THE PREVALENCE OF HEPATITIS A, B, C

AND HIV SEROPOSITIVITY

AMONG PATIENTS WITH ACUTE ICTERIC HEPATITIS

AT THE KENYATTA NATIONAL HOSPITAL

A DISSERTATION SUBMITTED IN PART FULFILMENT OF THE

REQUIREMENTS FOR THE DEGREE OF MASTER OF MEDICINE

(INTERNAL MEDICINE) OF THE UNIVERSITY OF NAIROBI

BY

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DECLARATION

I declare that this is my original work and has not been presented for a degree elsewhere.

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DEDICATION

This work is dedicated to my wife, Gladys, my daughter, Bwari, and my

long-suffering mother, Esther Moraa.

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LIST OF ABBREVIATIONS

AIDS: Acquired immune deficiency syndrome

ALP: Alkaline phosphatase

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

DNA: Deoxyribose Nucleic Acid

ELISA: Enzyme linked immunosorbent assay

HAART: Highly active antiretroviral therapy

HAV: Hepatitis A virus

HBV: Hepatitis B virus

HCV: Hepatitis C virus

HBeAg: Hepatitis B e antigen

HBcAg: Hepatitis B core antigen

HBsAg: Hepatitis B surface antigen

HIV: Human immunodeficiency virus

IgM anti-HAV: ImmunoglobinM antibodies against hepatitis A virus

IgM anti-HBc: Immunoglobin M antibodies against hepatitis B core antigen

KEMRI: Kenya Medical Research Institute

KNH: Kenyatta National Hospital

PFC: Paediatric Filter Clinic

RNA: Ribose Nucleic Acid

SPSS: Statistical Package for Social Sciences

USA: United States of America

WHO: World Health Organization

ACKNOWLEDGEMENTS

I am grateful to the following for assisting me in various ways during the project: -

1. My supervisors Professor Ogutu, Professor Hardison and Professor Mumo for guiding me through the study.

2. Dr Anzala, Mr Miriti and Mr Kahara of the Immunomolecular Diagnostic Laboratory for running the liver function tests.

3. Mrs Kimani, Mrs Karimi and Mr Kilonzo of the Immunology Laboratory for giving me assistance with the serological assays.

4. Dr Okoth and Mr Kaiguri for the HBsAg assays that were done at the KEMRI hepatitis laboratory free of charge.

5. Aventis Pasteur SA (East Africa) and GlaxoSmithKline for financial assistance.

ABSTRACT

Objective: The aim of the study was to determine the prevalence of hepatitis A, B, C and HIV seropositivity among patients with acute icteric hepatitis at the KNH.

Study design & Setting: The study design was a cross-sectional descriptive survey, done at the Kenyatta National Hospital, Nairobi, Kenya.

Methods: Outpatients and inpatients aged above 6 months with a history of jaundice not exceeding 6 months were recruited. A history was obtained, physical examination done, and blood taken for determination of bilirubin, ALT, AST and ALP levels. Sera that had disproportionately greater transaminase than ALP elevation were then assayed for IgM anti-HAV, IgM anti-HBc, anti-HCV, anti-HIV antibodies and also for the HBsAg.

Results: 47 males and 37 females aged 8 months to 67 years with a median of 25 years were studied. Hepatitis A, B, and C were found in 41.7%, 26.2% and 7.1% of the patients respectively. 13.1% of the patients were HBsAg carriers, and 30.1% were also HIV positive. 32 patients did not have hepatitis A, B, or C. This group was significantly associated with HIV infection (p 0.003).

Conclusions: HAV was the commonest overall cause of acute icteric hepatitis at the KNH, and among patients aged 15 years and below. HBV was the leading cause of acute hepatitis among those aged over 15 years. HCV accounted for 7.1% of acute icteric hepatitis. 30.1% of all patients, and 50% of those admitted with acute hepatitis were also HIV positive.

LITERATURE REVIEW

1. INTRODUCTION

Acute viral hepatitis is an infection of the liver lasting no more than six months characterized by diffuse inflammation with widespread liver cell necrosis. It is most often caused by the hepatitis viruses A, B, and C. Rarer causes of the disease include the hepatitis viruses D, E, and G (1).

At Kenyatta National Hospital, a 2000 bed referral facility, acute viral hepatitis accounted for nearly 1% of all admissions into the medical wards in the last five years. On average 40% of these patients died (2).

Viral hepatitis is an important disease because of the amount of morbidity and mortality it causes. The emergence of HIV infection and AIDS may worsen the situation (3). Therefore studies to describe the current prevalence of the various viral aetiological agents are necessary.

2. PREVIOUS STUDIES

The contribution of HAV, HBV and HCV to clinical disease varies widely in different parts of the world.

Bagshawe and colleagues were the first to study the prevalence of acute viral hepatitis in Kenya. They looked at the HBsAg among patients with liver disease at the KNH in 1971, and found the antigen in 54% of acute hepatitis cases, and in 6% of healthy Nairobi blood donors (4). The second and last study in this country, done by Greenfield in 1982, looked at adults with acute sporadic hepatitis at the KNH and it found HAV in 12%, HBV in 70% and non-A non-B in 18% of them (5). It is important to note that neither study looked at hepatitis C because the disease was not recognized at the time.

Tsega and colleagues in a study done in Ethiopia found that HCV accounted for 19% of acute sporadic hepatitis cases (6). Another African study by Topley and colleagues from Zimbabwe got rates of 21% and 65% for HBV and non-A non-B hepatitis respectively in patients with acute hepatitis (7).

Hazra from India studied HBV infection in high-risk groups in Calcutta in 1999, and found that 30.5% of infections were due to this virus. Among the controls 8% showed HBV reactivity (8). Further East, Saat in a four-year review of acute viral hepatitis cases in peninsular Malaysia found HAV rates of 26.1% in 1994, 47.8% in 1995, 66.4% in 1996 and 20% in 1997. Sera received in 1996 were also screened for other types of hepatitis, and rates of 1.4% and 5.4% for HBV and HCV respectively were found (9).

Researchers from the Far East have reported comparable rates. Chu from Taiwan in a 1999 study of the etiology of acute sporadic hepatitis found HAV in 36%, HBV in 5.1%, and non-A non-B in 38.3% of cases. Among the non-A non-B 54.7% had acute HCV infection (10). Wang in 1997 studied acute viral hepatitis in China and found that HAV infection made up 61.3% of the cases and occurred mainly in youngsters decreasing with age. HBV accounted for 26.2% of cases mostly in the middle aged and the elderly with its prevalence increasing with age. HCV accounted for 9.9% without any obvious age difference (11). A national survey of acute viral hepatitis carried out in 1999 in Japan and reported by the WHO found type A hepatitis in 53%, type B in 36.6%, and type C in10.3% of cases (12).

In Romania Balan studied hospitalized patients with acute viral hepatitis in 1998 and found 75% of them had HBV, 20% HAV and 8.3% non-A non-B infection (13). And in the United States of America, in 1997, acute hepatitis A accounted for 66.2%, HBV for 28.7%, and HCV for 4.4% of all sporadic hepatitis cases as reported by Alter (14).

3. VIROLOGY

3.1 HEPATITIS A

Hepatitis A virus is a heat, acid and ether resistant single stranded RNA virus in the hepatovirus genus of the picornavirus family. In spite of a 20% nucleotide variation among isolates, all strains are immunologically indistinguishable and belong to the same serotype (15).

The virus is shed in faeces from the time of prodromal symptoms and this continues for many weeks. Hence, it is transmitted mainly by the feco-oral route by ingestion of contaminated water, food or milk (16), or eating raw or undercooked shellfish or oysters from contaminated water masses. Outbreaks have followed breakdowns in sanitation, floods or other natural disasters (17). Outbreaks have also occurred among institutionalized children and adults (18).

Male homosexuality (19) intravenous drug addiction (20) and exposure to children in day care centers (21) have also been shown to be epidemiological sources of infection.

A short period of viraemia lasting 24-48 hours occurs. Transmission by blood and blood products has been reported if blood is donated during the viraemic period (3).

The host makes IgM anti-HAV within 10 days of the viraemia. This is followed in about 8 weeks by an IgG antibody response, which is life-long and confers protective immunity (3).

The typical pattern of clinical and laboratory features is shown in figure 1.
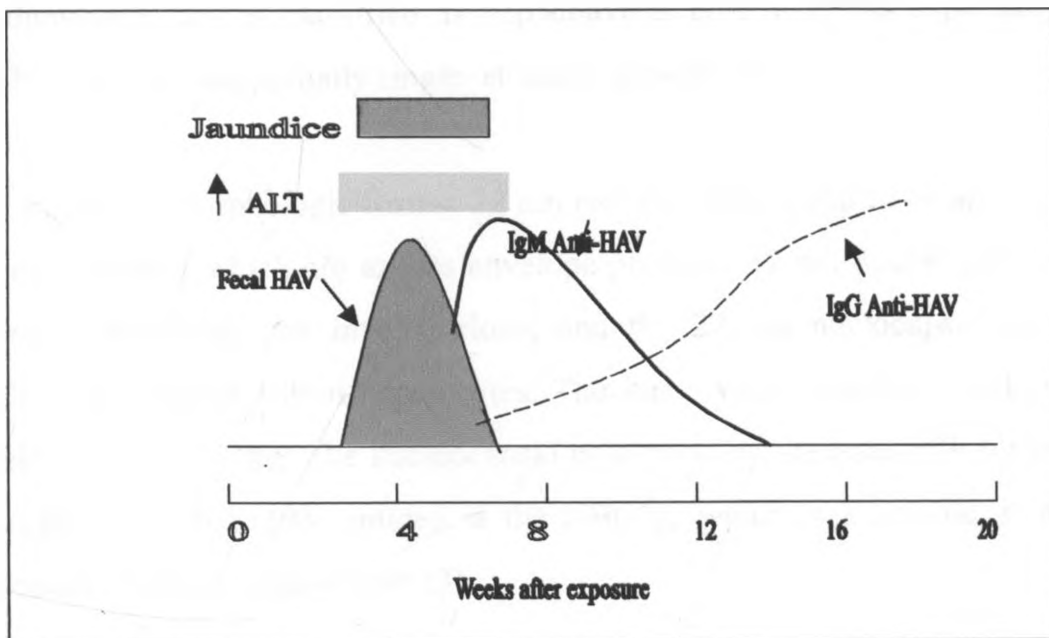
FIGURE 1: Scheme of typical clinical and laboratory features of acute hepatitis A (3)

A heat inactivated whole virus vaccine for family contacts of infected people and persons in high-risk occupations such as primary school teachers, sewage workers and attendants in mental health institutions is available (22).

3.2 HEPATITIS B

The hepatitis B virus is a DNA virus in the family of animal viruses called hepadnaviruses and is classified as hepadnavirus type 1. It has a partially double- stranded and partially single- stranded genome (3).

HBV has three morphologic forms; 22 nm particles which appear as spheres or long filaments which are excess envelope proteins, 42 nm double shelled spheres representing the intact virions, and the 27 nm nuclocapsid core which is only found within hepatocytes. The intact virion and the envelope protein express HBsAg. The nucleocapsid core protein expresses HBcAg on its surface. A third HBV antigen is the HBeAg, which is a soluble, non-particulate, nucleocapsid protein (3).

During replication, which occurs in the liver, the HBcAg particles remain in the hepatocyte, and only leave the cell after encapsulation with HBsAg. In contrast HBeAg is secreted into the circulation where it provides a convenient, readily detectable, qualitative marker of HBV replication and relative infectivity (3).

HBV is mainly transmitted percutaneously via tattooing, ceremonial scarification, blood transfusion, and injections. Significant transmission of the virus also occurs during sexual intercourse (23). Perinatal transmission is another important mode of spread with 50% of children born of infected mothers getting infected and 90% of these becoming chronic carriers. However, horizontal transmission is responsible for most spread in Africa. Studies in Kenya have shown that, unlike South East Asia, vertical transmission is rare here (24,25).

Soon after infection HBsAg appears in the circulation preceding elevation of aminotransferases in serum and clinical symptoms. It disappears during convalescence at about the 24th week from the time of exposure when anti-HBs antibodies become detectable. HBsAg persists beyond 6 months if chronic disease or carriage follows the acute infection (26).

Anti-HBc antibodies are also elaborated by the host; initially of IgM type but later replaced by IgG, which last indefinitely. HBeAg appears concurrently with or soon after HBsAg and disappears when peak transaminase levels are reached before HBsAg becomes undetectable. Anti-HBe becomes detectable from then onward (3). Figure 2 depicts the typical clinical and laboratory features of acute hepatitis B infection.
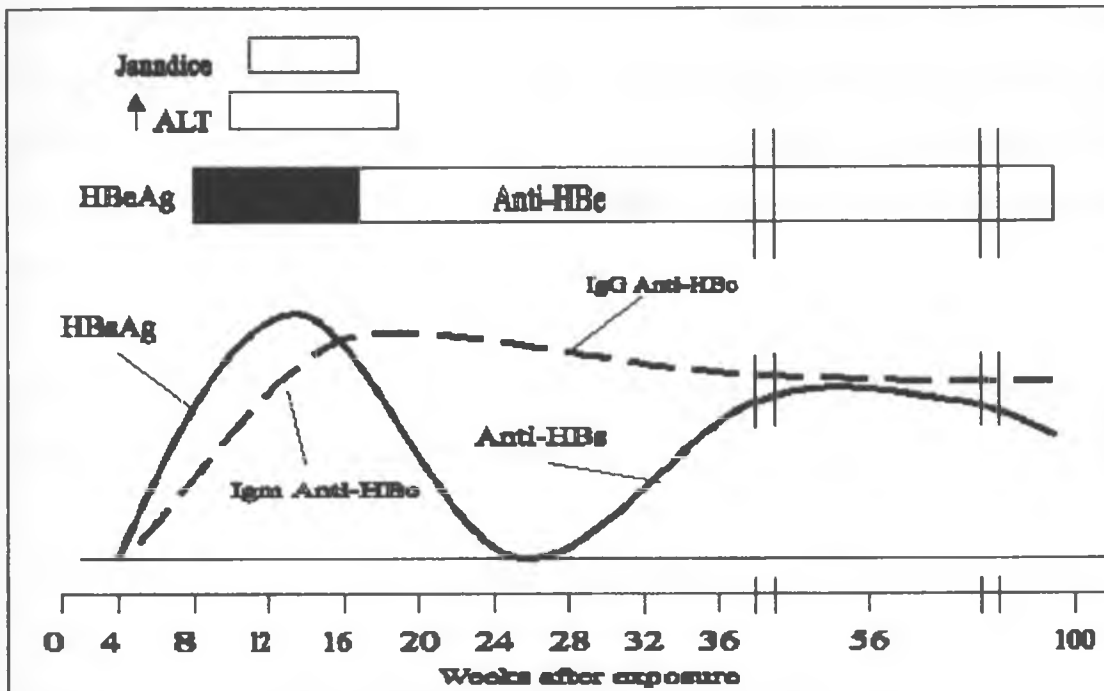
FIG 2: Scheme of clinical and laboratory features of acute hepatitis B (3)

Effective vaccines are available for protection against hepatitis B (27).

3.3 HEPATITIS C

The hepatitis C virus (HCV) is a linear, single-stranded RNA virus that constitutes its own genus in the family Flaviviridae. Direct visualization of the virus has been difficult because it circulates in very low titre. Similarly in vitro culture has not been convincingly achieved (3).

No fewer than six genotypes have been demonstrated by nucleotide sequencing. This high genotypic diversity, which is due to the high mutation rate of the virus, interferes with the development of effective humoral immunity after acute infection. Variation in pathogenicity and response to antiviral therapy has been shown among genotypes (28,29,30).

HCV transmission occurs by blood and blood products, and illicit drug use with sharing of needles. Occupational exposure to blood and its products can transmit the virus. The probability of infection is high in haemodialysis units (31). The risk of HCV infection is increased in organ transplant recipients and in patients with AIDS (3).

HCV replicates in hepatocytes though the virus has been demonstrated in peripheral blood lymphocytes as well (3).

There are no effective vaccines available for protection against HCV infection so far (3).

4. CLINICAL FEATURES

4.1 TYPICAL CLINICAL SYNDROME

The clinical syndrome of acute viral hepatitis is divided into four phases; incubation period, pre-icteric phase, icteric phase, and convalescence (32). The incubation period is asymptomatic and varies according to the agent responsible; 15-45 days (average 4 weeks) for HAV, 30-180 days (average 4-12 weeks) for HBV, and 15-160 days (average 7 weeks) for HCV (3).

The prodrome of acute hepatitis sets in suddenly with HAV, and insidiously with HBV and HCV infection. Malaise is the first symptom and occurs in 75% of patients. Other symptoms include fatigue, arthralgia, myalgia, headache, pharyngitis, cough, and coryza. Anorexia, dysgeusia, nausea and vomiting occur in 80% of patients while abdominal pain occurs in 60%. Mild weight loss is common and low-grade fever may occur (32).

Then jaundice and dark urine set in as the prodromal symptoms subside. 6-16% of children and 66% of adults who contract hepatitis A develop jaundice. 10% of children and 30-50% of adults getting hepatitis B become jaundiced. Less than 20% of all hepatitis C sufferers become icteric. Pruritus occurs in 40% of patients at the peak of the jaundice. Tender hepatomegaly occurs. Splenomegaly and cervical lymphadenopathy may occur in 10-20% of patients. Rarely a few spider naevi appear during the icteric phase (32).

During the recovery phase the constitutional symptoms disappear. The duration of the post-icteric phase is variable. Complete chemical and biochemical recovery is to be expected 1-2 months after HAV and 3-4 months after HBV and HCV infection (3).

4.2 COMPLICATIONS AND SEQUELAE

4.2.1 HEPATITIS A

Most HAV infections recover completely with only 0.1% progressing to fulminant hepatic failure. This complication of hepatitis A occurs mainly in older adults and in persons with underlying chronic liver disease (3,33)

A small proportion of hepatitis A patients experience relapsing hepatitis weeks to months after apparent recovery from acute hepatitis. Relapses are characterized by recurrence of symptoms, aminotransferase elevation, and occasionally jaundice and faecal excretion of HAV (34).

Prolonged cholestatic hepatitis appearing late in the acute phase and lasting several months may be seen. It is characterized by protracted jaundice and pruritus. Rarely liver enzyme abnormalities persist for many months even up to a year (35).

4.2.2 HEPATITIS B

The case fatality rate in hepatitis B is 0.1% with fulminant hepatitis accounting for the deaths. Among admitted patients with acute HBV infection the case fatality rate rises to 1% (3).

During the acute phase of acute hepatitis B a serum sickness-like syndrome characterized by arthralgia or arthritis, rash and angioedema may occur in 5-15% of patients. In children papular acro-dermatitis or the Gianotti- Crosti syndrome may occur (36).

5-10% of hepatitis B patients fail to clear HBsAg and become chronic carriers. The likelihood of becoming a chronic carrier is high among neonates and infants, people with Down's syndrome, chronically haemodialyzed patients, and immunosuppressed patients including persons with HIV infections (3). Only 2% of children under two years of age contracting hepatitis B clear the acute infection with 98% getting chronic infection. In contrast 95% of adults and children over two years clear the acute infection with only 5% going on to chronic infection. Chronic hepatitis may ultimately give rise to liver cirrhosis and/or hepatoma (37).

4.2.3 HEPATITIS C

Acute HCV infection is milder than HBV infection and is more likely to be anicteric. Fatalities are rare but the precise case fatality rate is unknown (3).

85% of patients with acute hepatitis C never clear the virus and become chronic carriers. About two thirds of these develop the clinical and histologic picture of chronic active hepatitis. About ten percent of those with chronic active hepatitis develop cirrhosis after 20 to 30 years. Hepatoma eventually occurs in some of the patients with cirrhosis and also in some of those with chronic hepatitis (37).

Acute hepatitis C, like acute HBV infection, may be complicated by mixed cryoglobulinemia (38) and glomerulonephritis (39).

5. HEPATITIS A, B, AND C IN HIV-INFECTED PERSONS

Hepatitis and elevated liver enzymes are common in HIV infected persons. HIV shares some modes of transmission with the hepatitis viruses. Moreover, HIV and the hepatitis viruses may interact by paracrine means or by co-infecting the same cells; HIV, HBV and HCV are all capable of infecting peripheral blood lymphocytes as well as hepatocytes (40).

5.1 HEPATITIS A

Several outbreaks have been reported in homosexual men and intravenous drug users who are also HIV positive (41). Nevertheless, HAV has not been shown to be more virulent in HIV infected people, and recovery with long-term immunity is common despite antecedent HIV infection. Although the HAV vaccine remains immunogenic in HIV infected patients, seroconversion rates are lower than in uninfected persons (42). 68% of HIV positive people with CD4+ counts above 200 develop effective antibody levels in response to the standard two dose hepatitis A vaccine while only 9% of those with counts below 200 do so (43). In addition, HAV super-infection has recently been reported to lead to a marked and prolonged increase in HIV-1 viral load (44).

5.2 HEPATITIS B

Hepatitis B is common in HIV infected persons; in one study over 90% of patients with HIV had HBV markers of current or past infection (45). HIV infected people are more likely to become chronically infected with HBV. Co-infected patients who are also intravenous drug users have a higher frequency of liver cirrhosis than patients without co-infection. Furthermore

the soluble HBV X protein has been shown to stimulate HIV replication in vivo, which may accelerate the progression of HIV infection (46).

HBV infected people who get HIV infection may demonstrate reduction in HBs antibody and the HBsAg may re-appear (47). Co-infected patients have high levels of HBV DNA and HBeAg because of enhanced HBV replication; hence their body fluids are more infectious (48). In addition response to the hepatitis B vaccine, which is T-cell dependent, is generally impaired and short-lived in HIV infected people although there may be adequate protection if immunization occurs before significant immunosuppression occurs (49).

Persons with chronic hepatitis B, co-infected with HIV, have poor response to interferon-α (50). These patients, when given lamivudine as a component of anti-HIV regimens, respond to therapy but resistant HBV strains emerge during treatment and after drug discontinuation (51,52). On the other hand HIV patients co-infected with HBV receiving protease inhibitor containing HAART regimens have higher rates of hepatic cytolysis and liver enzyme elevation than patients without co-infection (53,54).

5.3 HEPATITIS C

An estimated 20-30% of HIV positive patients are co-infected with HCV in developed countries (43). These patients have higher HCV titers and are therefore more infectious. Women are more likely to transmit the infection to their offspring (55,56). Severer and more rapidly progressive disease results with the duration to cirrhosis being as low as 3 years (57). Co-infected people are also more likely to develop liver failure (58).

HIV co-infected chronic hepatitis C patients respond poorly to α-interferon therapy (59). HCV loads of co-infected patients put on HAART increase during treatment (60,61). Moreover these patients when given HAART are at higher risk of developing liver enzyme elevation (52,53,62) and also have a smaller CD4+ T cell recovery compared to HIV patients without HCV co-infection (63).

6. LABORATORY FEATURES

The serum aminotransferases rise variably during the prodrome, peak at 400-4000 units or more in the icteric phase, and diminish progressively thereafter. The AST to ALT ratio is usually less than 1 in acute viral hepatitis (3). Other liver enzymes such as ALP, gamma glutamyl transferase and 5'-nucleotidase are only mildly elevated (32). The serum bilirubin level rises to 85-340 millimoles/L in icteric cases. This continues to rise as the aminotransferases are falling. The bilirubin is equally divided between the conjugated and unconjugated forms (3).

Serological tests are used to confirm the diagnosis of acute viral hepatitis. Acute hepatitis A is diagnosed by demonstrating IgM anti-HAV antibodies. These antibodies are detected in the serum from the 4th week after exposure and remain detectable for 12 weeks, rarely for up to 6-12 months.

Detecting HBsAg in serum indicates infection with HBV. Demonstration of IgM, and IgG, anti-HBc antibodies is used to establish whether the infection is acute or chronic.

HCV infection is diagnosed by demonstrating anti-HCV antibodies in serum. First generation assays detect antibodies to the non-structural protein C100-3, between 1-3 months after the onset of acute hepatitis. Second generation assays detect antibody to C100-3, C22 and C33 and become positive from the fourth week (3). Third generation assays also detect anti-HCV from the fourth week but lack specificity for genotypes other than type 1 (64). Fourth generation assays are able to detect type 2 and 3a as well (65).

Supplementary tests such as the recombinant immunoblot assay (RIBA) and HCV RNA PCR are used to confirm the diagnosis (66). Detection of HCV RNA by PCR is the most sensitive method of detecting HCV infection. HCV RNA can be detected even before aminotransferase elevation and before the appearance of anti-HCV in acute hepatitis (3).

Typical laboratory features in acute HCV infection are shown in figure 3.
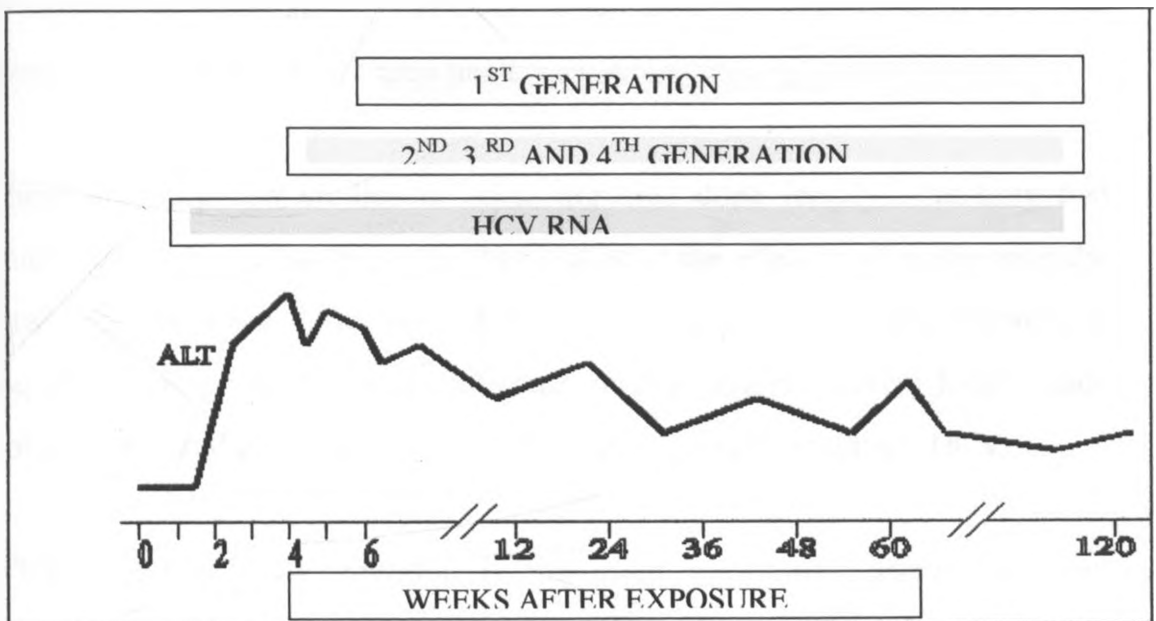
FIG 3: Scheme of typical laboratory features during acute hepatitis C (3)

The diagnosis of HIV infection depends on the demonstration of antibodies to HIV by ELISA and or the direct detection of HIV or one of its components by polymerase chain reaction. Antibodies to HIV generally appear 4-8 weeka after infection. A positive ELISA test is most commonly confirmed by the Western blot (3).

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JUSTIFICATION OF THE STUDY

Acute viral hepatitis is an important disease at the KNH and in this country by virtue of the amount of morbidity and mortality it causes. The proportion of resources devoted to the management of acute hepatitis and its long-term sequelae, and the amount of productivity lost by the economy is great.

Owing to scarcity of diagnostic resources the investigation of suspected hepatitis cases is incomplete. Indeed the only test that is regularly requested in such cases at the KNH is HBsAg, which alone is insufficient to diagnose even acute hepatitis B infection itself.

There are very few studies in acute hepatitis done locally. The only two studies available in the literature that looked at the etiology of acute sporadic viral hepatitis were done more than 15 years ago. Hence new research is required in this area to gather information that reflects current local trends not only of HAV and HBV but also of the increasingly important HCV.

While hepatitis virus infection is important in its own right, the most important viral disease currently in this country is HIV infection whose impact on the prevalence and course of hepatitis cannot be ignored. For this reason the extent of HIV co-infection with the hepatitis A, B and C viruses requires determination.

Quantifying the relative contribution of the hepatitis A, B and C viruses to the overall acute hepatitis burden, as well as the HIV seropositivity rate, will go a long way in improving the planning and provision of appropriate intervention measures in Kenya.

OBJECTIVES

MAIN OBJECTIVE

The main objective of the study was to determine the prevalence of hepatitis A, B, and C virus infection, and HIV seropositivity, among patients with acute icteric hepatitis seen at the Kenyatta National Hospital.

SPECIFIC OBJECTIVES

The specific objectives of the study were: -

1. To determine the prevalence of acute HAV infection among patients with acute icteric hepatitis at the KNH

2. To determine the prevalence of acute HBV infection among patients with acute icteric hepatitis at the KNH

3. To determine the prevalence of acute HCV infection among patients with acute icteric hepatitis at the KNH

4. To determine the HIV seropositivity among patients with acute icteric hepatitis at the KNH

PATIENTS AND METHODS

1. STUDY DESIGN

This was a hospital based descriptive cross sectional study conducted at the Kenyatta National Hospital.

1.1 SAMPLE SIZE

The minimum sample size for the study was 81 patients. This was calculated as shown in appendix 1.

1.2 STUDY PERIOD

The study was carried out over a duration of six months between the months of January and June 2001.

2. PATIENT SELECTION

2.1 SAMPLING

Patients were recruited consecutively from the all the medical and paediatric wards as well as the liver clinic of Kenyatta National Hospital. Posters were placed in the Casualty Department and the Pediatric Filter Clinic asking the Clinicians there to refer patients suspected to have hepatitis to the investigator at the Liver clinic for review.

2.2 DEFINITION OF ACUTE HEPATITIS

Acute icteric hepatitis was defined as the presence of a history of jaundice for a period not exceeding six months together with a disproportionately greater transaminase (ALT and AST) than ALP elevation. The degree of

elevation was assesed by dividing the measured serum concentration by the upper limit of normal for each enzyme.

2.3 INCLUSION CRITERIA

Patients of both sexes aged over six months were included in the study if they had a diagnosis of acute hepatitis as defined above, and they (or their parents or guardians in the case of those under 18 years of age) gave informed written consent to participate in the study.

2.4 EXCLUSION CRITERIA

Patients who had features of chronic liver disease including liver neoplasms and those who declined to give consent were excluded from the study.

3. METHODS

3.1 CLINICAL METHODS

3.1.1 HISTORY

The principal investigator interviewed all recruited patients. The name, age, sex, occupation, marital status, and residence of each patient were recorded on an already prepared data sheet (Appendix 2). The kind of sanitary facilities they used, habit of eating unwashed fruits and/or salad (patient recall) as well as history of recent travel (travelers are likely to eat unclean or not-freshly-cooked foods without washing hands en route) were noted. History of contact with anyone suffering from diarrhea and/or vomiting was also sought.

History of contact with a person with jaundice, blood transfusion, injections with reusable needles, and illicit intravenous drug use with sharing of needles in the previous six months was sought. Tattooing, ear piercing, and scarification with shared implements in the same period were also asked for. Sexual orientation (homosexual, heterosexual, bisexual) and the number of sexual partners in the 24 weeks prior to the onset of symptoms were sought. History of exposure to hepatotoxic chemicals, ingestion of wild mushrooms or hepatotoxic drugs was taken with a view to determining other possible causes of acute hepatitis.

3.1.2 PHYSICAL EXAMINATION

The principal investigator examined all patients. The general condition, as well as the presence of jaundice, pallor, lymphadenopathy, hepatomegaly (and tenderness), splenomegaly, ascites and hepatic encephalopathy was noted and recorded in the already prepared data sheet.

Hepatic encephalopathy was defined as the presence of an altered level of consciousness and behavior, with or without flapping tremor occurring within two weeks of the onset of jaundice (67). It was assigned clinical grades I-V as shown below (68.).

I	Confused. Altered mood or behaviour. Psychometric defects.
II	Drowsy. Inappropriate behaviour.
III	Stuporous but speaking and obeying simple commands. Inarticulate speech. Marked confusion.
IV	Coma
V	Deep coma with no response to painful stimuli

3.2 LABORATORY METHODS

The principal investigator drew 4 millilitres of venous blood from each patient in a biochemically clean bottle and took it to the Immuno-Molecular Diagnostic Laboratory where the serum was separated from the cells by centrifugation and the biochemical tests run.

3.2.1 BIOCHEMICAL TESTS

CARO GmbH diagnostic kits were used. The serum AST level was determined using the Karmen technique (69) as modified by Bergmeyer (70). The serum ALT and ALP levels were determined using the Wroblewski technique (71) as modified by Bergmeyer (70), and the ALP Study Group recommended method (72) respectively. The total serum bilirubin was determined using the method of Van den Bergh and Muller (73) as modified by Pearlman (74); the direct using the method of Keller (75), and the indirect by subtraction (76).

3.2.2 SEROLOGICAL TESTS

All but one serological test were done at the Immunology laboratory, using ELISA based (77) kits. The principal investigator ran all the tests with assistance from the technologists working in the laboratory, and supervision from an Immunologist who also verified the results. IgM anti-HAV was done using the HAV ImmunoComb II kit made by Orgenics which has a sensitivity of 99.5% and a specificity of 99%, IgM anti-HBc using the HBc ImmunoComb II kit from Orgenics which has a sensitivity of 100% and a specificity of 99%, and HCV antibodies using the 4th generation kit HCV AB IV from Innogenetics which has a sensitivity of 100% and a specificity of 99.8%.

Assays for antibodies against HIV-1 and HIV-2 were done using the Innotest kit manufactured by Innogenetics, which has a sensitivity of 100% and a specificity of 99.7%.

The remaining sera were taken to KEMRI hepatitis laboratory where HBsAg was assayed for using the Hepcell II kit, a reverse passive haemagglutinition test kit, with a sensitivity of 98% and a specificity of 99% (78).

4. ETHICAL CONSIDERATIONS

The study was carried out with the approval of the Ethical and Scientific Review Committee of the KNH (see copy of approval letter on page 66). Patients enrolled in the study were given a full explanation of the study and informed written consent sought from them (Appendix 3) or from relatives or guardians/parents for those patients who were unconscious or below 18 years of age. The principal investigator did pre- and post-test counselling for the HIV test. All information obtained about patients was handled with confidentiality. Biochemical and serological results were made available to the patients.

5. DATA MANAGEMENT & ANALYSIS

Data was gathered using a questionnaire (Appendix 2). It was verified and analysed with SPSS 10.0 computer software. Frequencies, percentages, means, ranges and standard deviations were calculated. The results were presented in tables and bar charts. The 95% confidence level was used to assess statistical significance. The Pearson chi-square test was utilized in assessing the statistical significance of association.

RESULTS

1.1 BASELINE CHARACTERISTICS

101 patients with history and physical findings suggestive of acute hepatitis were recruited. The blood specimens of 2 patients were misplaced in the laboratory and these patients were therefore excluded from the study. 15 patients had liver enzyme levels not compatible with a diagnosis of acute hepatitis and were also excluded. 84 patients were therefore enrolled into the study and their baseline characteristics are shown in table 1.

CHARACTERISTIC		NUMBER (N)	PERCENTAGE (%)
SEX	Male	47	56
	Female	37	44
AGE	15 years & below	24	28.6
	Above 15 years	60	71.4
SITE OF MANAGEMENT	Inpatient	56	66.7
	Outpatient	28	33.3
MARITAL STATUS (of patients aged > 15 years)	Married	34	40.5
	Single	26	31
RESIDENCE	Rural	21	25
	Urban	63	75

Table 1: Baseline patient characteristics

1.2 AGE DISTRIBUTION

The mean age of all patients was 25.98 years with a median of 25 and a standard deviation of 16.59 years. The age range was 8 months to 67 years. The mean age for male patients was 25.69 years with a standard deviation of 15.83, whereas female patients had a mean age of 26.35 years with a standard deviation of 17.73. There was no statistically significant difference in the mean age between males and females ($p = 0.409$).

The age distribution of the studied patients is shown in figure 4.
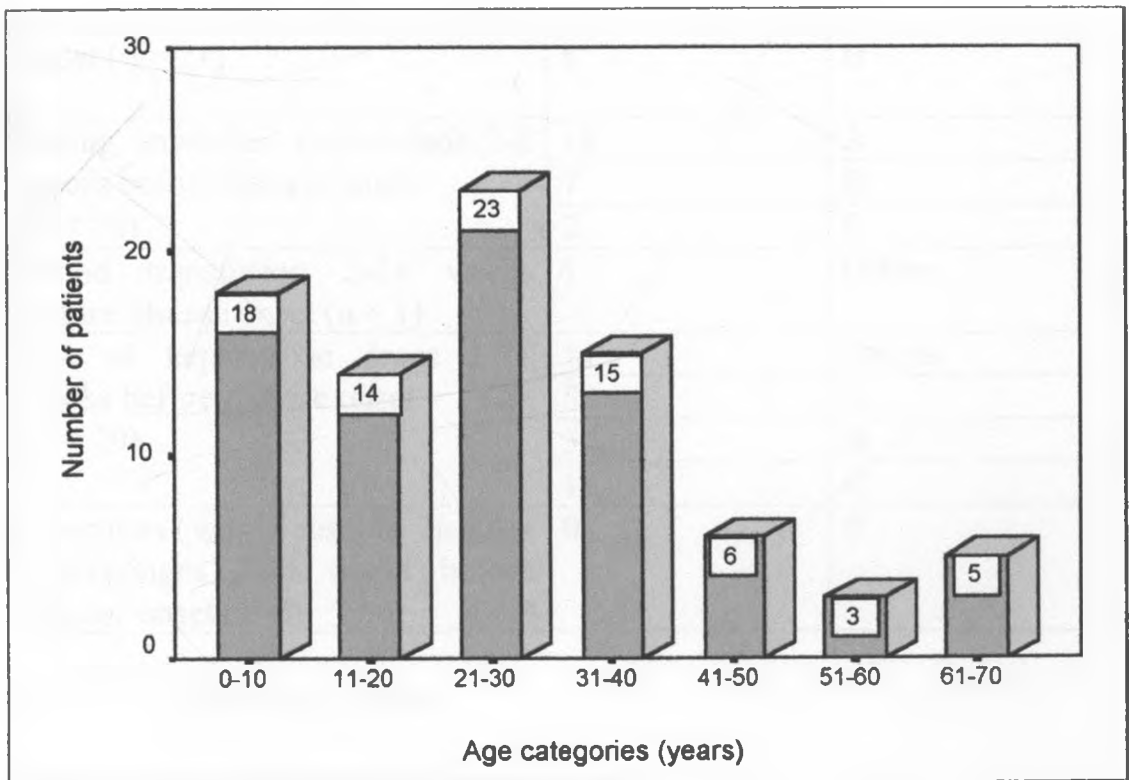
Figure 4: Distribution of patients by age category

2. RISK FACTORS FOR CONTRACTING HEPATITIS

Table 2 illustrates the various risk factors for contracting acute hepatitis looked for in the study.

RISK FACTOR	NUMBER	TYPE OF HEPATITIS
Contact with jaundiced person 2-24 weeks before onset of disease (n = 4)	3	A
	1	Others
Contact with person(s) with diarrhoea and/vomiting 2-7 weeks before disease onset (n = 4)	3	A
	1	B
Travel 2-7 weeks before disease onset (n = 21)	13	A
	8	B
Eating unwashed fruits/salads 2-7 weeks before disease onset (n = 26)	18	A
	7	B
	2	C
Blood transfusion 2-24 weeks before disease onset (n = 1)	1	Others
Use of hepatotoxic drugs 1-24 weeks before disease onset (n = 20)	11	Others
	5	A
	3	B
	1	C
Injections with reusable needles and/syringes 2-24 weeks before disease onset (n=0)	0	0

n = Number of patients

Table 2: Risk factors for contracting hepatitis

The figures were too small to test for statistical association between the risk factors and the various types of hepatitis.

3. CLINICAL FEATURES

3.1 DURATION OF JAUNDICE

The mean duration of jaundice from the time it was first noticed to the time the patients were recruited into the study was 17.43 days. The mean for female patients was 15.39 and for male patients 19.11 days.

The mean duration of jaundice for the various types of hepatitis is shown in figure 5.
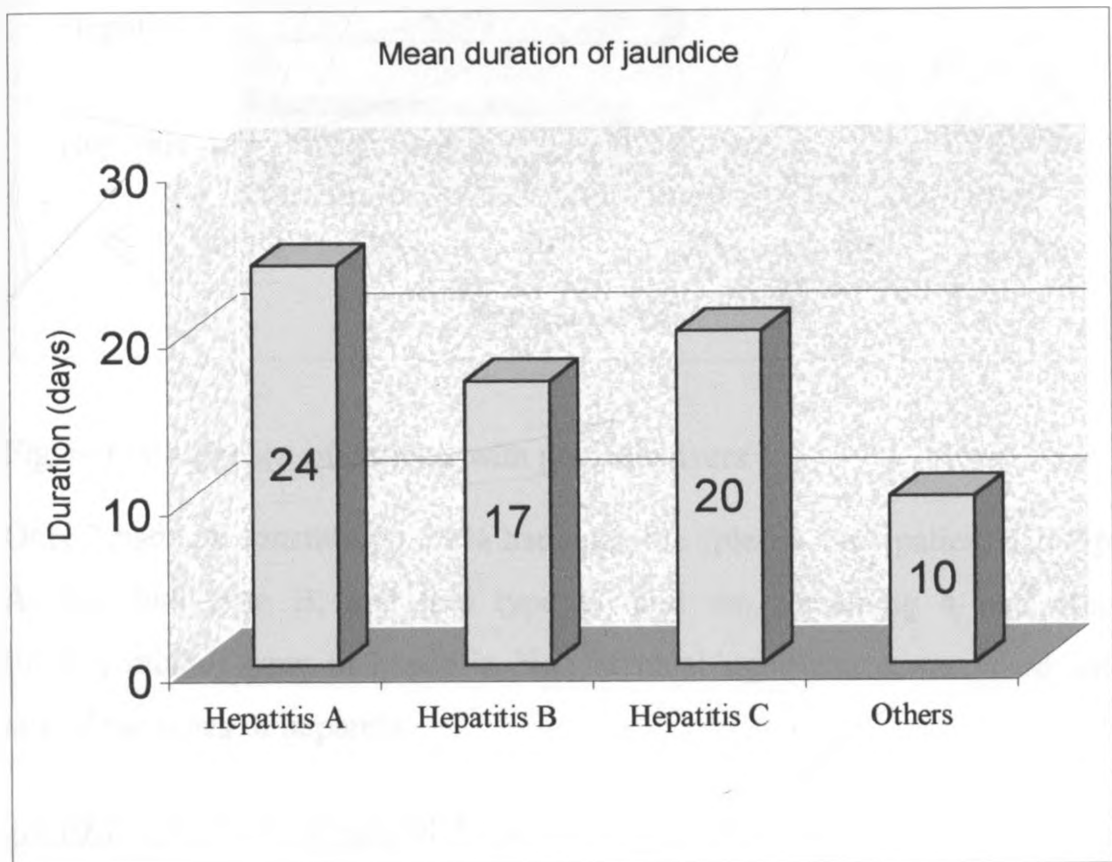
Figure 5: Mean duration of jaundice

3.2 PALPABLE LIVER AND SPLEEN

72.6% of patients had palpable livers. 54.8% of these organs were tender. The proportions of patients with palpable livers among those who had various types of hepatitis are shown in figure 6.
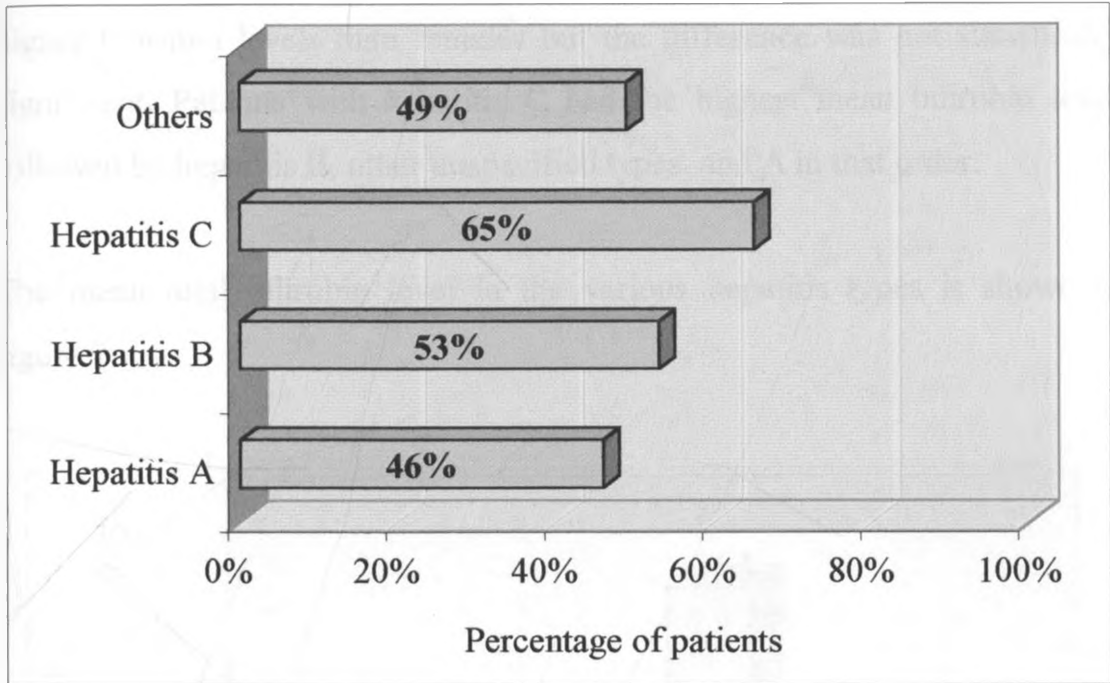
Figure 6: Proportion of patients with palpable livers

Only 7 patients constituting 8.3% had palpable spleens. One patient had type A, two had type B, one had type C, and the remaining 4 had other (undetermined) types of hepatitis. No statistical significance was noted with any of the types of hepatitis.

3.3 HEPATIC ENCEPHALOPATHY

Six patients (7.1%) were in hepatic encephalopathy; four in grade I, one in grade II and another one in grade III. One patient had hepatitis B, another hepatitis C and the remainder other undetermined types of hepatitis.

4. LIVER FUNCTION TESTS

4.1 SERUM BILIRUBIN LEVELS

The mean total, direct, and indirect bilirubin levels were 182.3 mmol/l, 75.5 mmol/l, and 104.3 mmol/l respectively. Male patients on the whole had higher bilirubin levels than females but the difference was not statistically significant. Patients with hepatitis C had the highest mean bilirubin level followed by hepatitis B, other unspecified types, and A in that order.

The mean total bilirubin level in the various hepatitis types is shown in figure 7.
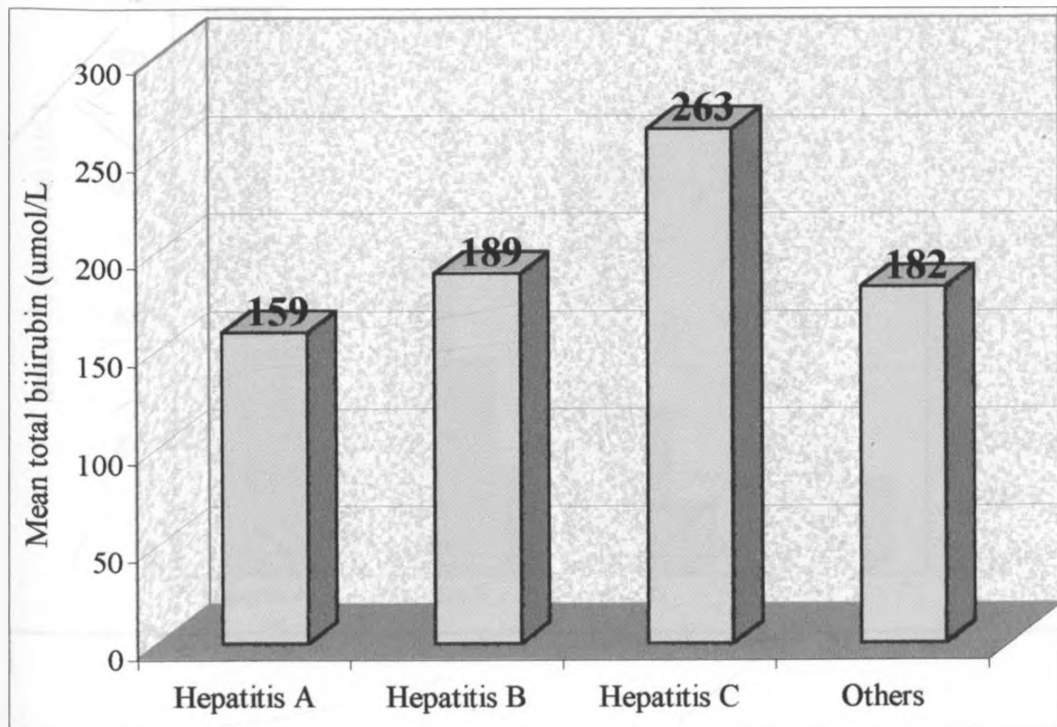
Figure 7: Mean total bilirubin levels in the various types of hepatitis

4.2 LIVER ENZYMES

The mean alanine aminotransaminase, aspartate aminotransaminase, and alkaline phosphatase levels in the study were 144.97 U/L, 271.4 U/L, and 593.24 U/L respectively. There was no statistical difference between mean levels in male and female patients.

The variation of the mean enzyme levels with the type of hepatitis is shown in figure 8.
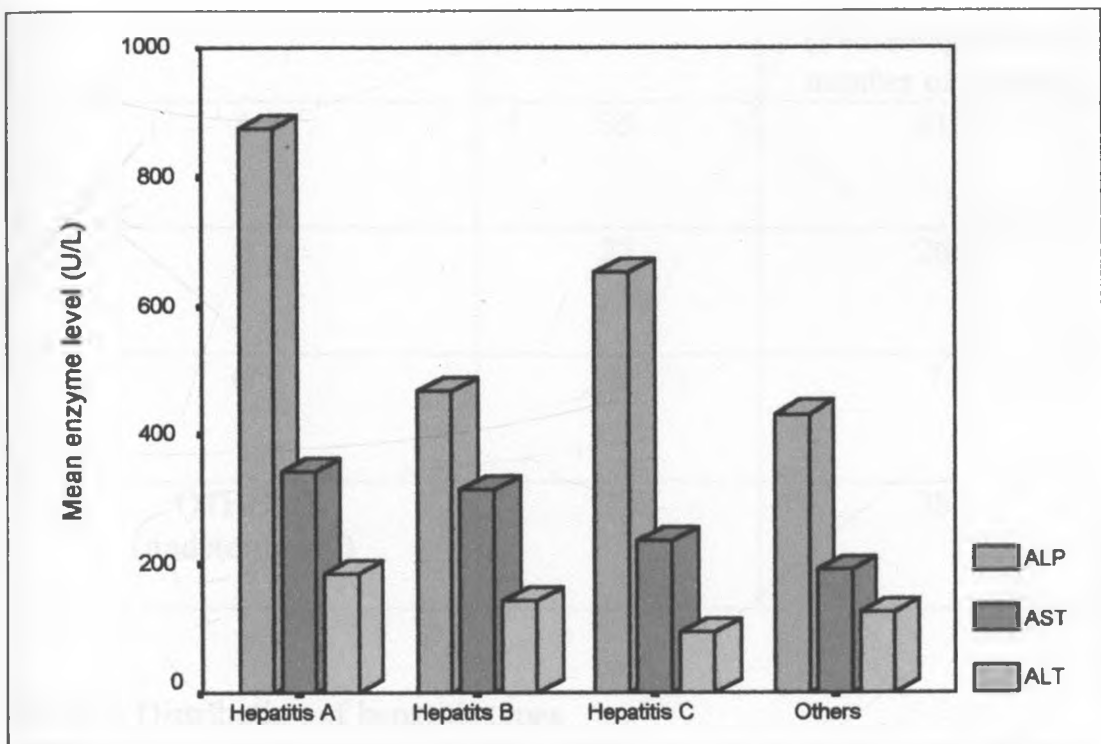
Figure 8: Mean liver enzyme levels

5. SEROLOGY

5.1 HEPATITIS PREVALENCE

5.1.1 OVERALL HEPATITIS PREVALENCE

63 Patients tested positive for one of the markers of acute hepatitis. No markers were detected in 21 patients. The number of patients testing positive for the various markers of acute infection is shown in table 3.

HEPATITIS TYPE	NUMBER	PREVALENCE (%) (Percentage of total number of patients)
A	35	41.7
B	22	26.2
C	6	7.1
OTHERS (undetermined)	32	38.1

Table 3: Distribution of hepatitis types

There were more cases of acute hepatitis recorded than the total number of patients enrolled in the study because some patients had infection with more than one type of hepatitis virus.

5.1.2 PREVALENCE IN PATIENTS AGED 15 YEARS AND BELOW, AND THOSE ABOVE 15 YEARS

The distribution of the various hepatitis types in patients aged 15 years and below and in those aged above 15 years is shown in the table 4.

Type Of Hepatitis	Percentage in Patients 15 Years and Below	Percentage in Patients Above 15 Years
A	67.9	23.9
B	7.1	29.9
C	10.7	4.5
Others (undetermined)	14.3	41.8

Table 4: Hepatitis prevalence in patients 15 and below, and above 15 years

5.1.3 HEPATITIS VIRUS CO-INFECTION

Eleven patients tested positive for markers of acute infection of more than one hepatitis virus. Seven patients had both hepatitis A and B, two had A and C, while one had B and C. One patient had triple infection with hepatitis A, B, and C.

5.2 HEPATITIS A

There were 35 patients with acute hepatitis A. 54.3 % of them were aged 15 years and below. The majority of the patients with hepatitis A were outpatients (65.7%).

The distribution of patients with type A hepatitis according to age category is shown in figure 9.
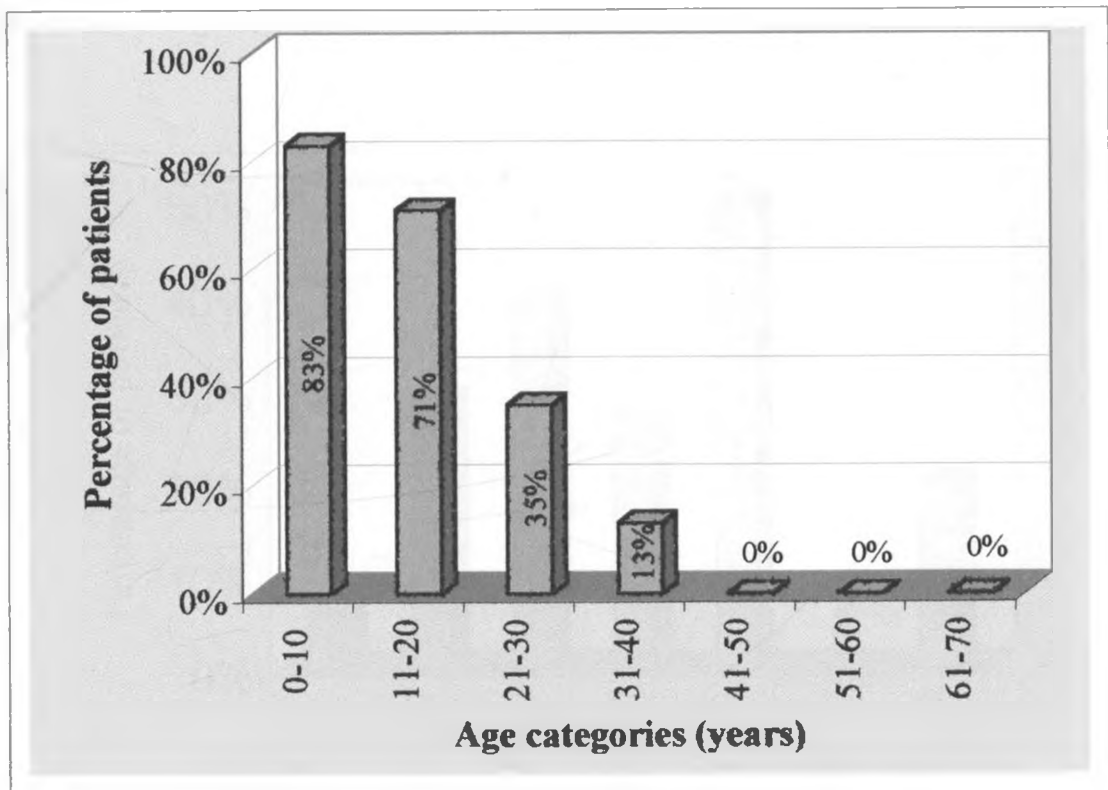
Figure 9: Age distribution of patients with acute hepatitis A

5.3 HEPATITIS B

5.3.1 ACUTE HEPATITIS B

There were 22 patients with acute hepatitis B, diagnosed by the presence of IgM anti-HBc antibodies, representing 26.2% of the total. 90.1% of these patients were aged above 15 years whereas only 9.9% were aged 15 years and below. Most of the patients with hepatitis B were inpatients (77.3%).

The distribution of patients with acute hepatitis B according to age category is shown in figure 10.
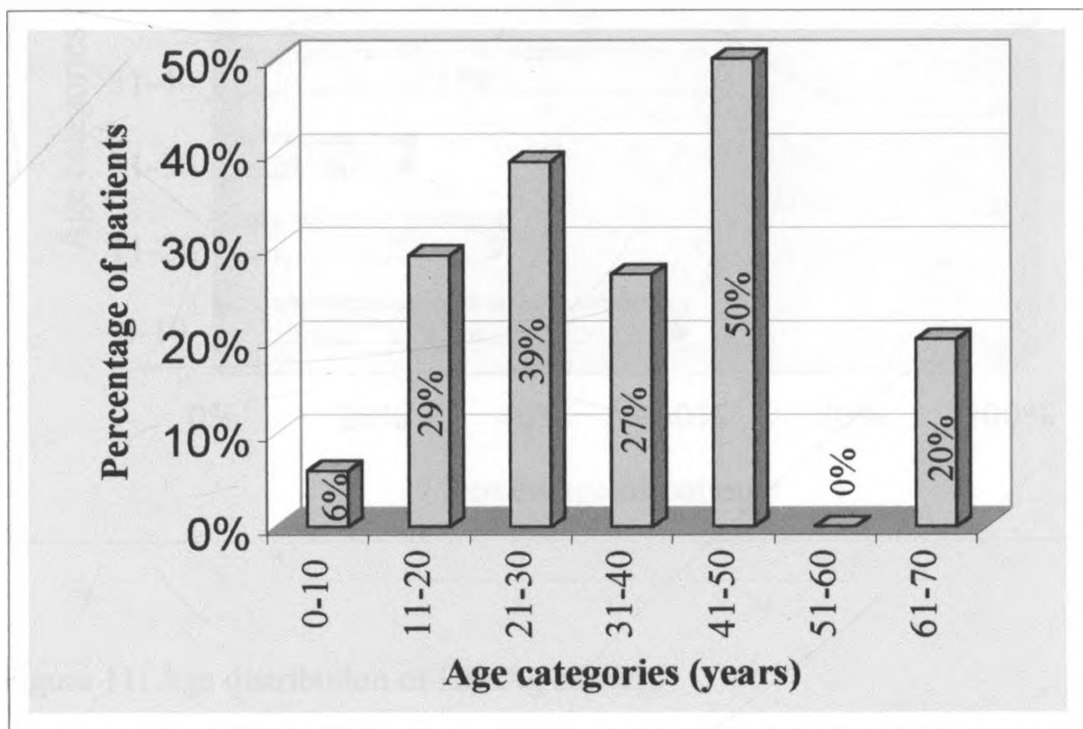
Figure 10: Age distribution of patients with acute hepatitis B

The hepatitis B surface antigen was detected in 21 patients representing 25% of all patients studied.

Figure 11 shows the age distribution of hepatitis B surface antigen carriers.
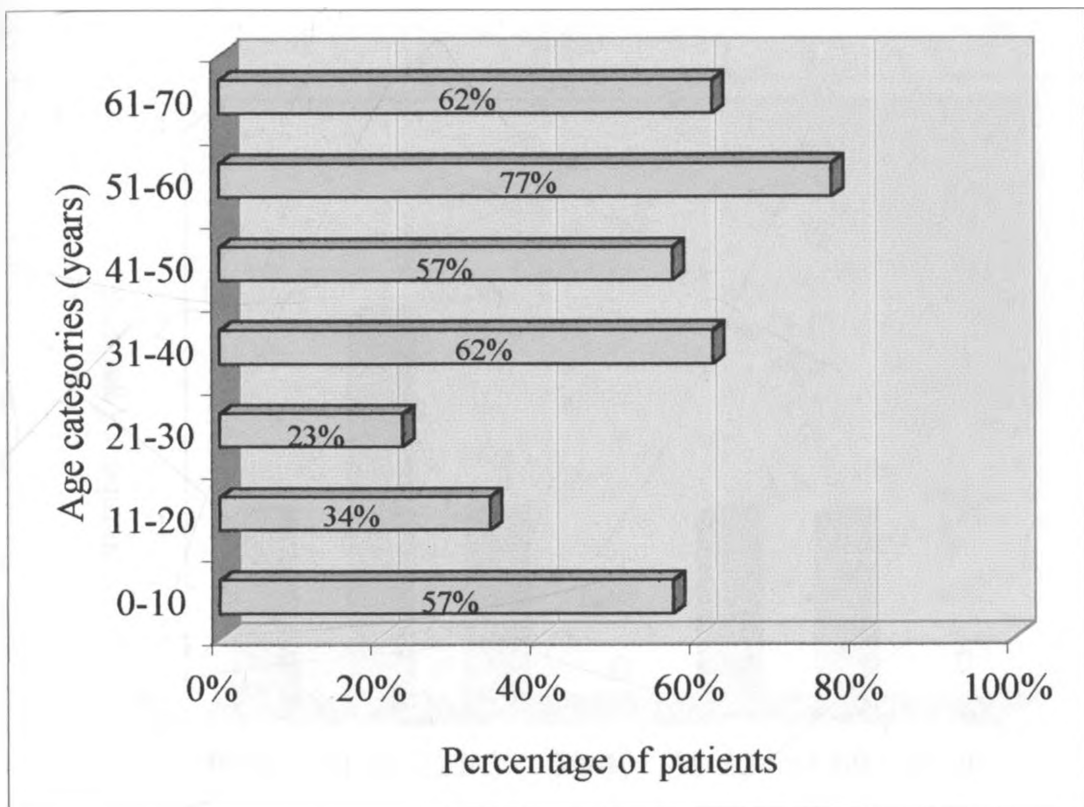
Figure 11: Age distribution of HBsAg carriers

11 patients had the HBsAg with a negative IgM anti-HBc test. These made up 13.1% of all patients and were considered carriers.

5.4 HEPATITIS C

There were 6 patients with acute hepatitis who tested positive for anti-HCV antibodies, representing 7.1% of all cases. Half of the patients were aged 15 years and below. There was also equal distribution of patients with HCV infection between inpatient and outpatient areas.

The age distribution of these patients is shown in figure 12.
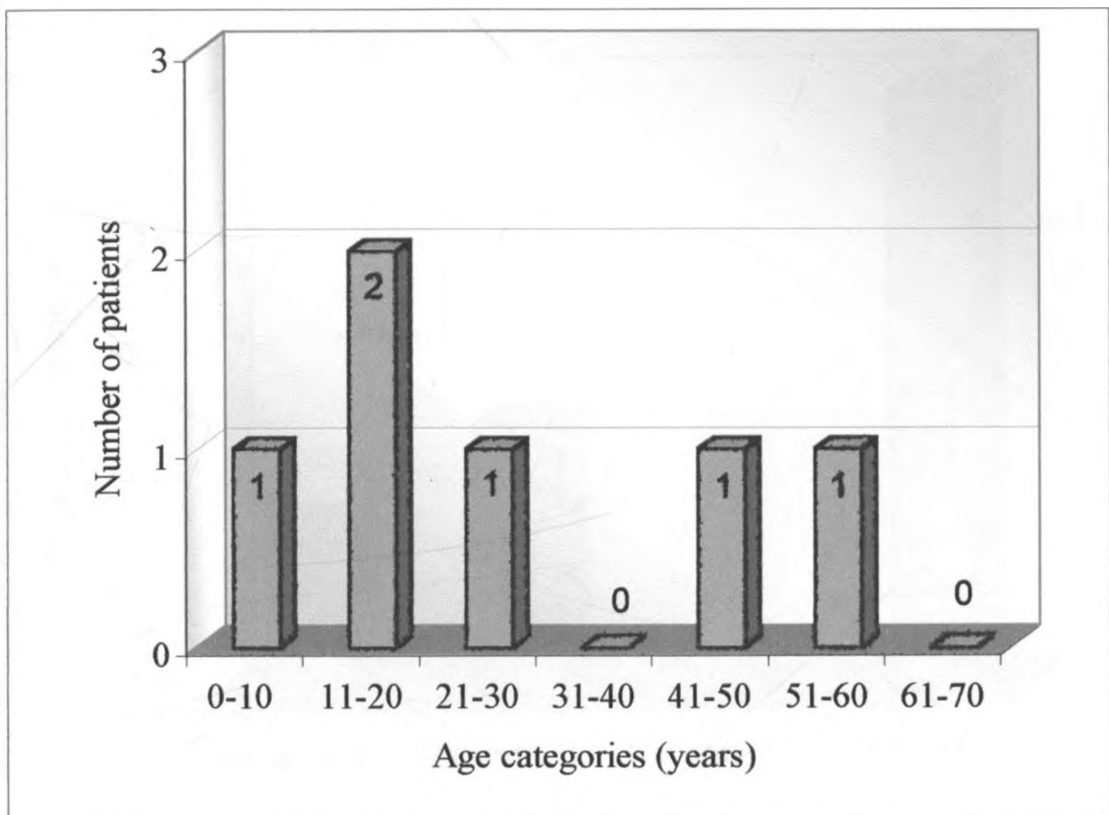
Figure 12: Age distribution of patients with acute hepatitis C

Four of the patients with acute hepatitis in this study were males while the remaining two were females.

5.5 HIV CO-INFECTION

26 Patients were also HIV positive. 5 of these patients had hepatitis A, 5 hepatitis B, and 16 other undetermined types of hepatitis. None of the patients with hepatitis C antibodies were HIV positive.

The distribution of HIV seropositivity among patients with various hepatitis types is shown in figure 13.
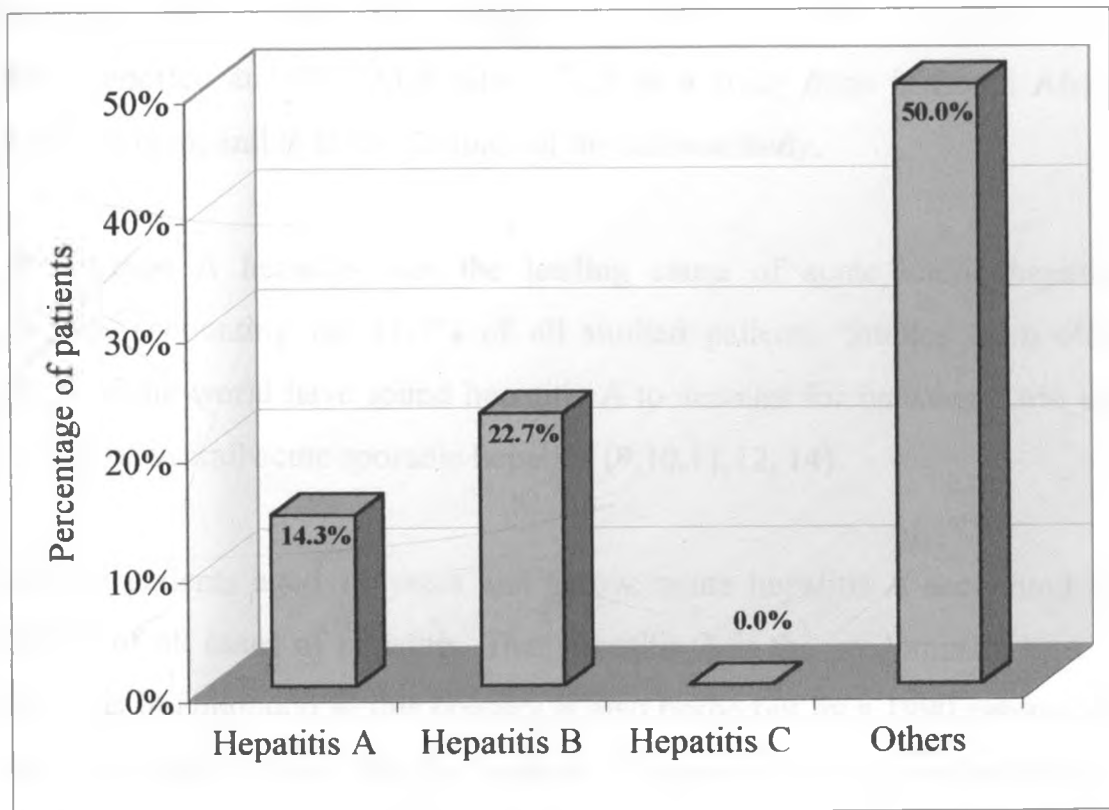
Figure 13: HIV status in patients with various hepatitis types

There was a statistically significant association between HIV seropositivity and the category of patients who had undetermined (others) causes for their hepatitis (p = 0.003)

DISCUSSION

The mean ALT and AST levels in this study were 144.97 and 271.4 U/L respectively, which were much lower than figures reported in literature (32). Transaminase levels are known to peak at the onset of jaundice and fall progressively thereafter (32). These low levels could be attributed to the rather long average duration of jaundice of 17.43 days before patients were recruited into the study. The AST to ALT ratio is typically less than 1 in acute viral hepatitis (79) though occasionally the ratio may be higher and even exceed 2 (80). The AST to ALT ratio in this study was 1.9. Lodenyo et al (81) have reported an AST: ALT ratio of 1.8 in a study done in South Africa, which is comparable to the findings of the current study.

Acute type A hepatitis was the leading cause of acute icteric hepatitis overall, accounting for 41.7% of all studied patients. Studies from other parts of the world have found hepatitis A to account for between 3.6% and 66.4% of overall acute sporadic hepatitis (9,10,11,12, 14).

Among patients aged 15 years and below, acute hepatitis A accounted for 67.9% of all cases of hepatitis. That hepatitis A is the predominant type of hepatitis in childhood in this country is also borne out by a 1990 community sero-prevalence survey for the markers of hepatitis A infection in Kiambu district, which borders Nairobi, where 80% of children below 4 years of age were found to have evidence of previous disease (82). Studies from other parts of the developing world have also found hepatitis A to be a disease of the young as was found, for instance, by Wang et al (11) in Taiwan where the disease occurred mainly in youngsters and its prevalence decreased with increasing age.

Acute hepatitis A accounted for 23.9% of all cases of hepatitis among patients older than 15 years, almost twice the 12% rate reported by Greenfield (5). This could perhaps be attributed to the marked population growth in the city of Nairobi, where most of the study patients came from, which has led to mushrooming of slums, overcrowding and sanitation inadequacies in the two decades since the Greenfield study was done.

Acute hepatitis B was the second most common type of hepatitis overall, being found in 26.2% of all the patients. This is comparable to rates reported from other parts of the developing world. Topley has reported 21% from Zimbabwe (7), Wang 26.2% from China (11), Alter 28.7% from the USA, and Hazra 30.5% from India (8).

Among people over 15 years of age acute hepatitis B was the leading cause of hepatitis. It was diagnosed in 29.9% of these patients. This is markedly different from the 70% Greenfield found (5). The decline in the proportion of patients with hepatitis B seen at the KNH is probably due to the gradual falling of such risky traditional practices as mass circumcision with shared knives, and tribal scarification into disfavour. Greater availability and use of blood screening facilities, and increased vaccination against hepatitis B are other possible explanations.

The mean age of patients with hepatitis B in the current study was 31 +/- 11.52 years whereas it was 24.1 +/- 3.0 years in the Greenfield study, which may indicate a tendency to affect older people. In some parts of the world hepatitis B occurs in much older people as has been found in Taiwan where hepatitis B mainly afflicts middle aged and elderly people (10).

Eleven (13%) of all patients were HBsAg positive but IgM anti-HBc antibody negative. These patients were probably hepatitis B carriers. Assaying for IgG anti-HBc antibodies would have clarified the true nature of these patients' infection status but this was beyond the scope of the present study. This figure is comparable to the 14% found by Greenfield in 1983 (5) and lies well within the 5-15% HBsAg carriage rate reported by Okoth and colleagues for Nairobi province (83). Reports from South Africa indicate that between 8 and 15 percent of Blacks are carriers (81). Chu et al have reported a rather high HBsAg carrier rate in Taiwan of 53% (10). Hazra et al from India reported a carrier rate of 8% in healthy controls (8).

Six patients (7.1%) tested positive for anti-HCV antibodies. One of these patients was in hepatic encephalopathy, and passed away soon after being recruited. These results confirm what has been reported elsewhere that HCV rarely causes acute icteric hepatitis (84). However they do show that the disease can be serious and even fatal.

The overall proportion of patients with acute hepatitis C compares very well with figures reported from the rest of the world. Alter (14) has reported 4.4% from the USA, Saat (9) 5.4% from Malaysia, Wang (11) 9.9% from China, and the WHO (12) 10.3% from Japan. Ethiopia, Kenya's northern neighbour, has been reported to have a much higher hepatitis C prevalence of 19%.

Hepatitis virus co-infection was present in eleven patients. Retesting of sera from these patients, preferably with different test kits, would have been desirable but was not possible because of logistical limitations. Co-infection

with hepatitis B and C may occur because of the shared modes of transmission. However dual infection involving hepatitis A with B or C may have little more than chance to explain it. Literature on hepatitis virus co-infection is scant. Previous studies done in this country (5, 90) did not report dual infection. A study done in Thailand in HIV positive people reported a HBV/HCV co-infection rate of 2.2% (85).

Serological testing did not reveal the etiology in 32 patients. This category probably comprised infection by agents like the hepatitis E, hepatitis G, cytomegalovirus, *Leptospira*, and *Brucella*. This group had a statistically significant association ($p = 0.003$) with HIV seropositivity. This suggests the possibility of affliction by such organisms as *Mycobacteria, Pneumocystis, Histoplasma, Cryptococcus, Penicicillosis* and other opportunistic conditions, which have been shown to affect the liver in HIV positive individuals (85). Drugs could also account for some of the cases since 57.9% of these patients had used various drugs although the association was not significant.

Twenty-six patients were infected with the HIV virus. Among admitted adults with hepatitis the HIV seropositivity rate was 50%. This was much higher than the 19% seroprevalence rate for admitted patients in the medical wards at the KNH reported by Gilks et al (86). Similarly high HIV seroprevalence rates have been found among admitted patients with various disorders in the same hospital: Owino got 40.9% among patients with exudative pleural effusion (87), and Hooker got 80% among patients with tuberculous meningitis (88). Among the admitted children in the study only

one was HIV seropositive, representing 8.3%. This is similar to the 12.7% reported by Wafula et al (89) among admitted children at the KNH.

HIV infection was significantly associated with age greater than 15 years (p value 0.005), which reflects the predominantly sexual mode of spread of the virus in this country. The HIV co-infection rates with the various types of hepatitis were 19.2%, 22.7%, 0%, and 50% for hepatitis A, B, C, and other unspecified types respectively. A South African study by Lodenyo et al (81) found a hepatitis B/HIV co-infection rate of 41%. The Hepatitis C/HIV co-infection rate was 1% in that study. These figures may reflect the higher HIV burden obtaining in that country. Studies from the West report HBV/HIV and HCV/HIV co-infection rates of 90-95% and 11.7%-30% respectively (43, 45, 90, 91, 92).

72.6% of the study patients had palpable livers, 95.8% of which were tender. The proportion of patients with palpable livers were 30%, 25%, 8% and 38% in hepatitis A, B, C, and other unspecified types respectively. This finding agrees closely with the hepatomegaly rates of 30%, 39% and 33% for hepatitis A, B and non-A non-B found in the Greenfield study (5). Jindani et al in an earlier study at the KNH found hepatomegaly in 50% of their patients (93).

Splenomegaly was found in 8.3% of all the patients. The splenomegaly rates among patients with hepatitis A, B, C and other (undetermined) types of hepatitis were 2.9%, 9.1%, 16.7% and 19% respectively. This is comparable to the findings of the Greenfield study (5) in which the splenomegaly rates in

hepatitis A, B and other unspecified types of hepatitis were 10%, 14% and 9% respectively (5). Jindani et al found no patient with splenomegaly (93).

7.1% of the patients in this study were found to have flapping tremor with or without an altered level of consciousness or behaviour. Electro-encephalograms would have been useful in ascertaining the diagnosis of hepatic encephalopathy but were not logistically feasible. Nevertheless the figure obtained in this study was markedly higher than the 3.2% Greenfield (5) found. This may be attributed to the fact that 66.7% of the patients in the current study were in-patients, and therefore had more severe disease, whereas only 10% of the patients in the Greenfield study were admitted.

STUDY LIMITATIONS

1. It was not logistically possible to rule out all the other viruses and micro-organisms known to cause hepatitis.

2. Supplemental tests such as the recombinant immunoblot assay (RIBA) or the HCV RNA polymerase chain reaction were not done owing to logistical limitations.

CONCLUSIONS

1. Hepatitis A virus infection was the commonest cause of acute icteric hepatitis overall. It was also the leading cause of hepatitis among patients aged 6 months to 15 years at the KNH.

2. Hepatitis B infection was the most common type of acute hepatitis seen among patients older than 15 years at the KNH.

3. Hepatitis C virus infection accounted for 7.1% of acute icteric hepatitis among patients seen at the Kenyatta National Hospital.

4. 30.1% of all the study patients, and 50% of those admitted in the medical wards, with acute icteric hepatitis also tested positive for HIV.

5. 32 patients in the study did not have markers of acute infection for hepatitis A, B or C. This group was significantly associated with HIV seropositivity ($p = 0.003$).

RECOMMENDATIONS

1. Assaying for IgM anti-HAV, IgM anti-HBc, and anti-HCV antibodies should be part of the diagnostic work-up of patients with acute icteric hepatitis at the KNH where this is not currently being practised.

2. Patients admitted with acute icteric hepatitis at the KNH should be advised to undergo testing for HIV.

3. Vaccination against hepatitis A and B, given as early as possible in life, should be encouraged among Kenyans.

4. A larger study is recommended to confirm the low hepatitis B prevalence in adults with acute icteric hepatitis.

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APPENDIX 1: SAMPLE SIZE CALCULATION

$$N = \frac{(Z - a/2)^2 \; X \; p(1 - p)}{d^2}$$

Where:

N = 81 (minimum sample size)

Z1 – a/2 = 1.96, corresponding to a significance level of 0.05

P = estimated prevalence {70%, the prevalence of acute B hepatitis in the Greenfield study (5)}

d = desired degree of accuracy (10%)

APPENDIX 2: PROFORMA

I.   HISTORY

1. Name ....

2. Age..

3. Sex...

4. Marital status..

5. Residence..

6. Occupation..

7. Mode of sewage disposal at home...

8. Duration of jaundice and/or dark urine..

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9. Recent travel in the 7 weeks before onset of illness (Yes/No).............................

10. Outbreak of diarrhoea and/vomiting in family /institution in the 7 weeks before onset of illness (Yes/No)..

11. Outbreak of jaundice in family /institution in the in the 7 weeks before onset of illness Yes/No)..

12. Ingestion of raw/undercooked seafood in the 7 weeks before onset of illness (Yes/No)...

13. Method of food storage:

      Grains (Granary/Other)...

      Cooked food (Fridge/Open/Other)..

14. Habit of eating unwashed fruits/ vegetables/salads (Yes/No)..............................

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15. Contact with jaundiced person in the 24 weeks before onset of illness (Yes/No)..

16. Injections with reusable needles in the 24 weeks before onset of illness (Yes/No)..

17. Intravenous drug use with sharing of needles in the 24 weeks before onset of illness (Yes/No) ....

18. Blood or blood product transfusion in the 24 weeks before onset of illness (Yes/No)

....

19. Tattooing/Scarification/Ear piercing with shared equipment in the 24 weeks before onset of illness Yes/No)...

20. Sexual habit (homosexual, heterosexual, bisexual)..

21. Multiple sex partners in the 24 weeks before onset of illness (Yes/No)..

----

22. Exposure to industrial metals and solvents one week prior to the onset of symptoms (Yes/No)..

23. Ingestion of wild mushrooms one week prior to the onset of symptoms (Yes/No)..

24. Exposure to the following drugs 6 months prior to the onset of symptoms (Yes/No): -

     Anaesthetic drugs e.g. halothane..

     Anti-TB drugs e.g. isoniazid and rifampicin..

     CNS drugs e.g chlorpromazine,Amitriptylline,imipramine..

     Anticonvulsants e.g phenytoin,carbamazepine..

     NSAIDs e.g. indomethacin, diclofenac..

     Antifungals e.g. ketoconazole, fluconazole, itraconazole..

     Antivirals e.g. AZT, DDI...

     Antihypertensives e.g. aldomet, , chlorthiazide...

     Calcium channel blockers e.g nifedipine,verapamil, diltiazem..

     Oral cotraceptives and androgens (Yes/No)...

     Antibiotics e.g erythromycin, trimethoprim-sulphamethoxazole(Yes/No)............

     Any other drug not listed above and considered hepatotoxic............................

II. EXAMINATION

General condition..

Jaundice (Yes/No)..Degree.............................

Pallor (Yes/No)...Degree..............................

Palpable lymph nodes (Yes/No)..........................Area(s)............................

Palpable liver (Yes/No)................................. ..Tender (Yes/No).................

       Span...

Palpable spleen (Yes/No)..

       Size in cm below costal margin...

Ascites (Yes/No)..

Flapping tremor (Yes/No)..

Coma (Yes/No)..

       Grade..

III. LAB RESULTS

Bilirubin

       Total (umol/L)..

       Direct (umol/L)...

       Indirect (umol/L)..

AST (U/L)..

ALT (U/L)..

ALP (U/L)..

IgMantiHAV(Positive/Negative))..

HbsAg (Positive/Negative)..

IgM anti-HBcAg (Positive/Negative)..

Anti-HCV (Positive/Negative)..

ELISA for HIV (Positive/Negative)..

APPENDIX 3: INFORMED CONSENT

This is to confirm that I have agreed to participate in the study entitled **"The prevalence of hepatitis A, B, and C and HIV seropositivity among patients with acute icteric hepatitis at the Kenyatta National Hospital."**

As explained to me, this will involve undergoing a physical examination and having blood samples taken from me for viral hepatitis markers as well as a test for HIV. While the results remain the confidential property of the investigator, significant findings that may influence further management of my condition may be made available to me.

I understand that I am free to withdraw from the study at any time without forfeiting any medical benefits due to me.

Patient's Name...

Signature.. (Parent / Guardian if under 18 years)

Date..

Witness...

Name...

Date..

APPENDIX 4: IgM ANTI-HAV ASSAY

TEST PRINCIPLE

The Immnocomb II IgM anti-HAV test is a solid-phase enzyme immunoassay, based on an immunocapture principle. The solid phase is a comb with 12 projections (teeth). Each tooth is sensitised with rabbit antibodies to human IgM (lower spot), and monoclonal antibody to HAV (upper spot), which acts as the internal control. The developing plate has 6 rows (A-F) of 12 wells, each row containing a reagent solution ready for use at a different step in the assay. The test is performed stepwise by moving the comb from row to row, with incubation at each step.

PROCEDURE

1. Serum is pre-diluted 1:50 and added to diluent in the wells of row A. It is mixed and incubated for 2 hours at 37° C. Human IgM will be captured by the anti-IgM antibodies on the teeth.

2. The comb is inserted into the rows of row B which contains wash solution, that washes away unbound components.

3. The comb is inserted into row C, mixed and incubated for 30 minutes at 37^{0} C. HAV antigen in the wells will react with anti-HAV antibodies captured on the teeth.

4. The comb is inserted into the wells of row D, mixed and incubated at 37^{0} C for 20 minutes. ALP-labeled monoclonal anti-HAV antibody binds to the bound HAV.

5. The comb is washed for 2 minutes in the wells of row E.

6. The comb is inserted into row F wells, which contain chromogenic substrate solution (5-bromo-4-indolyl phosphate and nitro blue tetrazolium), mixed and incubated for 10 minutes at 37° C.

7. The reaction is stopped by re-inserting the comb into row E, and incubating for 1 minute. The comb is then dried in air.

8. Results are read by comparing the intensity of the lower spot of each specimen tooth with that of the lower spot of the control tooth; a positive test will produce a spot with an intensity equal to or higher than that of the positive control whereas negative tests will produce no spot or one with an intensity lower than that of the positive control.

APPENDIX 5: IgM ANTI-HBc ASSAY

TEST PRINCIPLE

The Immnocomb II IgM anti- HBc test is a solid-phase enzyme immunoassay, based on an immunocapture principle. The solid phase is a comb with 12 projections (teeth). Each tooth is sensitised with goat antibodies to human IgM (lower spot), and rabbit anti-HBc (upper spot), which acts as the internal control. The developing plate has 6 rows (A-F) of 12 wells, each row containing a reagent solution ready for use at a different step in the assay. The test is performed stepwise by moving the comb from row to row, with incubation at each step.

PROCEDURE

1. Serum is pre-diluted 1:50, added to diluent in the wells of row A, mixed, and incubated for 1 hour at 37° C. IgM will be captured by the anti-IgM antibodies on the teeth.

2. The comb is inserted into the rows of row B which contains HBcAg, that reacts with any bound IgM anti- HBc.

3. The comb is inserted into row C, mixed and incubated for 20 minutes at 37^0 C. The bound HBcAg will react with rabbit anti-HBc antibody labelled with alkaline phosphatase.

4. The comb is inserted into the wells of row D, and agitated for 2 minutes. 5. The comb is similarly washed for 2 minutes in the wells of row E.

6. The comb is inserted into row F wells, which contain chromogenic components which react with the bound alkaline phosphatase, and left for 10 minutes.

7. The reaction is stopped by re-inserting the comb into row E, and incubating for 1 minute. The comb is then dried in air.

8. Results are read by comparing the intensity of the lower spot of each specimen tooth with that of the lower spot of the control tooth; a positive test will produce a spot with an intensity equal to or higher than that of the positive control whereas negative tests will produce no spot or one with an intensity lower than that of the positive control.

APPENDIX 6: HBsAG ASSAY

PRINCIPLE

The KEMRI Hepcell II test is a reverse passive hemagglutination method for detecting HbsAg. Test serum is mixed with anti- HBs coated sheep erythrocytes in a microtitre plate. The cells will agglutinate if the test serum contains HBsAg. If the test serum does not contain HBsAg, the cells will settle and form a button-like precipitate at the bottom of the plate.

PROCEDURE

1. Hepcell diluent is added to the microtitre wells.
2. Test serum is added to the diluent producing a dilution of 1:16.
3. The reagent, which contains coated sheep erythrocytes, is added, the contents mixed, and incubated at room temperature for 1-2 hours.
4. Results are read by looking for the presence of agglutination.
5. Sera showing positive or indeterminate results are confirmed by repeating the test at greater dilutions of up to 1:64.

APPENDIX 7: HCV ANTIBODY ASSAY

PRINCIPLE

The INNOTEST HCV Ab IV test is an enzyme immunoassay for the detection of antibodies to HCV. Microplate wells coated with a mixture of HCV antigens are utilized. Test sera are incubated in the wells. Viral specific antibodies to HCV, if present, will bind to the solid phase antigens. Subsequently an affinity-purified rabbit anti-human IgG labelled with the enzyme horseradish peroxidase is added. Upon a positive reaction this labelled antibody becomes bound to any solid-phase antigen-antibody complex previously formed. Incubation with enzyme substrate produces a blue colour, which turns yellow when the reaction is stopped with sulphuric acid.

PROCEDURE

1. A 1:10 dilution of test serum is made by adding diluent to serum in the well and mixing.
2. The wells are incubated at 37^0 C for 60 minutes (to capture antibody).
3. Each well is washed 6 times with wash solution.
4. Conjugate (containing enzyme labelled anti-human IgG) is added to each well, mixed and incubated at 37^0 C for 60 minutes.
5. Each well is washed 6 times with wash solution.
6. Substrate is added and the wells incubated for 30 minutes in the dark, at room temperature.
7. The reaction is stopped by adding stop solution and mixing thoroughly.
8. The absorbance of the solution in the wells is read at 450 nm with a microplate reader.
9. The cut-off value is calculated by dividing the mean absorbance of positive controls by
    2.75. A test sample is positive or negative if its absorbance is greater or less than the
    2.76. cut-off value respectively.

APPENDIX 8: HIV TEST

PRINCIPLE

The Innotest HIV-1/HIV-2 test is an enzyme immunoassay. Wells coated with synthetic peptides representing envelope antigens of HIV-1 and HIV-2 are used. Test sera are incubated in the wells. Viral specific antibodies to HIV-1 and HIV-2 if present will bind to the solid phase antigens. Subsequently an affinity-purified rabbit anti-human IgG labelled with the enzyme horseradish peroxidase is added. Upon a positive reaction this labeled antibody becomes bound to any solid-phase antigen-antibody complex previously formed. Incubation with enzyme substrate produces a blue colour, which turns yellow when the reaction is stopped with sulphuric acid.

PROCEDURE

1. A 1:20 dilution of test serum is made by adding diluent to serum and mixing.
2. The wells are incubated at 37^0 C for 30 minutes (to capture antibody).
3. Each well is washed 5 times with wash solution.
4. Conjugate (containing enzyme labelled anti-human IgG) is added to each well, mixed and incubated at 37^0 C for 30 minutes
5. Each well is washed 5 times with wash solution.
6. Substrate is added and the wells incubated for 30 minutes at 20-25^0 C.
7. The reaction is stopped by adding 1-2 mol/l sulphuric acid and mixing thoroughly.
8. The absorbance of the solution in the wells is read at 450 nm.
9. The cut-off value is calculated by adding the mean absorbance of the negative and positive controls, and then dividing by 6. A sample is positive or negative if its absorbance is greater or less than the cut-off value respectively.