receipt of organs or blood}

HBV may also be transmitted less frequently through the receipt of organs or blood products and haemodialysis.\textsuperscript{40}

\textbf{1.2.2: Hepatitis C}

HCV is spread primarily by direct contact with human blood,\textsuperscript{17} such as, through blood transfusion with infected blood, needle stick injuries and intravenous drug use.

WHO estimates that 4.7 million HCV infections are spread annually through unsafe injections practices.\textsuperscript{26} Needle stick injuries in the health care setting continue to result in nosocomial transmission of HCV. Following a needle stick injury the risk of transmitting HCV is 3\%.\textsuperscript{27} This number is influenced by the size of the inoculum, the size of the needle and the depth of inoculation\textsuperscript{27}. HCV is however not efficiently transmitted through occupational exposure to blood.\textsuperscript{28}
Maternal fetal transmission occurs but it is infrequent and is often associated with coinfection with HIV in the mother.\textsuperscript{29,30}

Sexual transmission of the virus appears to be inefficient.\textsuperscript{31} However, co-infection with HIV-1 appears to increase the risk of both sexual and maternal-fetal transmission.\textsuperscript{29,32,33}

HCV can be recovered from saliva of infected persons.\textsuperscript{34} However, casual house hold contact and contact with the saliva of infected persons appear to be very inefficient modes of transmission.\textsuperscript{34,35}

Blood transfusion, before 1990, was an important route of transmission of HCV.\textsuperscript{36}

The introduction however, in 1990 and 1992 of improved blood screening measures based on the detection of HCV antibodies, has dramatically decreased the risk of transfusion associated HCV infection.

1.3: Clinical features of HBV infection

1.3.1: Acute infection

Acute HBV infection is usually mild and the risk of chronicity in immune competent adults is less than 5%. However this risk is greatly increased in the newborn and the immune-compromised.\textsuperscript{45}

About 30% of persons have no signs or symptoms of infection and this number is less in children.\textsuperscript{46}

In patients with clinical illness, the onset is usually insidious with tiredness, anorexia vague abdominal discomfort, nausea, vomiting and sometimes arthralgias and rash. This often progresses to jaundice.\textsuperscript{51}

The icteric phase of acute hepatitis usually begins within 10 days of the initial symptoms
with the appearance of dark urine followed by pale stools and yellow discoloration of the mucous membranes, conjunctivae, sclera, and skin. It is accompanied by mild splenomegaly. As many as 30-50% of adults will present with jaundice.\textsuperscript{51} Fever is mild or absent.\textsuperscript{51}

The hallmark of acute viral hepatitis is the elevation of serum transaminases which varies from 3-10 fold to a striking increase of > 100 fold.\textsuperscript{51} Acute infection may on occasion lead to fulminant liver failure in 0.1-0.5% of adults.\textsuperscript{47,48} This will present as encephalopathy and coagulopathy and has a case fatality of 80%.\textsuperscript{49,45}

In acute infection, the illness resolves in 4-12 weeks after onset of jaundice with development of natural protective antibodies in about 95% of adults.\textsuperscript{50}

1.3.2: Chronic infection

Chronic hepatitis B is defined as the persistence of HBsAg in the serum of a patient for at least six months.\textsuperscript{51} Although most adult patients recover completely from acute hepatitis B in 5-10% of adults the virus persists in the body, while in children, 70-90% of infants infected in their first few years of life become chronic carriers of HBV.\textsuperscript{52,53} Of the persistent HBsAg carriers 70% have chronic persistent hepatitis and 30% have chronic active hepatitis. There are many factors that influence the probability of developing a chronic infection. Age is important and transmission at birth nearly always results in chronic infection. Other risk factors for chronic infection include, male gender, homosexual orientation, and having an altered immune system.\textsuperscript{54}

There may also be a genetic component with certain racial groups having a higher risk for
with the appearance of dark urine followed by pale stools and yellow discoloration of the mucous membranes, conjunctivae, sclera, and skin. It is accompanied by mild splenomegaly. As many as 30-50% of adults will present with jaundice.\textsuperscript{51}

Fever is mild or absent.\textsuperscript{51}

The hallmark of acute viral hepatitis is the elevation of serum transaminases which varies from 3-10 fold to a striking increase of > 100 fold.\textsuperscript{51}

Acute infection may on occasion lead to fulminant liver failure in 0.1-0.5% of adults.\textsuperscript{47, 48} This will present as encephalopathy and coagulopathy and has a case fatality of 80%.\textsuperscript{49, 45}

In acute infection, the illness resolves in 4-12 weeks after onset of jaundice with development of natural protective antibodies in about 95% of adults.\textsuperscript{50}

1.3.2: Chronic infection

Chronic hepatitis B is defined as the persistence of HBsAg in the serum of a patient for at least six months.\textsuperscript{51} Although most adult patients recover completely from acute hepatitis B in 5-10% of adults the virus persists in the body, while in children, 70-90% of infants infected in their first few years of life become chronic carriers of HBV.\textsuperscript{52, 53}

Of the persistent HBsAg carriers 70% have chronic persistent hepatitis and 30% have chronic active hepatitis. There are many factors that influence the probability of developing a chronic infection. Age is important and transmission at birth nearly always results in chronic infection. Other risk factors for chronic infection include, male gender, homosexual orientation, and having an altered immune system.\textsuperscript{54}

There may also be a genetic component with certain racial groups having a higher risk for
chronicity, but this has yet to be proven.

Most people with chronic persistent hepatitis are asymptomatic carriers and do not develop severe liver disease while patients with chronic active hepatitis, although also usually asymptomatic, are at an increased risk of severe liver disease and are very infectious.\textsuperscript{54}

About 33\% of people with chronic hepatitis B have symptoms and these include general malaise, fatigue, weakness, loss of appetite, nausea, general abdominal discomfort or "just feeling unwell". Fever and jaundice are variable.\textsuperscript{54}

Others develop diseases such as ulcerative colitis, pulmonary fibrosis, nephritis, acne and haemolytic anaemia.\textsuperscript{54}

Patients with chronic infection with active replication, typically have abnormal transaminases and higher viral loads. While those in the non replicative state have decreased markers of liver inflammation and damage and lower viral loads.

Transaminases may be normal or increased anywhere from 1-10 times the upper limit of normal in chronic infection. Serum bilirubin and gamma globulin values are mild to markedly elevated and autoimmune antibodies such as antinuclear antibody, anti smooth muscle antibody and anti mitochondrial antibody may be present\textsuperscript{55}.

Up to 20\% of the chronic persistent hepatitis cases progress to cirrhosis. Globally, 30\% of cirrhosis is attributed to HBV\textsuperscript{57}. 
1.3.3: HBV and HCC

It has been recognized for some time that HCC occurs most frequently in certain under developed parts of the world. In particular, HCC is one of the most frequent causes of cancer death in sub-Saharan Africa and the Far East.\textsuperscript{56} Patients with chronic hepatitis B infection have a risk of HCC that is 100 times as high as that for non carriers.\textsuperscript{58} Globally, HBV causes 60-80\% of the world’s primary liver cancer.\textsuperscript{60}

The frequency of HCC follows the same general geographic distribution pattern as that of persistent HBV infection. The age distribution of patients with clinically recognized tumours suggests that these tumours appear after a mean duration of about 35yrs of HBV infection.\textsuperscript{55,53}

Within the HBsAg – positive group, HBeAg – positive carriers have the highest risk of HCC but even carriers with anti-HBe antibodies have a substantial risk of HCC.\textsuperscript{59}

Persons at increased risk of developing HCC include adult males, and chronic hepatitis B patients with cirrhosis who contracted hepatitis B in early childhood.\textsuperscript{52} Only 5\% of patients with cirrhosis develop HCC while between 60-90\% of HCC patients have underlying cirrhosis.

1.3.4: HBV and Cirrhosis

Up to 20\% of the chronic persistent hepatitis cases progress to cirrhosis.

In cirrhosis, liver cells die and are progressively replaced with fibrotic tissue leading to nodule formation. The internal structure of the liver is deranged leading to the obstruction of blood flow and decrease in liver function. This damage is caused by recurrent immune
responses stimulated by the presence of the virus. Because liver inflammation can be totally symptomless, progression of inflammation can occur without the knowledge of patient.

1.4: Clinical features of Hepatitis C

1.4.1: Acute infection.

In the USA, hepatitis C represents approximately 20% of cases of acute hepatitis. The incubation period of acute hepatitis C averages 6-10 weeks. Approximately 80% of patients who develop acute hepatitis C have no symptoms. The onset of disease is usually insidious with anorexia, vague abdominal discomfort, nausea, vomiting, fever, and fatigue progressing to jaundice in about 25% of patients. Rarely acute hepatitis C may progress to rapid fulminating liver failure. As many as 70-90% of infected people fail to clear the virus during the acute phase of the disease and become chronic carriers. The course of acute hepatitis C is variable although elevation in serum ALT levels often in a fluctuating pattern are its most characteristic feature. After acute infection 15-25% of persons resolve their infection without sequelae.

1.4.2: Chronic Infection

Chronic hepatitis C can be defined as a continuing disease without improvement for at least six months. Sixty to eighty percent of persons with chronic HCV have no symptoms.

In chronic HCV infection, fatigue is the most frequent complaint. Other complaints may include depression, nausea, anorexia, abdominal discomfort and difficulty with concentration.
Common extra hepatic manifestations of HCV infection include mixed cryoglobulinaemia and porphyria cutanea tarda. Membrano-proliferative glomerulonephritis, leucocytoclastic vasculitis, focal lymphocytic sialadenitis and idiopathic pulmonary fibrosis may occur in rare cases. Cirrhosis develops in about 10-20% of persons with chronic infection and liver cancer develops in 1-5% of persons with chronic infection over a period of 20-30yrs. HCV associated cirrhosis leads to liver failure and death in 20-25% of cirrhotic patients. HCV associated cirrhosis now represents a leading indication for liver transplantation.

HCV also exacerbates the severity of the underlying liver disease when it coexists with other hepatic conditions. In particular, liver disease progresses more rapidly among persons with alcoholic liver disease and HCV infection.

1.4.3: HCV and HCC

An important late complication of HCV is primary HCC. It usually occurs in patients with cirrhosis. The mechanism by which HCV may lead to HCC is unknown. The feature that links HCV with cancer may be repeated cycles of hepatocyte destruction and regeneration cycles which may cause neoplastic changes which then progress to carcinoma.

The yearly incidence of HCC in patients with cirrhosis is 3-5%
1.5: Laboratory diagnosis of HBV

Definitive diagnosis of HBV is based on serological tests.

Acute hepatitis B infection is characterized by the presence of HBsAg in serum and the development of IgM-anti HBe. HBeAg is also detectable in acute infection. Detection of HBsAg has evolved from immunodiffusion methods to reversed passive haemagglutination assays to more sensitive immunoassays and radio assays which detect HBsAg at concentrations of ≥ 0.1 ng/ml. Rapid tests for HBsAg have been found to have lower sensitivity thus, more sensitive enzyme immuno assays may be necessary for diagnosing HBV infection. However these are more expensive and not readily available.

The cost of testing for HBsAg in private laboratories in Kampala is US $ 2.

During recovery, HBsAg and HBeAg are cleared and anti HBs, anti HBe and anti HBe antibodies develop. Anti HBs is a protective antibody that neutralizes the virus. Its presence, following acute infection, indicates recovery and immunity from re-infection.

It is also detected in vaccinated individuals.

Immunoassays for detection of total anti-HBe involve both IgM and IgG antibody to the core protein and indicate current or past exposure to the virus and viral replication. IgG anti-HBe appears shortly after HBsAg among people with disease and persist for life. Therefore, total anti-HBe is not a good marker for persons with acute disease.

Detection of IgM anti-HBe is diagnostic of acute HBV infection.

In persons with chronic HBV infection, HBsAg remains persistently detectable for life. HBeAg is variably present and IgM anti-HBe generally becomes undetectable 6 months after acute infection.
Hepatitis B viral DNA is detectable in both acute and chronic infection.\textsuperscript{69,70} Most slot or dot hybridization assays detects 1.5 pg of HBVDNA/ml and the branched DNA hybridization assay detects 2.5pg of HBV DNA per ml. It can be considered as an alternative for quantitative detection of HBVDNA in serum samples with relatively high titres of HBV viraemia. PCR is much more sensitive than direct hybridization and detects HBVDNA levels of $10^3$/ml. PCR has the same significance as HBsAg whereas hybridization indicates significant viral replication and a high probability of liver disease hence it is similar to HBeAg.

1.6: Laboratory diagnosis of HCV

Diagnosis of HCV is made using antibody and molecular tests for viral particles.\textsuperscript{71} Antibody tests

Antibodies can be detected within 4-10 weeks of infection. The primary HCV screening tests is Enzyme Immuno Assay (EIA) of which there are three consecutive generations with increasing sensitivity.

In high prevalence immunocompetent populations, sensitivity of EIA ranges from 98.8% to 100%. In immune compromised patients, the sensitivity of EIA is lower (50-95%) depending on the degree of immune suppression. Rapid HCV antibody tests can also be used to diagnose HCV infection however they have been found to yield many false positives. The cause for this is unknown but has been found to occur in African populations and makes it important to carry out confirmatory testing with other tests. The Recombinant Immunoblot Assay (RIBA) also in its third generation has been used to confirm positive EIA results in low risk populations. It also uses antigens similar to
those used by EIA but in an immunoblot format. A positive assay is defined by detection of antibodies against 2 or more antigens and an indeterminate assay by detection of antibodies against a single antigen.

**Molecular tests**

These may be quantitative or qualitative.

Qualitative HCVRNA tests are based on the PCR technique and have a lower limit of detection of 100 copies of HCVRNA/ml. Qualitative PCR assay should be used in patients with negative results on EIA in whom infection is suspected, in patients with hepatitis with no identifiable cause or in those with known reasons for false negative antibody tests.

Quantitative tests are used to determine HCV viral load in patients with persistent infection in antibody positive patients, to monitor response to treatment and to determine the presence of infection in immune-compromised patients.

The rapid HCV antibody test costs US $ 7 in private laboratories in Kampala.

**1.7: Treatment of HBV.**

The goal of treating HBV infection is reduction of viral replication which then results in a reduction in liver inflammation and even fibrosis. Before starting treatment it is important to determine the presence of HBeAg, the HBV viral load, and serum aminotransferases. Imaging of the liver by ultrasonography is necessary to identify liver cirrhosis or liver masses. Features of liver cirrhosis on ultrasonography include diffuse liver surface irregularity and increased echogenicity of the liver parenchyma. Liver biopsy is also needed to determine if treatment is indicated.
Currently four drugs have been approved by the US Food and Drug Administration for treating chronic HBV. They include, lamivudine, interferon alfa-2b, adefovir dipivoxil and entecavir. Lamivudine and Interferon are available in Uganda at a cost of 18 US dollars per month and 350 dollars per week respectively. Entecavir and adefovir are not yet available in Uganda.

1.8: Treatment of HCV infection:

The aim of treating chronic HCV infection is elimination of the virus. Treatment is associated with stabilization or improvement in the clinical course of liver disease as well as the liver histology. There are two drugs that are used to treat HCV infection, namely, interferon alfa-2b and ribavirin. Interferon may be used singly or in combination with ribavirin. The best outcomes are seen when these two drugs are combined.
CHAPTER TWO
2.0: PROBLEM STATEMENT AND JUSTIFICATION.
2.1: STATEMENT OF THE PROBLEM

There is a high burden of HBV and HCV globally. Two billion people have been infected with HBV worldwide and more than 350 million are chronic carriers. HCV on the other hand, affects 170 million people.

Uganda is one of the countries endemic for hepatitis B virus infection and the HIV/AIDS sero-behavioral survey of 2004-2005 found HBV prevalence of 12% in men and 9% in women.

These viruses are some of the causes of acute hepatitis, chronic hepatitis, liver cirrhosis and HCC which account for one million deaths annually world wide.

Chronic infection with HBV appears to account for most cases of HCC in high-incidence areas such as sub-Saharan Africa; whereas chronic infection with the hepatitis C virus (HCV) is the underlying cause in at least two-thirds of cases of HCC in areas of intermediate incidence such as southern Europe and Japan.

HCC is one of the most frequent causes of cancer death in Africa and liver disease was the third most common cause of death on the medical wards of the University College Hospital of Ibadan, Nigeria.

Liver cirrhosis and HCC together accounted for 63.6% of the deaths due to liver disease in a Nigerian hospital and in both diseases HBV was the commonest cause.\(^{73}\)

In Uganda, however, we do not know yet how much these viruses contribute to liver disease.
2.2: JUSTIFICATION OF THE STUDY

No recent study in Uganda has addressed the prevalence of Hepatitis B and C among patients with liver disease. It is therefore not possible to make inferences about these viruses in this population.

Patients infected with hepatitis B or hepatitis C, who have chronic hepatitis and liver Cirrhosis, are the ones who benefit from treatment of HBV and HCV, because it halts progression of liver disease and averts mortality. However, they can only receive treatment if these infections are identified. This study will help us to find out the burden of infection among these patients with liver disease so that we can advocate for the procurement of drugs for HBV and HCV which are currently not available to these patients in Mulago hospital.

HBsAg testing and Hepatitis C antibody enzyme immuno assays are not readily available in Mulago hospital. We therefore need to know the burden of Hepatitis B and C among patients with liver disease to decide if it is cost effective, in our resource limited setting, to start routine screening for all patients with hepatitis, liver cirrhosis and hepatocellular carcinoma for these infections.
2.3: RESEARCH QUESTION

What is the prevalence and associated factors of Hepatitis B and Hepatitis C among patients with liver disease in Mulago hospital?

2.4: STUDY OBJECTIVES

2.4.1: GENERAL OBJECTIVES

To determine the prevalence and associated factors of Hepatitis B and Hepatitis C among patients with liver disease in Mulago hospital.

2.4.2: SPECIFIC OBJECTIVES

1. To determine the prevalence of HBsAg among patients with liver disease in the gastroenterology ward and clinic of Mulago hospital.

2. To determine the prevalence of antibodies to hepatitis C among patients with liver disease in the gastroenterology ward and clinic of Mulago hospital.

3. To determine the factors associated with hepatitis B and hepatitis C among patients with liver disease in the gastroenterology ward and clinic of Mulago hospital.
CHAPTER THREE

3.0: METHODOLOGY

3.1: Study design

This was a cross sectional study

3.2: Study setting

The study was conducted in the gastroenterology ward and gastroenterology

Out patient clinic of Mulago Hospital. Mulago Hospital serves as the National referral

and University teaching hospital. The gastroenterology ward and clinic were chosen as

the study sites because of their higher turn over of patients with liver disease.

Patients with liver disease are admitted on the GE ward and followed up on discharge in

the GE clinic. Other new patients with liver disease are also evaluated and treated

from the GE clinic.

An average of 1 patient with suspected liver disease is admitted on the GE ward

every day and about 20 new patients with liver disease are seen in the GE clinic each

month.

3.3: Study population

All patients admitted to the GE ward or clinic with provisional or confirmed diagnosis of

liver disease who meet the inclusion criteria

3.4: Accessible population

All patients admitted to the GE ward or clinic.
3.5: Selection Criteria

3.5.1: Inclusion criteria

- Patients 13yrs and above.
- Patients who met the definition criteria for acute hepatitis, chronic hepatitis liver cirrhosis or HCC as outlined in the operational definitions
- Patients who gave written and informed consent or assent.

3.5.2: Exclusion criteria

- Patients who had grade 3 or grade 4 encephalopathy
- Patients below 13yrs of age.

3.6: Study measurements

For objective (1) HBsAg

For objective (2) anti-HCV antibodies

For objective (3) socio-demographic factors, history of: blood transfusion, needle stick injury, therapeutic or cosmetic cuts, injection drug use, liver disease in the mother, sexual contact, family history of liver disease, occupations at risk, such as health workers, clinical features of liver disease, liver function tests and HIV sero status

3.7: Sampling Method

Consecutive sampling

3.8: Sample size estimation

Using the Kish and Leslie formula for total population less than 10,000

\[ n = \frac{K}{1 + \frac{K}{N}} \]
Where
n = sample size

\[ K = \frac{Z^2 P (P-1)}{d^2} \]

\( Z = 1.96 \) (standard deviation at 95% confidence interval)
\( d = 0.05 \) (acceptable error for the study.)

N is the number of patients with liver disease admitted on the GE ward and seen in the GE clinic over a period of four months.

N = 200

P = 73% (from prevalence of hepatitis B among patients with chronic hepatitis, cirrhosis and HCC in Niger\(^5\))

K = 302.87
n = 120

Using a prevalence of Hepatitis C among patients with Chronic hepatitis, liver cirrhosis and HCC in Niger of 19.1% and using the Kish and Leslie formula for populations less than 10,000.

K = 236.196
n = 108

A sample size of 120 was chosen for the study because it was the bigger sample size

**3.9: Recruitment**

The principle investigator and research assistant recruited patients on the GE ward from Monday to Friday of each week and the patients in the GE clinic only on Wednesday.

The clinicians on the GE ward and clinic were approached to assist in identifying patients admitted with suspected or confirmed liver disease. The PI and research assistant briefed
the patients and attendants about the study including its objectives benefits and risks and also informed them of the need to have LFTs, Abdominal Ultra sound and where necessary alpha feto protein and liver biopsy before they could be recruited in the study. The costs for LFTs, and alpha feto protein were covered by the PI. The other necessary tests were covered by the PI if the patient could not afford them.

Following the above tests, those patients who met the definition criteria for acute hepatitis, chronic hepatitis, liver cirrhosis and HCC were requested to participate in the study. Patients who did not meet the definition criteria were forwarded to the attending team to continue with patient management. Patients eligible to participate in the study and who had consented were enrolled into the study. Enrollment was done consecutively until the sample of 120 was attained. The PI and research assistant then took a detailed clinical history from the patient. Information obtained included selected socio-demographic characteristics, sexual history, history of blood transfusion, surgery, needle stick injury, therapeutic and cultural cuts, history of alcohol use, history of liver disease in the family, and symptoms of liver disease. Physical exam included general examination for jaundice, oedema, stigmata of chronic liver disease, other signs of liver disease.

A venepuncture was then performed and 5mls of blood were taken off for HBsAg and HCV antibody testing. The HIV serostatus of patients was obtained from the the HIV routine counseling and testing programme currently running on all medical wards and in the medical out patient clinics.
3.11: Data collection:

The principal investigator and research assistant collected data using a pre-coded pre-tested questionnaire. Variables to be measured included socio-demographic characteristics, history of possible exposure to HBV and HCV, and symptoms of liver disease. Physical examination for clinical features of liver disease was also done. Laboratory investigations including HBsAg and hepatitis C antibody tests were done. All the above findings were recorded in the questionnaire.

**Laboratory procedures**

HbsAg testing was done using SD Bioline HbsAg Fast test. The details of this test are outlined in appendix (IV). HCV antibody tests were done using the SD Biolline HCV Fast test. The details of this test are also in the appendix (IV).

3.12: Data management and analysis

The data collected was checked for completeness, organized and coded. Raw data was stored safely to prevent loss. The data was then entered using EPI-INFO version 3.2. Data analysis was done with the help of an epidemiologist using STATA version 8. Prevalence and descriptive variables were analysed using Univariate analysis. Categorical data was summarized using frequencies, and percentages and the results were presented as tables. Bivariate analysis was used to determine association and P values ≤ 0.05 were interpreted as statistically significant association. Backward logistic regression was done to ensure that there were no confounders among the factors that were found to be significantly associated with hepatitis B or Hepatitis C.
3.14: Quality assurance

The internal validity was ensured as follows;

- The questionnaire was pre tested on a few patients to ensure clarity and was subsequently standardized.

- The research assistant was a qualified doctor who was taught how to recruit the patients and how to administer the questionnaire.

- Questionnaires were checked for completeness before leaving the study site

- All specimens were placed in appropriate specimen bottles and labeled with study number.
- A competent laboratory technologist was used to perform the laboratory investigations

3.15: Ethical considerations

Approval to carry out the study was sought from the Department of Internal medicine, Makerere University Faculty of Medicine Research and Ethics Committee, and the Mulago Research Ethics Committee.

The nature and benefit of the study was explained to patient or guardian in a language they could understand.

The participants were assured of confidentiality of all the information that was collected Informed consent or assent was obtained by signature or thumb print on the consent form. The results of investigations done were passed on to the attending doctor to assist with the management of the patient.

HBsAg and Hepatitis C antibody tests were done at no cost to the patient. The initial tests that had to be done prior to enrolling in the study such as LFTs, and alpha feto protein were done free for the participants.

The results of the investigations done were passed on to the attending team to assist with patient management.
3.16: PROCEDURE:

From September 2007 to March 2008, all patients admitted on the gastroenterology unit (GE ward and clinic) of Mulago hospital with suspected liver disease were screened for inclusion in the study. In all, two hundred and seven patients were seen during the study period out of whom one hundred and twenty were recruited. Fifty six patients were excluded because they did not meet any of the definition criteria for acute hepatitis, chronic hepatitis, liver cirrhosis or HCC, sixteen patients had already been tested for hepatitis B and C in the last six months and did not wish to be retested, five patients had grade three hepatic encephalopathy, two patients were minors without an accompanying adult, four patients did not want to participate because they had had several blood draws already, and four patients preferred to wait until they had recovered before enrolling in the study.

Sixty seven patients were recruited from the gastroenterology ward and fifty three patients were recruited from the gastroenterology clinic. Eighteen patients had acute hepatitis diagnosed as history of right upper quadrant pain for less than six months, with or without jaundice and elevated ALT (>41U/L). Four patients had chronic hepatitis diagnosed as right upper quadrant pain for more than six months with or without jaundice with elevated ALT (>41U/L). In addition all the four patients had a liver biopsy done which confirmed chronic hepatitis. Thirty six patients had HCC diagnosed as a palpable hard irregular liver with ultrasound findings of a liver mass(es) and serum alfa feto protein >200U/L. In addition liver biopsy was done in eighteen of these patients and confirmed HCC.
Sixty two patients had liver cirrhosis diagnosed as abdominal ultrasound showing a reduced liver span(<12cm), with ascites, with diffuse liver surface irregularity and increased echogenicity of the liver parenchyma. In addition, fourteen patients had liver biopsy done which confirmed liver cirrhosis.

The above information is summarized in the patient flow chart shown in figure.1 below.

Fig.1: Patient flow chart:

207 screened for liver disease using:
- Clinical features
- LFTs
- Ultrasonography
- Alfa fet protein
- Liver biopsy

120 recruited as follows:
- Acute Hepatitis = 18
- Chronic Hepatitis = 4
- HCC = 36
- Liver cirrhosis = 62

87 Excluded as follows:
- 56- did not meet inclusion criteria
- 16- already tested for HBV and HCV
- 5- had grade 3 encephalopathy
- 2- were minors
- 4- did not want to participate
- 4- preferred to wait to recover first
CHAPTER FOUR.

RESULTS:

Table 1: Socio demographic characteristics of the participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. respondents: N= 120</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (yrs)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13-39</td>
<td>60</td>
<td>50</td>
</tr>
<tr>
<td>≥ 40</td>
<td>60</td>
<td>50</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>78</td>
<td>65</td>
</tr>
<tr>
<td>Female</td>
<td>42</td>
<td>35</td>
</tr>
<tr>
<td><strong>Region of origin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>North</td>
<td>19</td>
<td>15.8</td>
</tr>
<tr>
<td>East</td>
<td>20</td>
<td>16.7</td>
</tr>
<tr>
<td>West</td>
<td>16</td>
<td>13.3</td>
</tr>
<tr>
<td>Central</td>
<td>65</td>
<td>54.2</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>52</td>
<td>43.3</td>
</tr>
<tr>
<td>Married</td>
<td>52</td>
<td>45.8</td>
</tr>
<tr>
<td>Divorced</td>
<td>06</td>
<td>5</td>
</tr>
<tr>
<td>Widowed</td>
<td>07</td>
<td>5.8</td>
</tr>
<tr>
<td><strong>Education level</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Primary</td>
<td>53</td>
<td>44.2</td>
</tr>
<tr>
<td>Secondary</td>
<td>32</td>
<td>26.7</td>
</tr>
<tr>
<td>High school</td>
<td>03</td>
<td>2.5</td>
</tr>
<tr>
<td>Tertiary</td>
<td>20</td>
<td>16.7</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Health worker</td>
<td>04</td>
<td>3.3</td>
</tr>
<tr>
<td>Peasant farmer</td>
<td>42</td>
<td>35</td>
</tr>
<tr>
<td>Civil servant</td>
<td>14</td>
<td>11.7</td>
</tr>
<tr>
<td>Other</td>
<td>60</td>
<td>50</td>
</tr>
</tbody>
</table>
4.2: Prevalence of Hepatitis B and Hepatitis C

The prevalence of Hepatitis B and Hepatitis C is shown in tables 2 and 3 and figures 2, 3 and 4 respectively.

Hepatitis B was the more common infection with 54(45%) of the patients infected with this virus. Hepatitis C antibodies were uncommon with a prevalence of 4.2% (5 patients). All infections occurred singly there was no co-infection.

Fig 2: Pie chart showing the prevalence of HBV and HCV infections
Table 2: Prevalence of HBsAg.

<table>
<thead>
<tr>
<th>Diagnosis (N=120)</th>
<th>HBsAg positive (n)</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute hepatitis (n=18)</td>
<td>8</td>
<td>44.4</td>
</tr>
<tr>
<td>Chronic hepatitis (n=4)</td>
<td>2</td>
<td>50.0</td>
</tr>
<tr>
<td>Liver cirrhosis (n=62)</td>
<td>27</td>
<td>43.5</td>
</tr>
<tr>
<td>HCC (n=36)</td>
<td>17</td>
<td>47.0</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>54</td>
</tr>
</tbody>
</table>

Overall prevalence 45.0

Fig 3: Prevalence of HBsAg
Table 3: Prevalence of antibodies to Hepatitis C

<table>
<thead>
<tr>
<th>Diagnosis (N=120)</th>
<th>HCV antibody positive (n)</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute hepatitis (n=18)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chronic hepatitis (n=4)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Liver cirrhosis (n=62)</td>
<td>2</td>
<td>3.2</td>
</tr>
<tr>
<td>HCC (n=36)</td>
<td>3</td>
<td>8.3</td>
</tr>
<tr>
<td>Total = 120</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

Overall prevalence 4.2

Fig 4: Prevalence of antibodies to Hepatitis C
4.3: Factors associated with HBV and HCV

4.3.1: Socio demographic and behavioral factors associated with HBV and HCV infection.

As illustrated in table 4, young age group (< 40yrs), coming from northern Uganda and having had tertiary education were the sociodemographic factors significantly associated with HbsAg positivity. Figure 4 also shows the high rates of HBsAg positivity among patients from northern Uganda as compared to patients from other regions of Uganda. Blood transfusion and therapeutic cuts were the risk factors significantly associated with HBsAg positivity. Other factors such as HIV serostatus, alcohol use and occupation were not significantly associated with HbsAg positivity. On the other hand, hepatitis C antibody positivity was seen in only 5 patients and none of the known risk factors for transmission of hepatitis C were significant in this patient population.
<table>
<thead>
<tr>
<th>Variable</th>
<th></th>
<th>HBV(+) n(%)</th>
<th>95% CI</th>
<th>P value</th>
<th>HCV(+) n(%)</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Region of Origin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>North (N=19)</td>
<td>18(94.7)</td>
<td>4.5-390</td>
<td>0.001</td>
<td>0(0)</td>
<td>0.05-7.3</td>
<td>0.688</td>
<td></td>
</tr>
<tr>
<td>West (N=16)</td>
<td>06(37.5)</td>
<td>0.3-5.6</td>
<td>0.636</td>
<td>1(6.25)</td>
<td>0.04-2.1</td>
<td>0.226</td>
<td></td>
</tr>
<tr>
<td>Central (N=65)</td>
<td>24(36.9)</td>
<td>0.4-4.0</td>
<td>0.572</td>
<td>2(3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>East (N=20)</td>
<td>06(30)</td>
<td></td>
<td></td>
<td>2(10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertiary (N=20)</td>
<td>12(60)</td>
<td>1.2-43.7</td>
<td>0.025</td>
<td>0(0)</td>
<td>0.03-1.4</td>
<td>0.104</td>
<td></td>
</tr>
<tr>
<td>Secondary (N=32)</td>
<td>13(40.6)</td>
<td>0.6-18.2</td>
<td>0.15</td>
<td>2(6.25)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary (N=53)</td>
<td>24(45.3)</td>
<td>0.8-20.7</td>
<td>0.084</td>
<td>0(0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None (12)</td>
<td></td>
<td></td>
<td></td>
<td>3(25)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age (yrs)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13-39 (N=60)</td>
<td>34(56.7)</td>
<td>1.2-5.4</td>
<td>0.011</td>
<td>1(1.6)</td>
<td>0.02-2.2</td>
<td>0.204</td>
<td></td>
</tr>
<tr>
<td>&gt;40 (N=60)</td>
<td>20(33.3)</td>
<td></td>
<td></td>
<td>4(6.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Transfusion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (N=10)</td>
<td>1(10)</td>
<td>0.01-0.9</td>
<td>0.047</td>
<td>1(10)</td>
<td>0.3-29.2</td>
<td>0.356</td>
<td></td>
</tr>
<tr>
<td>No (N=110)</td>
<td>53(48.2)</td>
<td></td>
<td></td>
<td>4(3.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>H/o Surgery</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (N=18)</td>
<td>5(27.8)</td>
<td>0.1-1.3</td>
<td>0.119</td>
<td>1(5.5)</td>
<td>0.2-13.7</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>No (N=102)</td>
<td>53(51.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Therapeutic cuts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (N=19)</td>
<td>14(73.7)</td>
<td>1.4-12.5</td>
<td>0.009</td>
<td>0(0)</td>
<td>0.1-5.8</td>
<td>0.664</td>
<td></td>
</tr>
<tr>
<td>No (N=101)</td>
<td>40(39.6)</td>
<td></td>
<td></td>
<td>5(4.95)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sexual contact</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (103)</td>
<td>45(43.7)</td>
<td>0.3-2.0</td>
<td>0.637</td>
<td>4(3.88)</td>
<td>0.1-5.8</td>
<td>0.664</td>
<td></td>
</tr>
<tr>
<td>No (17)</td>
<td>8(50)</td>
<td></td>
<td></td>
<td>1(5.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Family h/o liver disease</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (N=11)</td>
<td>8(72)</td>
<td>0.6-2.5</td>
<td>0.068</td>
<td>0(0)</td>
<td>0.1-15.5</td>
<td>0.968</td>
<td></td>
</tr>
<tr>
<td>No (N=106)</td>
<td>45(42)</td>
<td>0.9-14.4</td>
<td>0.754</td>
<td>5(4.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Don’t know (N=3)</td>
<td>1(33)</td>
<td></td>
<td></td>
<td>0(0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single (N=52)</td>
<td>23(44.2)</td>
<td>0.6-2.6</td>
<td>0.615</td>
<td>1(1.92)</td>
<td>1(1.8)</td>
<td>0.968</td>
<td></td>
</tr>
<tr>
<td>Married (N=55)</td>
<td>27(49.1)</td>
<td></td>
<td></td>
<td>1(1.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Divorced (N=6)</td>
<td>2(33.3)</td>
<td>0.1-3.8</td>
<td>0.612</td>
<td>0(0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Widowed (N=7)</td>
<td>2(28.5)</td>
<td>0.1-2.8</td>
<td>0.438</td>
<td>3(42)</td>
<td>3.2-457.4</td>
<td>0.004</td>
<td></td>
</tr>
</tbody>
</table>
Figure 5  Region of Origin and HBsAg Positivity

<table>
<thead>
<tr>
<th>Region</th>
<th>HBsAg positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>North</td>
<td>94%</td>
</tr>
<tr>
<td>East</td>
<td>30%</td>
</tr>
<tr>
<td>Central</td>
<td>36.9%</td>
</tr>
<tr>
<td>West</td>
<td>37.5%</td>
</tr>
</tbody>
</table>
4.3.2: Clinical features of liver disease associated with HBV and HCV infection.

As shown in table 5, jaundice was significantly associated with Hepatitis C antibody positivity.

<table>
<thead>
<tr>
<th>Table 5: Clinical features associated with HBV and HCV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>History of yellow eyes</td>
</tr>
<tr>
<td>Yes (N=75)</td>
</tr>
<tr>
<td>No (45)</td>
</tr>
<tr>
<td>History of haematemesis</td>
</tr>
<tr>
<td>Yes (N=20)</td>
</tr>
<tr>
<td>No (N=100)</td>
</tr>
<tr>
<td>Jaundice</td>
</tr>
<tr>
<td>Yes (N=87)</td>
</tr>
<tr>
<td>No (33)</td>
</tr>
<tr>
<td>Oedema</td>
</tr>
<tr>
<td>Yes (N=55)</td>
</tr>
<tr>
<td>No (N=65)</td>
</tr>
<tr>
<td>Splenomegaly</td>
</tr>
<tr>
<td>Yes (N=30)</td>
</tr>
<tr>
<td>No (N=90)</td>
</tr>
<tr>
<td>Stigmata of liver disease</td>
</tr>
<tr>
<td>Yes (N=19)</td>
</tr>
<tr>
<td>No (N=101)</td>
</tr>
</tbody>
</table>
4.3.3: Liver function tests and HBV and HCV infection

Significant liver enzyme elevation in HBV and HCV infection which is likely to be an indicator for treatment is usually taken as 2x upper limit of the normal range for ALT and AST. As shown in table 6, AST >2xULN was significantly associated with HBsAg positivity. Other abnormal liver function tests were not associated with HBV or HCV infection.

Table 6: Liver function tests and HBV and HCV

<table>
<thead>
<tr>
<th>Variable</th>
<th>HBV(+) n(%)</th>
<th>95% CI</th>
<th>P value</th>
<th>HCV (+) n(%)</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST 2x ULN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (N=72)</td>
<td>38(52.7)</td>
<td>1.1-4.8</td>
<td>0.037</td>
<td>4(5.5)</td>
<td>0.3-25.5</td>
<td>0.370</td>
</tr>
<tr>
<td>No (N=48)</td>
<td>34(70.8)</td>
<td></td>
<td></td>
<td>1(2.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GGT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated (N=75)</td>
<td>33(44)</td>
<td>0.4-1.7</td>
<td>0.612</td>
<td>2(2.7)</td>
<td>0.1-2.4</td>
<td>0.304</td>
</tr>
<tr>
<td>Normal (N=45)</td>
<td>21(45)</td>
<td></td>
<td></td>
<td>3(6.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (N=87)</td>
<td>37(42.5)</td>
<td>0.6-1.1</td>
<td>0.162</td>
<td>4(4.6)</td>
<td>0.1-1.8</td>
<td>0.199</td>
</tr>
<tr>
<td>Normal (33)</td>
<td>17(51.5)</td>
<td></td>
<td></td>
<td>1(3.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (N=87)</td>
<td>37(42.5)</td>
<td>0.7-1.2</td>
<td>0.446</td>
<td>4(4.6)</td>
<td>0.1-1.8</td>
<td>0.188</td>
</tr>
<tr>
<td>Normal (33)</td>
<td>17(51.5)</td>
<td></td>
<td></td>
<td>1(3.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. Bilirubin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated (N=78)</td>
<td>37(47)</td>
<td>0.8-3.0</td>
<td>0.21</td>
<td>2(2.6)</td>
<td>0.1-2.1</td>
<td>0.251</td>
</tr>
<tr>
<td>Normal (42)</td>
<td>17(40)</td>
<td></td>
<td></td>
<td>3(7.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated (N=82)</td>
<td>36(44)</td>
<td>0.4-1.9</td>
<td>0.723</td>
<td>2(2.4)</td>
<td>0.1-1.8</td>
<td>0.188</td>
</tr>
<tr>
<td>Normal (38)</td>
<td>18(47)</td>
<td></td>
<td></td>
<td>3(7.9)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER FIVE:

5.0: Discussion:

5.1: Prevalence of HBV and HCV infections.

The results of this study have demonstrated that the burden of HBV infection among patients with liver disease is quite high with almost half of the study patients infected with this virus. This finding is in favour of testing every patient who has liver disease for HBV infection because one of every two patients is likely to be infected and may therefore benefit from treatment of this infection.

The prevalence of HBV of 45% in this study is comparable to the prevalence of 42.9% found among patients with liver cirrhosis in Ghana but is slightly higher than the 38.5% found by Olweny among patients with HCC in Mulago. This could simply be because HCC is not representative of the larger spectrum of liver disease and therefore not comparable, or it could mean that the prevalence of HBV in liver disease has actually increased.

Anti hepatitis C antibody positivity was demonstrated in 5(4.2%) patients. This means that HCV may not be causing much liver disease in this population. Indeed, this prevalence is consistent with the prevalence of 4.1% that was found among blood donors in central Uganda presumably without clinically evident liver disease. However, it was much less than the 19.6% found among patients with liver disease in Niger.

It should be noted that due to issues of false positive testing for hepatitis C antibodies, the 4.2% positivity may not reflect the true prevalence of HCV infection.
5.2: Factors associated with HBV and HCV infections.

5.2.1: Sociodemographic and behavioral factors associated with HBV and HCV infections.

The age group less than 40yrs was significantly associated with HBsAg positivity. This suggests that most Hepatitis B infection in Uganda is acquired in early life probably perinatally or horizontally in the first five years as has been shown in other areas with high HBV prevalence.1,3,37,44

The northern part of Uganda has been shown to have the highest prevalence of hepatitis B infection by the Uganda National Sero-behavioral survey of 2005. Being of northern Uganda origin was significantly associated with Hepatitis B infection among the patients presenting with liver disease this study. Life styles, including scarification and therapeutic cuts may contribute to this high rate of occurrence of HBV infection. In addition sexual transmission may also be playing a role in this high prevalence of HBV infection given the high prevalence of sexually transmitted diseases that was found in northern Uganda by the national serobehavioral survey of 2005.

History of blood transfusion was found to be significantly associated with Hepatitis B infection. Although nation wide screening of donor blood by the Uganda National Blood transfusion Service started in 1990, some patients may have been transfused with HBV infected blood before that time considering the association of HBV and blood transfusion in this study. In addition, there is still a chance of transfusion of HBV infected blood during the window period when HBsAg, the screening marker is still not detectable. Blood transfusion as a risk factor for HBV transmission was also described in patients with liver cirrhosis in Ghana.6
Being a health worker was not significantly associated with either hepatitis B or hepatitis C infection.

5.2.2: Clinical features of liver disease and HBV and HCV infections.
Jaundice was the only clinical feature significantly associated with HCV antibody positivity. However, only one patient had both HCV antibody and Jaundice. This makes interpretation of this result is very restricted.
None of the clinical features in this study were significantly associated with hepatitis B infection.

5.2.3: HBV, HVC infections and Liver function tests
Elevated AST of clinical significance was associated with HBV infection. This suggests that a significant proportion of HBV infected patients with liver disease may need treatment.
There was no significant difference in the other abnormal liver function tests between patients infected with HBV compared to those without this infection.
None of the abnormal liver function tests was associated with HCV infection.

Limitations of the study:
The various rapid tests used for testing for HCV and to a lesser extent HBV are associated with false positive and false negative results. This may cause an over estimation or under estimation of the prevalence rates.
We were not able to do liver biopsies for all patients with acute hepatitis, chronic hepatitis, liver cirrhosis and HCC in order to confirm these diseases. Hence, some
patients without the above liver diseases may have been enrolled which could result in an under estimate of the prevalence rates.

The number of patients with HCV infection, were very few such that we did not have enough numbers to analyse for some of the associated factors for HCV.
CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS.

6.1: Conclusions.

1. This study has shown that almost half (45%) of the patients with liver disease in the Gastroenterology ward and clinic of Mulago hospital are infected with HBV.

2. Hepatitis C virus infection was demonstrable in 5 (4.2%) of the patients with liver disease in Mulago hospital.

3. Younger patients with liver disease are more likely to be infected with HBV and should be vigilantly screened for this infection.

4. Patients from the northern part of Uganda with liver disease are more likely to be infected with HBV than those from other parts of Uganda.

5. History of blood transfusion and therapeutic cuts and scarifications are risk factors significantly associated with HBV infection.
6.2: **Recommendations:**

1. In view of the high burden of HBV among patients with liver disease, it is important that all patients with liver disease are screened for this infection, because some of these patients may benefit from treatment of HBV which may halt the progression of their liver disease.

2. All health workers and medical students should be immunized against Hepatitis B as there is a significant risk of acquiring this infection while working in the hospital due to the high prevalence of HBV.

3. Due to the much lower prevalence of HCV among patients with liver disease, it may not be cost effective to screen all patients with liver disease for this infection. It would be more cost effective to screen for HCV after excluding HBV infection.

4. The significant association of HBV infection with patients coming from the northern part of Uganda necessitates intensification of the hepatitis B control programmes as well as further investigation of the risk factors for HBV infection in this particular group of people.

5. Diagnostic facilities especially for active HBV and less so for HCV should be made available in the national referral hospital to improve patient management.

6. Drugs for the treatment of HBV should be availed to Mulago hospital to handle the high proportion of patients with HBV associated liver diseases. Smaller quantities of drugs for treating HCV should also be made available.

7. Although being a health worker was not significantly associated with HBV or HCV infection, we recommend that all health workers who are HBV positive
should be transferred to areas or units where they do not actively interact with patients in order to avoid transmission of HBV from the health worker to patient.
REFERENCES


2. WHO media centre. Hepatitis B fact sheet no. 204. 2000


17. WHO media centre. Hepatitis C fact sheet


20. Hepatitis C global infection rates. 2006


45. Wright TL, Lau JY. Clinical aspects of hepatitis B virus infection. Lancet 1993; 342: 1340-4

46. CDC: Hepatitis B fact sheet [www.cdc.gov/ hepatitis]


46


54. Hepatitis B V4.1. Virology and Immunology


63. EASL international consensus statement. Journal of Hepatology, 1999, 31: 3-8


APPENDIX 1: PATIENT INFORMATION AND CONSENT FORM

STUDY TITLE: THE PREVALENCE AND ASSOCIATED FACTORS OF HEPATITIS B AND HEPATITIS C AMONG PATIENTS WITH LIVER DISEASE IN MULAGO HOSPITAL.

Purpose and Background
My name is Dr Nazziwa Esther Rosette. I am performing a research on the GE ward and clinic of Mulago hospital in order to find out the frequency of certain viruses that affect the liver. These viruses are called hepatitis B and Hepatitis C. They attack the liver, injure the liver cells and cause liver disease. As a result of this injury the liver fails to carry out its functions.

The aim of this study is to find out how many patients with liver disease have these viruses. This will help us to know whether it is necessary to look for these viruses in every patient with liver disease. It will also help us to advocate for drugs for the treatment of these viruses if we realize that they contribute significantly to liver disease.

You have been identified as one of the patients that can participate in this study. Your doctors will look for the cause of your current illness and treat it appropriately. However, if you accept to participate in this study, we shall be able to test you for these viruses at no cost. The results of these tests shall be released to you if you wish to know them. We shall however require your contact so that we can relay these results to you.

These results will be useful to your doctors to help them make decisions about your treatment.

The tests to detect these viruses will require obtaining a maximum of 5mls of blood from you.

This will be in addition to blood taken off for other tests required for the management of your current condition.

Procedures
During this study, the following may be done to you;

1. You will be asked some questions, some of which are aimed at identifying the risk factors for acquiring the above viruses and we shall also need to know your HIV sero status.

2. About 5mls of blood will be removed from your vein to measure the following,
   - HBsAg
   - HCV antibodies
   - Liver function tests

RISKS AND DISCOMFORTS
Having blood drawn from you may cause some little discomfort such as, bleeding where the needle enters the body a little pain and very rarely may cause infection. The amount of blood drawn however will be too small to affect your health. All in all this procedure has very low risk.
BENEFITS
This study will help us get more knowledge about these viruses in patients with liver disease. You will personally benefit by having all the above viral tests done promptly and results forwarded to you at no cost and will help with choosing treatment for your illness. Drugs for treating hepatitis B and C will not be offered freely. In case you are unable to afford them, supportive treatment will be offered.

ALTERNATIVE (Right to Refuse)
If you choose not to participate in this study, you will not be denied any treatment and you will not be victimized at all. Your participation is entirely voluntary.

CONFIDENTIALITY
All information collected will remain confidential. Medical information relating to hepatitis B or C with your consent will be forwarded to your attending doctors to assist in treating you appropriately.

FUTURE STUDIES ON COLLECTED BLOOD SAMPLES
I would like to request to have your blood stored in a refrigerator for further studies on the above mentioned viruses. Some of these studies may be done abroad because we lack the technical expertise to do them here.
This blood will not be used for any other purpose other than studies on these viruses. These studies will help us identify viruses that are present in very low levels and the various serotypes and genotypes of these viruses.

QUESTIONS
If you have any problems or questions relating to this study, you may ask them now or at any time during the study or contact Dr Nazziwa Esther at Tel 0772648859 or check with the Department of Medicine, 4th floor Mulago hospital
STATEMENT OF CONSENT

A copy of this consent form will be provided for me to keep at my request. The purpose and nature of this study has been explained to me and I have understood. I understand that my participation in this study is entirely voluntary and that no untoward consequences will result if I choose not to participate or to withdraw from the study even after consenting.
I have a right to know the results of the tests. By signing this form I am willing to participate in the study described above.
I hereby document my agreement by signing below

Name of participant ........................................ Signature of participant/ thumb print
Date .........................................................

Name of witness .................................................. Signature of witness/ thumb print
Date .........................................................

I have explained the purpose of the study to the participant and they have understood.
Name of principal investigator or research assistant ..............................................................
Date .........................................................

Signature of principal investigator or research assistant.

Telephone number of the participant or next of kin. ..................................................

CONSENT FOR STORAGE AND FUTURE TESTS
I ........................................ have understood the purpose of this study and agreed to participate in it. I consent that my blood samples be stored for further studies on hepatitis B and C viruses some of which may be done abroad
I hereby document my agreement by signing below

Name of participant ........................................ signature/ thumbprint of participant
Date .........................................................

Name of witness .................................................. signature/thumb print of participant
STATEMENT OF ASSENT

A copy of this assent form will be provided for me to keep at my request. The purpose and nature of this study has been explained to me and my attendant and we have understood it. I understand that participation in this study is entirely voluntary and that no untoward consequences will result if I choose not to participate or withdraw from the study even after assenting. I have the right to know the results of the tests. By signing this form, I agree to participate in the study described above. I hereby document my agreement by signing below.

.................................................. .................................................. 
Name of participant                          Signature of participant/ thumb print

Date .................................................................

I have explained the nature and purpose of the study and I am convinced that the patient and attendant have understood it.

.................................................. .................................................................
Name of Investigator/ Authorised representative. Signature of investigator/ Authorised representative

CONSENT FOR STORAGE AND FUTURE TESTS

I ................................................................. have understood the purpose of this study and agreed to participate in it. I consent that my blood samples be stored for further studies on hepatitis B and C viruses some of which may be done abroad. I hereby document my agreement by signing below.

.................................................. .................................................................
Name of participant                          signature/ thumbprint of participant

Date .................................................................

.................................................. .................................................................
Name of witness                              signature/thumb print of participant
APPENDIX 11: CONSENT FOR THE PARENT/GUARDIAN

STUDY TITLE: THE PREVALENCE AND ASSOCIATED FACTORS OF HEPATITIS B AND HEPATITIS C AMONG PATIENTS WITH LIVER DISEASE IN MULAGO HOSPITAL

Purpose and Background

My name is Dr. Nazziwa Esther Rosette. I am performing a research on the GE ward and clinic of Mulago hospital to help us find out the frequency of certain viruses that affect the liver. These viruses are called hepatitis B and Hepatitis C. They attack the liver, injure the liver cells and cause liver disease. As a result of this injury the liver fails to carry out its functions.

The aim of this study is to find out how many patients with liver disease have these viruses. This will help us to know whether it is necessary to look for these viruses in every patient with liver disease. It will also help us to advocate for drugs for the treatment of these viruses if we realize that they contribute significantly to liver disease.

Your child has been identified as one of the patients that can participate in this study. Your doctors will look for the cause of his/her current illness and treat it appropriately. However, if he/she accepts to participate in this study, we shall be able to test him/her for these viruses at no cost.

The results of these tests shall be released to you if you wish to know them. We shall however require your contact so that we can relay these results to you. These results will be useful to your doctors to help them make decisions about your child’s treatment.

The tests to detect these viruses will require obtaining a maximum of 5mls of blood from your child.

This will be in addition to blood taken off for other tests required for the management of your child’s current condition.

Procedures

During this study, the following may be done to your child:

1. Your child will be asked some questions, some of which are aimed at identifying the risk factors for acquiring the above viruses and we shall also need to know his/her HIV sero status.

2. About 5mls of blood will be removed from his/her vein to measure the following,
   - HBsAg
   - HCV antibodies
   - Liver function tests

RISKS AND DISCOMFORTS

Having blood drawn from him/her may cause some little discomfort such as, bleeding where the needle enters the body a little pain and very rarely may cause infection. The amount of blood drawn however will be too small to affect his/her health. All in all this procedure has a very low risk.
BENEFITS
This study will help us get more knowledge about these viruses in patients with liver disease. Your child will personally benefit by having all the above viral tests done promptly and results forwarded to you at no cost and will help with choosing treatment for his/her illness. Drugs for treating hepatitis B and C will not be offered freely. In case you are unable to afford them, supportive treatment will be offered.

ALTERNATIVE (Right to Refuse)
If you choose that your child should not participate in this study, he/she will not be denied any treatment and will not be victimized at all. His/her participation is entirely voluntary.

CONFIDENTIALITY
All information collected will remain confidential. Medical information relating to hepatitis B or C with your consent will be forwarded to your attending doctors to assist in treating you appropriately.

FUTURE STUDIES ON COLLECTED BLOOD SAMPLES
I would like to request to have your child’s blood stored in a refrigerator for further studies on the above mentioned viruses. Some of these studies may be done abroad because we lack the technical expertise to do them here. This blood will not be used for any other purpose other than studies on these viruses. These studies will help us identify viruses that are present in very low levels and the various serotypes and genotypes of these viruses.

QUESTIONS
If you have any problems or questions relating to this study, you may ask them now or at any time during the study or contact Dr Nazziwa Esther at Tel 0772648859 or check with the Department of Medicine, 4th floor Mulago hospital
STATEMENT OF CONSENT

A copy of this consent form will be provided for me to keep at my request. The purpose and nature of this study has been explained to me and I have understood. I understand that my child’s participation in this study is entirely voluntary and that no untoward consequences will result if I choose that my child does not participate or to withdraw from the study even after consenting.
I have a right to know the results of the tests. By signing this form I am willing to have my child participate in the study described above.
I hereby document my agreement by signing below

Name of participant ................................................................. Signature of participant/ thumb print
Date .............................................................

Name of witness ................................................................. Signature of witness/ thumb print
Date .............................................................

I have explained the purpose of this study to the parent/guardian and they have understood.

Name of principal investigator or research assistant ................................................................. Signature of principal investigator or research assistant
Date .............................................................

Telephone number of the participant or next of kin. .............................................................

CONSENT FOR STORAGE AND FUTURE TESTS

I ................................................................. have understood the purpose of this study and agreed to have my child participate in it. I consent that his/her blood samples be stored for further studies on hepatitis B and C viruses some of which may be done abroad.
I hereby document my agreement by signing below

Name of participant ................................................................. signature/ thumbprint of participant
Date .............................................................

Name of witness ................................................................. signature/thumb print of participant
APPENDIX 111: Data collection tool

A: IDENTIFICATION (DEMOGRAPHIC CHARACTERISTICS)

DATE ..............................................

i) Study NO.............. ii) Age .............. iii) Gender: M F

iv) Nationality: Ugandan 1 = Yes 2 = No .............................................

If (2) above specify.................................................................

v) Tribe...........................................................

vi) Region of origin (if Ugandan) 1 = North 2 = East 3 = West 4 = Central

...........................................................

vii) Marital status

(a) single = 1 (b) married = 2 (c) Divorced = 3 (d) widowed = 4 .................

viii) Education level

a) None b) Primary c) Secondary (S1-S4) d) high school (S5-S6) e) Tertiary

B: History of exposure to HBV or HCV

1. Occupation

(a) Health worker (b) Peasant farmer (c) Civil servant (d) Other ...............

If (d) above specify.................................................................

2. History of sexual contact Yes = 1 No = 2 ...........................................

If yes, how many sexual contacts have you had in your life time?

(a) One = 1 (b) Two = 2 (c) Three = 3 (d) More than 3 = 4 ......................

3. Have you ever been transfused with blood or blood products?

Yes = 1 No = 2 .................................................................

If yes specify the year. ..........................................................
4. Have you ever been pricked by a needle that has been used on another patient?  
   Yes = 1  No = 2  Do not know = 3  

5. Have you ever been involved in any surgical operations?  Yes = 1  No = 2  

6. Have you ever used drugs for recreation which are injected directly into your Body?  
   Yes = 1  No = 2  

7. Ever had therapeutic cuts in the body?  Yes = 1  No = 2  

8. Have you ever had cosmetic cuts on your body?  Yes = 1  No = 2  

9. Was any member of your family ever diagnosed with liver disease?  
   Yes = 1  No = 2  Don't know = 3  

C: Alcohol use.  

1. History of alcohol consumption.  Yes = 1  No = 2  
   If yes,  
   i) What type of alcohol do you drink?  
      a) beer  b) wine  c) spirits  d) local brew  e) others  
   ii) How often do you drink?  
      a) every day  b) once a week  c) more than once a week but less than every day  
      d) once a month  e) others  
   iii) Have you ever felt you ought to cut down on your drinking?  Yes = 1  No = 2  
   iv) Have people ever annoyed you by criticizing your drinking?  Yes = 1  No = 2  
   v) Do you feel guilty about your drinking?  Yes = 1  No = 2  
   vi) Have you ever had a drink first thing in the morning to steady your nerves?
Yes = 1  No = 2

Interpretation

(a) No suspected alcohol problem (all no)
(b) Suspected alcohol problem (one yes)
(c) Alcohol abuse/dependency (more than one yes)

D: Symptoms of hepatitis, liver cirrhosis and HCC

(a) Have you ever had yellow eyes? Yes = 1  No = 2

If yes, for how long? Below six months = 1  Above six months = 2

(b) Have you ever had right upper quadrant pain? Yes = 1  No = 2

If yes, for how long? Below six months = 1  Above six months = 2

(c) Was (a) and or (b) above associated with nausea, vomiting or anorexia?

Yes = 1  No = 2

(d) Have you ever had swelling of the abdomen? Yes = 1  No = 2

If yes for how long?

(e) Have you ever vomited blood? Yes = 1  No = 2

(f) Have you ever passed blood stained stools/black stools? Yes = 1  No = 2

(g) Have you ever been tested for any of the viruses below?

(i) Hepatitis B Yes = 1  No = 2

Result: Positive = 1  Negative = 2

(ii) Hepatitis C Yes = 1  No = 2

Result: Positive = 1  Negative = 2
Physical Findings of hepatitis, liver cirrhosis and HCC

1. Jaundice  
   - Yes = 1  
   - No = 2

2. Oedema  
   - Yes = 1  
   - No = 2

3. RUQ tenderness  
   - Yes = 1  
   - No = 2

4. Hepatomegaly  
   - Yes = 1  
   - No = 2
   - if yes to (4) above is it tender or non tender?  
     - Tender = 1  
     - Non tender = 2
   - If yes to (4) above is it soft or hard?  
     - Hard = 1  
     - Soft = 2  
     - Others = 3
   - If yes to (4) above specify size  
     - cm BCM
   - If yes to (4) above is there a liver bruit?  
     - Yes = 1  
     - No = 2

5. Specify position of the upper border of the liver  
   - intercostal space

6. Splenomegaly  
   - Yes = 1  
   - No = 2
   - If yes, specify  
     - cm BCM

7. Ascites  
   - Yes = 1  
   - No = 2

8. Flapping tremor  
   - Yes = 1  
   - No = 2

9. Any other stigmata of liver disease such as palmar erythema, leuconychia, spider naevi, parotid enlargement, caput medusa, Dupuytrens contractures.
   - Yes = 1  
   - No = 2
   - If yes please specify
F: LABORATORY INVESTIGATIONS

LABORATORY NO ..................................................

1. HBV: POSITIVE = 1  NEGATIVE = 2 .........................
2. HCV: POSITIVE = 1  NEGATIVE = 2 .........................

3. LIVER FUNCTION TESTS

ALT: ......................... Elevated =1  Normal = 2 ......
AST: ......................... Elevated = 1  Normal = 2 ......
GGT: ......................... Elevated = 1  Normal = 2 ......
ALP: ......................... Elevated = 1  Normal = 2 ......
TOTAL PROTEIN ................. Normal = 2  Low = 3 ......
ALBUMIN ...................... Normal = 2  Low = 3 ......
TOTAL BILIRUBIN ............. Elevated = 1  Normal = 2 ......
DIRECT BILIRUBIN ............ Elevated = 1  Normal = 2 ......

4. Was alpha feto protein done? Yes =1  No = 2 ..............
   If yes specify results ........................................

G: ABDOMINAL ULTRASOUND FINDINGS

LIVER SIZE:  Normal = 1  Increased = 2  Reduced = 3 ......
LIVER SURFACE: Normal =1  Nodular/ irregular = 2 .......
LIVER MARGIN: Regular = 1  Irregular = 2 ...............
LIVER ECHOGENICITY: Normal =1  Increased = 2  Reduced = 3 ......
LIVER MASS/ MASSES Present = 1  Not present = 2 ...........
ASCITES:  Present = 1  Not present = 2 ...............
SPLENOMEGALY:  Present =1  Not present = 2 ............

61
H: LIVER BIOPSY RESULTS

Was liver biopsy done? Yes = 1  No = 2

If yes specify histopathological results

I: HIV SEROSTATUS. Positive = 1  Negative = 2

F: Final diagnosis based on the operational definitions:
APPENDIX IV: Laboratory tests

Hepatitis B testing:

SD BIOLINE HBsAg test was used.

Explanation of the test.

The SD BIOLINE HBsAg test is an in vitro immunochromatographic one step assay designed for qualitative determination of HBsAg in human serum or plasma. The test cassette contains a membrane strip, which is pre-coated with mouse monoclonal anti-HBS capture antibody on the test band region. The mouse monoclonal anti-HBS-colloid gold conjugate and serum sample moves along the membrane chromatographically to the test region (T) and forms a visible line as the antibody-antigen-antibody gold particle complex forms. The SD BIOLINE HBsAg test cassette has a letter T and C as “Test line” and “Control line” on the surface of the cassette. Both the Test line and control line in the result window are not visible before applying any samples. The control line is used for procedural control. The control line should always appear if the test procedure is performed properly and the reagents of the control line are working.

Procedure of the test.

Remove the test device from the foil pouch and place on a flat dry surface.

Add 100μl of specimen into the sample well.

As the test begins to work you will see purple color move across the result window in the center of the test device.

Interpret the test results at 20 minutes.
Interpretation of the test.

A color band will appear at the left section of the results window to show that the test is working properly. This band is the control band.

The right section of the results window indicates the test results. If another color band appears at the right section of the results window this the test band.

The presence of only one purple color band within the results window indicates a negative result. The presence of two color bands within the results window indicates a positive result. If no color band appears in the results window then the test is invalid.

Testing for Hepatitis C

The SD BIOLINE HCV FAST test will be used.

Explanation of the test.

The SD BIOLINE Fast is an immunochromatographic (rapid) test for the qualitative detection of antibodies specific HCV in human serum or plasma. The SD BIOLINE HCV Fast strip contains a membrane strip which is precoated with recombinant HCV capture antigen (core NS3, NS4 and NS5) on the test band region. The protein A-colloid gold conjugate and serum samples moves along the membrane chromatographically to the test region (T) and forms a visible line as the antigen-antibody-protein A gold particle complex forms with a high degree of sensitivity and specificity.

The test strip has a letter T for “test line” and a letter C for “control line” on the surface of the strip. Both the Test line and the control line in the result window are not visible before applying any samples.
The control line is used for procedural control and it should always appear if the test procedure is performed properly and the test reagents of the control line are working properly.

**Procedure of the test**

Remove the test strip from the container or foil pouch

Add 10μL of serum or plasma to the empty test tube and then add 3 drops of assay diluent to the test tube.

Put the SD BIOLINE HCV Fast strip into the test tube. Be sure the specimen level is below the stop line.

As the test begins to work you will see a purple colour move across the results window in the centre of the strip

Interpret the results at 5-20 minutes.

**Interpretation of the test:**

A color band will appear in the left section of the results window to show that the test is working properly. This is the control band. The right section of the results window indicates the test results. If another color band appears at the right section of the results window this is the test band. The presence of only one purple colour band within the results window indicates a negative result. The presence of two colour bands within the results window indicates a positive result. If no color band appears in the results window then the test is invalid.
APPENDIX V

LIVER BIOPSY PROTOCOL

- Patients had to avoid aspirin and all non steroidal anti-inflammatory medications for at least one week prior to the procedure.

- All patients had a complete blood count and PT/INR performed 1-2 days prior to the procedure.

- If the platelet count was < 80,000 or the INR > 1.3, the procedure was postponed until this was corrected.

- Patients were encouraged to have breakfast, preferably a fatty meal, to encourage gall bladder emptying.

- Immediately before the liver biopsy was done vital signs were measured and recorded and an intravenous line with normal saline was inserted and run to keep the vein open.

- The liver biopsy set was placed at the bedside and included the following:
  - Hibitane
  - Sterile drape
  - Sterile gauze
  - 5 ml syringe
  - 1 liver biopsy needle
  - 2% lignocaine
  - 1 scalpel blade
  - one bottle of sterile saline
  - 1 bottle of formalin for biopsy specimen
○ Bandages

After liver biopsy had been performed, the patient was told to lie supine or on their right side for a period of 6hrs

Vital signs were measured hourly, for the first 4 hours and then once four hourly up to the next day.

Patients were given panadol 500mg 1 to 2 tablets 4-6 hourly after the procedure

The PI was to be notified if the patient had any of the following problems

- Very severe or increasing abdominal pain
- Hypotension (drop of > 15mmHg from pre-biopsy blood pressure)
- Fever
- Vomiting.

**Circumstances under which liver biopsy was not done for patients with liver cirrhosis and HCC**

- Patients who were very ill
- Patients who were uncooperative
- Patients with coagulopathy (abnormal PT/INR)
- Patients with thrombocytopenia
- Patients with anaemia
- Patients with gross ascites