PREVALENCE OF HCV, GENOTYPIC SUBTYPES AND HCV-HIV CO-INFECTION IN VARIOUS BROAD SUBGROUPS OF THE KENYAN POPULATION.

A DISSERTATION IN PART FULFILLMENT FOR THE AWARD OF THE DEGREE OF MASTER OF MEDICINE (INTERNAL MEDICINE) IN THE FACULTY OF MEDICINE, UNIVERSITY OF NAIROBI.

BY DR J.W.KARURU

2004

DECLARATION

This dissertation is original work and has not been presented for a degree in any other university.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Virus
ARV	Antiretrovirals
CTL	Cytotoxic -T-lymphocytes
DNA	Deoxyribonucleic acid
ELISA	Enzyme Linked Immunosorbent Assay
HAART	Highly Active Antiretroviral Therapy
HAV	Hepatitis A Virus
HBV	Hepatiti B Virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HIV	Human Immunodeficiency Virus
IgG	Immunoglobulin G
IVDU	Intravenous drug users
KAVI	Kenya Aids Vaccine Initiative
KNH	Kenyatta National Hospital
Ksh	Kenya Shillings
NANB	Non A, Non B Hepatitis
NBTSC	National Blood Transfusion Services Centre
PCR	Polymerase Chain Reaction
RIBA	Recombinant immunoblot assay
RNA	Ribonucleic acid
RUQ	Right Upper Quadrant
SCD	Sickle Cell Disease
USA	United States of America
VCT	Voluntary Counseling and Testing
VL	Viral load

ACKNOWLEDGEMENTS

I would like to thank the following to whom I am greatly indebted and without whom this work would not have been possible.

1. My supervisors: Prof G N Lule, Dr M. Joshi, Dr O. Anzala and Dr J. Nyamongo for their guidance, supervision and support throughout this study.
2. KNH administration, in particular Mr. Micheni, for allowing me to organize and have education talks on HCV and this study in the hospital for the staff.
3. My assistants – Redemptar, Anastacia, Nduta, Chari, Jackie for helping in data collection and entry.
4. Mr. G. Ochenge and Mr. F. Njeru, who assisted me with the data analysis.
5. The phlebotomists – Mr. Ochieng and Mr. Gakuo, for their invaluable help in blood collection for this study.
6. The laboratory technologists Mr. Bakari and Mr. Onyango for assisting me with the storage and analysis of the samples in this study.
7. All the staff of the HIV-VCT in KNH for assisting me in collecting data in the VCT.
8. All the patients and staff who participated in this study.
9. Roche Pharma for the financial support in carrying out this study.

ABSTRACT

Objective: To determine the seroprevalence and genotypes of HCV infection and prevalence of HIV co-infection among various broad subgroups of the Kenyan population.

Study Design and Setting: This was a prospective cross-sectional descriptive study, done at KNH, a tertiary referral and teaching hospital, inpatient and outpatient departments and the National Blood Transfusion Services Center, Nairobi.

Subjects:

1. Volunteer blood donors
2. Hospital staff, KNH
3. HIV/AIDS and HIV negative in-patients at KNH medical wards
4. VCT Attendants

Methods: After recruitment, the above subjects were assessed for risk factors for HCV/HIV transmission through a questionnaire. Blood for determination of HCV seropositivitiy and genotypes was obtained.

Results: The prevalence of HCV/HIV co-infection among 6154 blood donors in the NBTSC was very low, at <0.02%. The prevalence of HCV infection among 977 KNH staff was 3.6%. Among 458 HIV/AIDS medical in-patients, the prevalence of HCV was 3.7% while in the 518 HIV negative patients, it was 4.4%. The prevalence of co-infection with HCV and HIV was 3.7%. The HIV prevalence among the 353 KNH HIV-VCT attendees was 9.3%, none of the clients tested positive for HCV. The genotypes for 5 patients was done; all were genotype 4. The incidence of risk factors in the persons with HCV and/or HIV infection(s) was low.

LITERATURE REVIEW

Introduction

The HCV was first isolated in 1989 and identified as the commonest cause of Post-Transfusion NonA, NonB (NANB) Hepatitis (1, 2). It accounts for about 80 - 90% of community acquired NANB Hepatitis (1, 2, 3). It is emerging as the cause of the second major epidemic of viral infection after HIV within the past two decades. Approximately 3% of the world's population is affected and viraemia persists in over 80% of these (4, 5). HCV is also recognized as one of the leading causes of chronic liver disease, and as a result, mortality attributable to HCV is expected to more than triple over the next two decades and to exceed the number of HIV related deaths (6, 7).

Epidemiology

HCV is an important global problem with about 170 million people affected and 3 to 4 million new infections annually. In the general population, the prevalence rates of HCV infection vary widely among different countries and geographical areas.

The figures are quite small in the Northern countries, ranging from 0.01 - 0.05% in Northern Europe and Canada (1) to 0.5 - 1.4% in N. America and 0.5% in New Zealand (8). The prevalence is estimated to be 1.2% in S. America (1), 0.3 - 1.3% in Asia and 1.2 - 1.5% in Japan (8, 9).

HCV infection is apparently endemic in some regions like Cameroon and Egypt as well as Thailand and Vietnam where the prevalence rates of 4 - 22% has been reported in low risk groups such as blood donors, school children and army recruits (1).

In Africa, there seems to be an increase in prevalence with southward movement down the Nile River. In Ethiopia, Tsega *et al* found that HCV accounted for 19% of acute sporadic hepatitis, while in Zimbabwe Topley *et al* got rates of 65% of NANB hepatitis.

In Kenya, the prevalence is low and estimated to be between 0.2 - 0.9% (10, 11). Lule *et al* in 1995 found the prevalence rate of HCV to be 2.8% among patients with chronic liver disease in KNH. Mwangi in 1995 found a prevalence rate of 0.9% among blood donors (12). However, the prevalence in some clinical groups, in different geographical regions is significantly higher as shown on the table below.

Prevalence Rates of HCV infections (in percentages) in different populations

Population	HCV
General population (Worldwide)	3.2% (4)
HIV positive Patients (Europe)	7-57% (5, 13)
IVDUsers (Southern Europe)	80-90% (13, 14, 15)
HIV positive haemophiliacs (U.S.A.)	98% (16, 17)
Patients with HCC (KNH)	9.7% (18)
Patients on maintenance dialysis (KNH)	6.1% (19)
Patients with SCD (KNH)	16.1% (20)
Patients with acute icteric hepatitis (KNH)	7.1% (21)

Virology of the Hepatitis C Virus

HCV is an enveloped, linear, single-stranded RNA virus, which is distantly related to flaviviruses. It has a genome of about 94 nucleotides (11, 22, 23), with an amino acid and a carboxyl group terminals, and multiple regions of the genome representing multiple viral protein antigens. This gives rise to the diverse

nucleotide sequencing which is found among HCV isolates from different geographic areas (24, 25).

There are six main genotypes, designated 1 to 6, and multiple subtypes (designated a, b, c etc.) and their global distribution varies widely (24, 26). There is a very high spontaneous nucleotide substitution rate of HCV at each nucleotide site each year, which explains the emergence of mutant strains of HCV and persistence of infection (2).

The clinical significance of genomic diversity of HCV is not completely understood, but it has been observed that infection with genotypes 1b and 4 are associated with severe liver disease.

Routes of infection and clinical presentation

HCV is transmitted mainly by intravenous drug use with 80% to 90% of long-term drug users being affected (16). Blood transfusion has been a major risk factor in the past before routine HCV screening was done, with infection rates among haemophiliacs being very high, upto 60% to 90% in some cases (17, 27, and 28).

Other routes include accidental needle pricks, sexual and vertical transmission (29).

HCV has an incubation period of 14 to 160 days, rarely causes jaundice but 50% of those affected go on to develop chronic hepatitis, a small percentage of who progresses to cirrhosis with 10% getting HCC in over 20 to 40 years (30-32).

Treatment in the acute phase is mainly symptomatic. In chronic HCV infection, sustained virologic response rates (SVR) of up to 62% are achieved using ribavirin in combination with pegylated interferon. This has an important positive impact on the quality of life of the patient (1) and prevents progression to the chronic complications of HCV infection.

Laboratory diagnosis

HCV infection is diagnosed by demonstrating anti-HCV antibodies in serum. First generation assays detect antibodies to the non-structural protein C100-3, between 1and 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. Third generation assays also detect anti-HCV from week four but lack specificity for genotypes other than type 1. Fourth generation ELISA assays can detect types 2 and 3a as well (33).

Thus fourth generation ELISA assays are better since they detect a wider spectrum of antibodies, genotypes and do so earlier in the course of the illness.

Supplementary tests such as the recombinant immunoblot assay (RIBA) and HCV RNA PCR are used to confirm the diagnosis. 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.

The fourth generation ELISA assay is the current gold standard in clinical practice and also for population screening. HCV RNA by PCR is used to confirm diagnosis, determine HCV genotype and monitor treatment (33).

Diagnosis of HCV infection (34)

	ACUTE	CHRONIC	RECOVERED
IgM anti HCV	+++	–	–
IgG anti HCV	+	+++	+++
PCR - HCV RNA	+++	+++	–

Prevention

There is no effective vaccine for HCV infection but vaccination against HAV and HBV prevents development of severe acute hepatitis in those with chronic HCV infection (35). Recognition of the risk factors for the acquisition of the infection in our country will go a long way in reducing the disease burden.

HIV-1-HCV Co-infection

Introduction/Prevalence

Co infection with HIV and HCV represents a growing population for the future. In the USA, it is estimated that there are 300,000 co infected subjects (36). This represents 15 - 30% of all HIV infected persons and 5 - 10% of all HCV infected persons. It is estimated that anywhere from 1% to over 30% of HCV patients may be co infected with HIV (37). The number is much higher in patients infected through IVDU or haemophiliacs who received contaminated blood products, than in patients who acquired the infections through sexual practices.

Since the introduction of HAART and the dramatic improvement in the life expectancy of HIV infected individuals, the impact of HCV on mortality and on the development of HCC has become more evident (38,39). More recent studies in those with HIV/HCV coinfection have demonstrated that HCV is the leading non-AIDS cause of death in these subjects, and end stage liver disease due to HCV infection accounts for up to 50% of all deaths (6, 7).

HIV and HCV share common routes of transmission and there is increasing evidence that sexual and mother to child transmission of HCV is facilitated by HIV infection (28, 40-42).
HCV is approximately10 times more infectious than HIV through percutaneous blood exposures (43).

Effect of HIV on HCV progression.

It has been noted that co infected patients have a faster progression to liver disease and death than those not infected with HIV (4.5 times greater increased risk of death in a recent study) (38). HIV infection also accelerates HCV hepatotoxicity sevenfold and increases the severity of liver fibrosis (44). This rise has been found to be inversely proportional to the CD4 cell count, with worse

results at CD4 counts below 500cells/mm3 (45-47). HIV is associated with an eight-fold rise in HCV replication (48). HIV seems to alter the natural history of HCV infection resulting in an aggressive course to end stage liver disease and liver failure.

Effect of HCV on HIV progression.

HCV infection appears to have a significant effect on the progression of HIV to AIDS defining illnesses and AIDS related mortality. In a recent Swiss study the risk of progression to AIDS defining illnesses or death was 3.54 compared with HCV uninfected individuals (49). Daar et al reported a detrimental effect of HCV viral load on HIV progression (50). For every 10 fold increase in baseline HCV viral load, the RR for clinical progression to AIDS was 1.66, even after controlling for CD4 cell count and HIV RNA level. It is evident that HCV is an independent risk factor associated with HIV progression to AIDS and AIDS related death. HCV also appears to blunt immune recovery in HIV infected persons on HAART (51).

Pathogenesis of HIV-1/HCV co infection.

Both HIV and HCV are RNA viruses whose genomes are transcribed frequently by polymerases. While the HIV genome is reverse transcribed and the complementary DNA integrated into the DNA of latent T cells leading to persistence and precluding HIV clearance, the HCV infection is sustained by ongoing replication.

Interaction of HIV and hepatitis viruses may occur via co infection of the same cell or by release of soluble factors from infected cells. The immune deficiency due to HIV infection is known to augment the replication of many viruses. The HIV, HBV and HCV are all capable of infecting peripheral blood lymphocytes as well as hepatocytes as demonstrated by William Sievert (52).

The mechanism by which HCV influences HIV progression remains speculative. HCV may down regulate proliferation of T cells or increase apoptosis of T cells by apoptotic pathways (53, 54). Patients with HCV infection express Fas on

peripheral blood mononuclear cells and HIV-RNA has preferentially been detected in these Fas positive cells (55). This may form the basis for a synergistic effect of HIV and HCV on CD4 cells both in terms of underproduction and apoptosis of T cells, which could in turn, explain the negative impact of HCV on HIV progression.

Another theory proposes that the two viruses may compete for replication, so that if the HIV is suppressed, in the absence of anti HCV medication, the HCV is therefore more able to replicate and produce further liver damage (56).

Effect of HAART on HCV progression.

There is no definite evidence to support a clear effect of HAART on the natural history of HCV infection, but HCV infection does appear to be an independent predictor of hepatotoxicity following the introduction of HAART (57). As stated above, introduction of HAART without anti HCV medication may give way to increased replication of HCV and consequent liver damage.

Effect of HCV treatment on HIV disease progression

Combination of ribavirin and interferon-alfa is the current gold standard in the treatment of HCV infection (58, 59). Based on three studies, coinfected patients treated with this combination showed a comparable sustained HCV viral response rate (60, 61). Treatment of HCV does not seem to have a direct effect on the progression of HIV but the resulting reduction in HCV viral load is associated with an improvement in the quality of life with reduction in symptoms such as fatigue, myalgia, flu-like symptoms and depression (62).

HCV Co infection and HAART-Associated Hepatotoxicity

Antiretroviral drug use has been associated with hepatotoxicity that can interrupt HIV therapy and cause significant mortality and morbidity. Some studies suggest that HAART-induced hepatotoxicity may be more common in persons with HCV co infection, particularly those taking HIV-1 protease inhibitors (62, 63, 57-66). The mechanisms of enhanced drug-induced hepatotoxicity among co infected patients are unknown but may include decreased drug metabolism, HCV-specific immune reconstitution, or increased susceptibility to mitochondrial dysfunction (67-69).

JUSTIFICATION

HCV is emerging as an important cause of morbidity and mortality in HIV positive patients in many studies that have been done in Europe and America. In Africa, an estimated 13.9 million persons have HCV infection representing 5.3% of the entire population. Although the impact of HCV deaths is more noticeable in co-infected patients who are on HAART, it is important to establish just what the prevalence of co-infection is. This is of greater urgency now with the possibility of more patients having access to HAART therapy. Some studies have been done locally to establish the prevalence of HCV in some patient groups such as those with HCC, SCD and on maintenance dialysis, but no recent study has addressed both high and low risk individuals. It is also not known with certainty what genotypes of HCV are prevalent in our country.

This study proposes to demonstrate the prevalence of HCV infection in various subgroups of the Kenyan population, determine the genotypes prevalent locally and suggest possible risk factors for this infection. Considering the burden of HIV we have and the known relationship between the two viruses, this study proposes to establish and examine the correlation between the two viruses.

OBJECTIVES

The broad objective of this study was to determine the prevalence of HCV and HIV co-infection in various broad subgroups of the Kenyan population and establish the HCV genotypes.

The specific objectives were:

1. To determine the prevalence of HCV/HIV co-infection among blood donors in the NBTSC.
2. To determine the prevalence of HCV infection among hospital staff in KNH.
3. To determine the prevalence of HCV infection among HIV/AIDS in-patients in KNH medical wards and how it compares this with HIV negative in-patients.
4. To determine the prevalence of HCV/HIV co-infection among HIV-VCT attendants at the KNH VCT center.
5. To determine the HCV genotypes prevalent in the above population subgroups.
6. To describe known risk factors for HCV infection in the persons with HCV and/or HIV infection(s).

PATIENTS AND METHODS

Study period:

This study was carried out between December 2003 and June 2004

Subject recruitment and sampling:

- Data on blood donors in the Nairobi NBTS center from October 2003 to March 2004 was collected using a standard form (appendix III) and analysed for the study variables. The data was in the form of a hand-written register and included: the donors name, age, sex, blood group (ABO and rhesus), location and date of blood collection, as well as results of HIV, HBV, HCV and VDRL serology. All the complete data was analysed.

- In the HIV-VCT, clients were recruited consecutively until the required number was obtained. The study was introduced to them by the counselor attending them and then those who agreed to participate in this study met the principal investigator. Permission was sought from each client by the investigator to know and confirm their HIV status, while the HCV status was determined.

- For the hospital in-patients arm of the study, consecutive sampling of medical in-patients on the first post admission day was done. The cases were patients with clinical features of HIV and an AIDS defining illness in whom the HIV serology was positive. The controls were age (margin of 5 years), sex and cohort- matched to the cases, *ie* admitted within at most three days of the admission of the cases, with other illnesses and with a negative HIV serology. The questionnaire was administered to those who gave consent and blood samples for HCV determination were collected.

- A stratified random sample of KNH hospital staff to include clinical and non-clinical personnel of different job groups was obtained and recruited in the study. Official hospital registers of employees were used.

Inclusion criteria for all in the prospective group

Persons aged above 13 years who gave a written informed consent. (Or their guardian for those under 18 years of age)

Exclusion criteria for all in the prospective group

Those who failed to give consent, had incomplete data or had acute icteric hepatitis were excluded from the study.

Clinical Methods

Written informed consent was obtained from each person. (Appendix IV). For those under 18 years of age, consent was obtained from their guardian. (Appendix V).

Each of the study subjects then completed the appropriate questionnaire. (Appendices I and II)

Laboratory Methods

Two milliliters of blood were obtained from each of the persons in the prospective arm by venepuncture and put in a biochemically clean bottle for serological tests for HCV as per the methodology outlined in appendix III-IV. The samples were collected and processed using standard methods and stored at the required –20°C and then analysed in batches. Assay of the samples was done by a senior laboratory technologist using 4th generation ELISA.

Determination of the HCV genotype was done in the positive samples using a PCR RNA qualitative assay (Cobas Amplicor, Roche Diagnostics).

Data base analysis

Data on HCV and HIV serology of blood donors in the NBTSC from October 2003 to March 2004 was analysed.

Data Analysis

The data obtained was cleaned, edited and coded. After verification it was entered into a computer datasheet. A nalysis was done using the Statistical Package for Social Sciences (SPSS) 11.5 software. Frequencies, percentages, means, ranges and standard deviations were calculated. The point prevalence estimate was obtained. The results were presented in tables, bar charts and pie charts. The 95% confidence interval was used to assess significance. The Pearson chi-square test was used to assess the statistical significance of association.

RESULTS

I. BLOOD DONORS

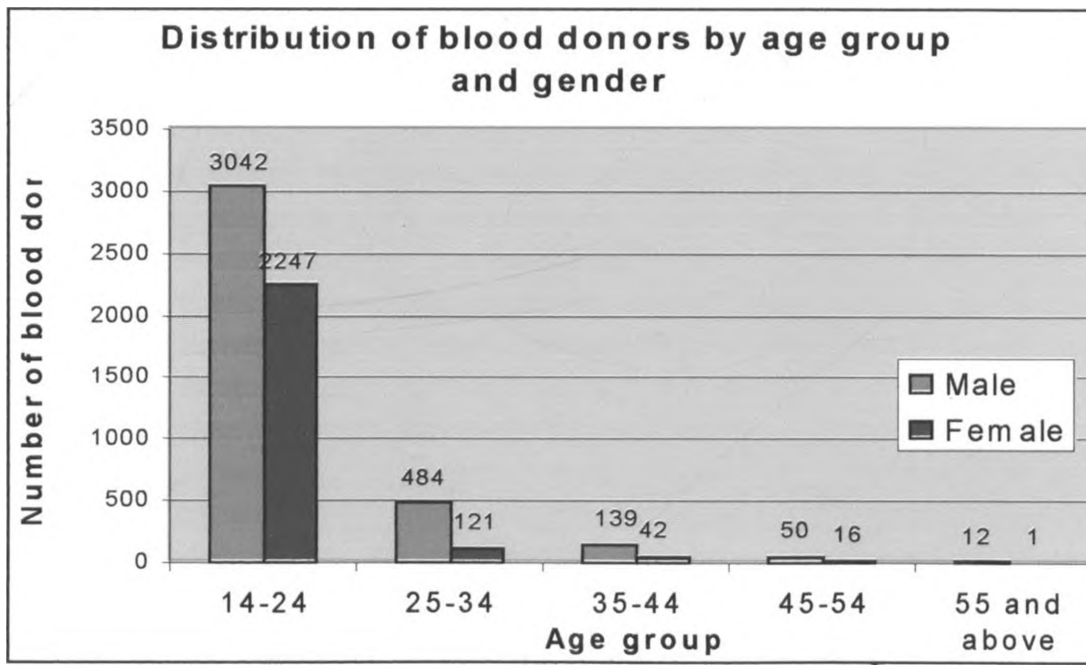
Analysis of data on blood donors at the National Blood Transfusion Services, Nairobi was done. The data covered a period of 6 months (Oct 2003 to Mar 2004) during which testing of donated blood for HIV and HCV had been done. Blood was collected mainly from learning institutions. Data available included the donors name, age, gender and serology results for the above named studies. It is presumed that this is a group at a considerably low risk for HIV, HCV, and HBV and other sexually transmitted infections and blood borne diseases.

Data from 6154 blood donors was analysed

Distribution of blood donors by Age and Gender

There were more male donors, 3727 (60.56%) than female, 2427 (39.43%). The age range was from 14 years to 66 years with a mean age of 24 years.

Figure I.1: Distribution of blood donors by Age and Gender



HIV positive blood donors

Of all the 6154 BTU clients, 66 were HIV positive, representing a prevalence of 1.07%.

HCV positive blood donors

Of the 6154 BTU clients, only 49 were HCV positive representing a prevalence of 0.79%.

HIV/HCV co-infected blood donors

One of these 6154 clients had HIV/HCV co-infection. This was a 24 year old male blood donor from a training institute in Kiambu, a district bordering Nairobi.

II. HOSPITAL STAFF

A total of 758 patients were screened; 8 were excluded: 3 had had jaundice in the past and 5 failed to give consent. The remaining 750 hospital staffs were recruited for the study.

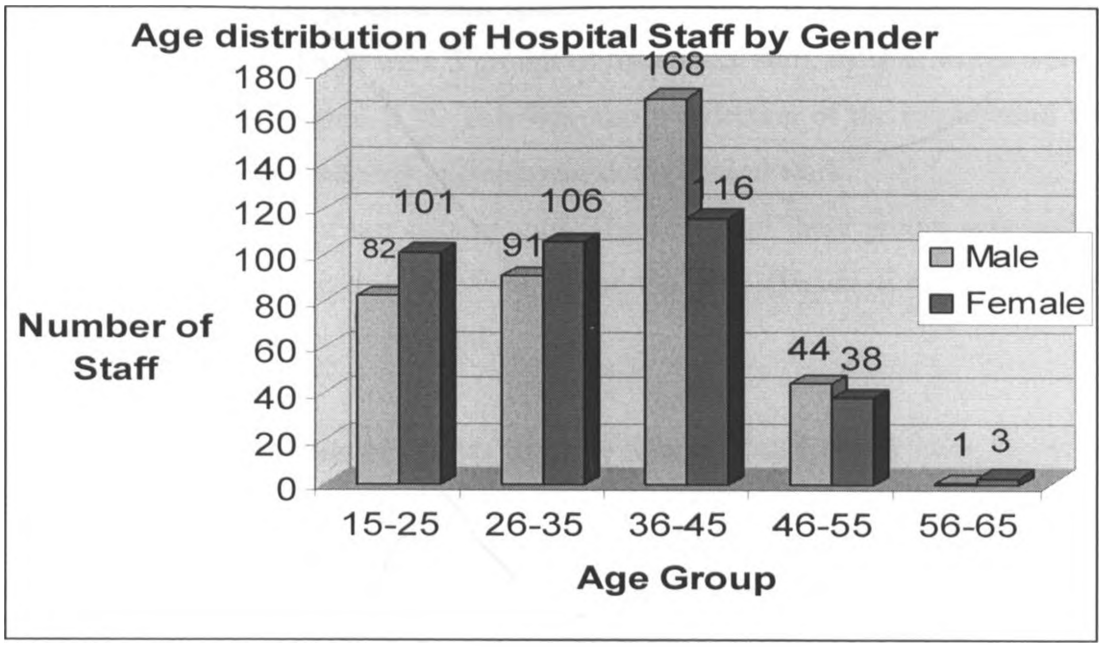
Distribution of hospital staff by Age and gender

There were more male subjects recruited; 386 vs 364 (51.46% vs 48.53 %) The age of the subjects ranged between 21 and 56 years, with a mean age of 33.89 years and a median of 35 years. (Table II.1, figure II.1)

Table II.1 Distribution of hospital staff by Age and gender

		Sex		Total
		Male (%)	Female (%)	
Age Group	15- 25	82(44.8)	101(55.2)	183
	26-35	91(46.2)	106(53.8)	197
	36-45	168(59.2)	116(40.8)	284
	46-55	44(53.7)	38(46.3)	82
	56-65	1(25)	3(75)	4
Total		386(51.5)	364(48.5)	750

Figure II. 1 Distribution of hospital staff by Age and gender

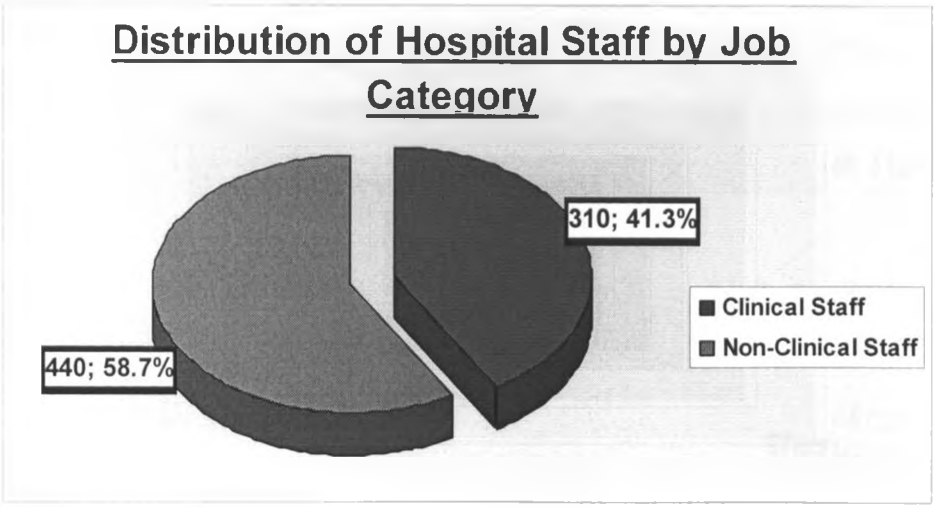


Distribution of hospital staff by Job Category

The patients were grouped into clinical and non-clinical categories. Non-clinical staff were more than the clinical staff (440 vs. 310 ie 58.7% vs 41.3%). This is a representation of the employee structure of the hospital.

(Figure II. 2)

Figure II.2: Distribution of hospital staff by Job Category



Distribution of hospital staff by Specific Job Groups

The staff were further grouped into specific job groups, as per the particular task they were entrusted with. There were 5 groups of the clinical staff, most of whom were nurses (20%) of the total (Figure II.3). This was also a reflection of the employment strata in which nurses form the majority of employees doing clinical work.

There were 6 groups of non-clinical staff. The largest of these groups was made up of subordinate staff and consisted of 17.3% of the total (Figure II.4). This reflects the employment structure in the hospital.

Figure II.3: Distribution of Clinical staff by Specific Job Groups

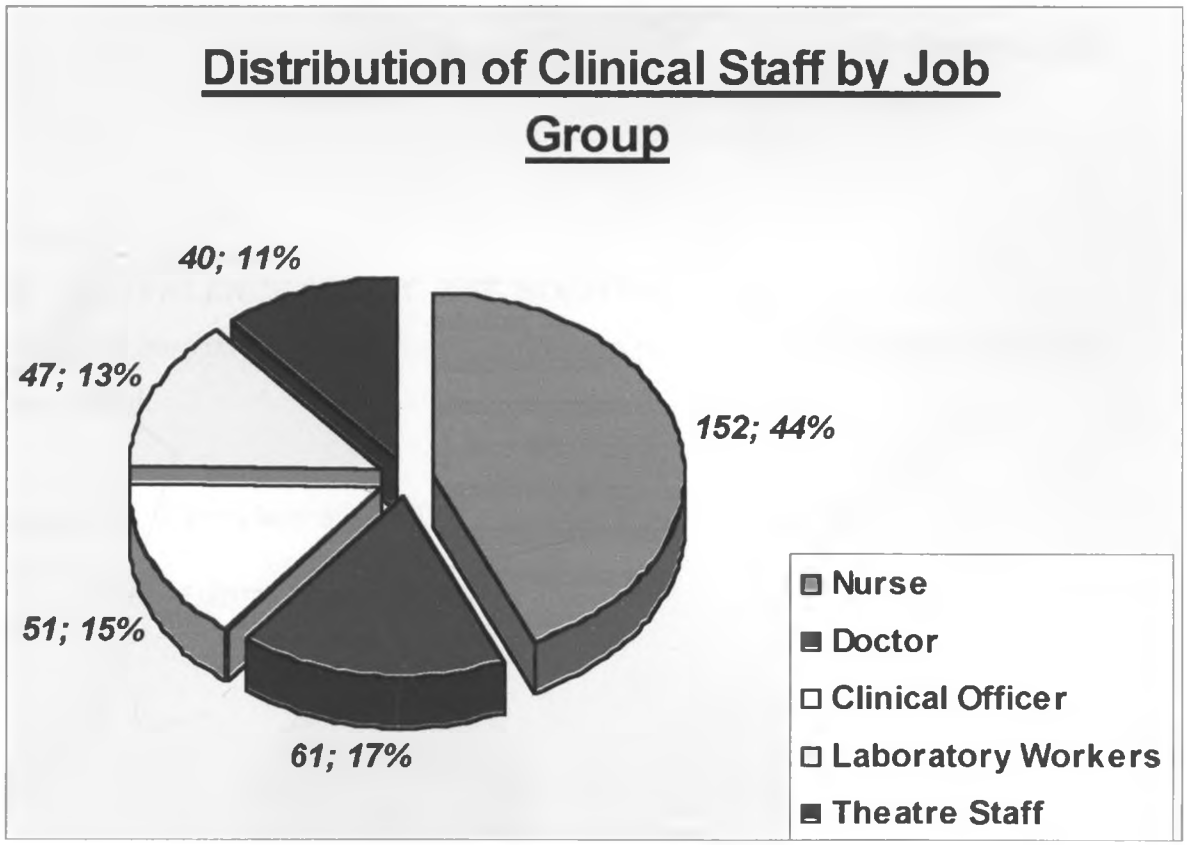
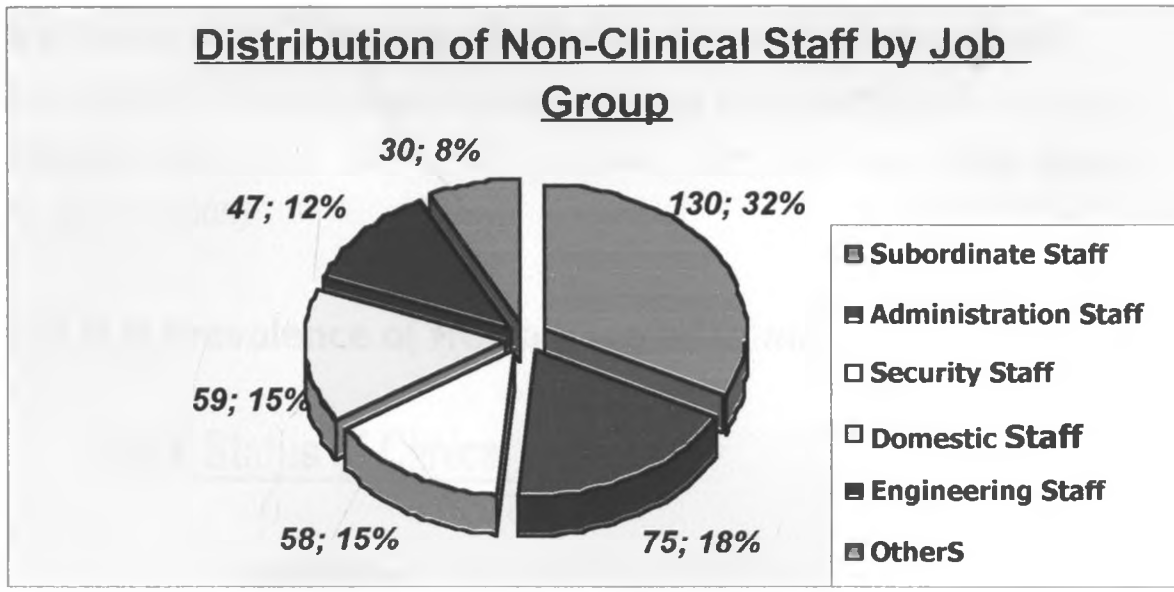


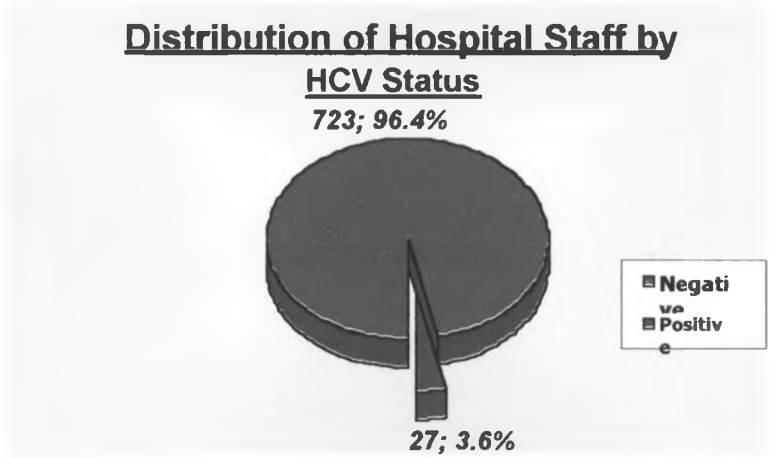
Figure II.4: Distribution of non-clinical staff by Specific Job Groups



HCV PREVALENCE AMONG THE HOSPITAL STAFF

Of the 750 hospital staff tested for HCV, 27 were positive. This represented 3.6% of all those tested.

Figure II.5: Prevalence of HCV among all hospital staff



Prevalence of HCV among all Clinical and non-clinical staff

The prevalence of HCV among the clinical staff was 5.2% (Figure II 6). This was significantly higher than that in the non-clinical staff which was 2.5% (figure II.7). ($p = < 0.005$)

Figure II.6: Prevalence of HCV among all Clinical staff

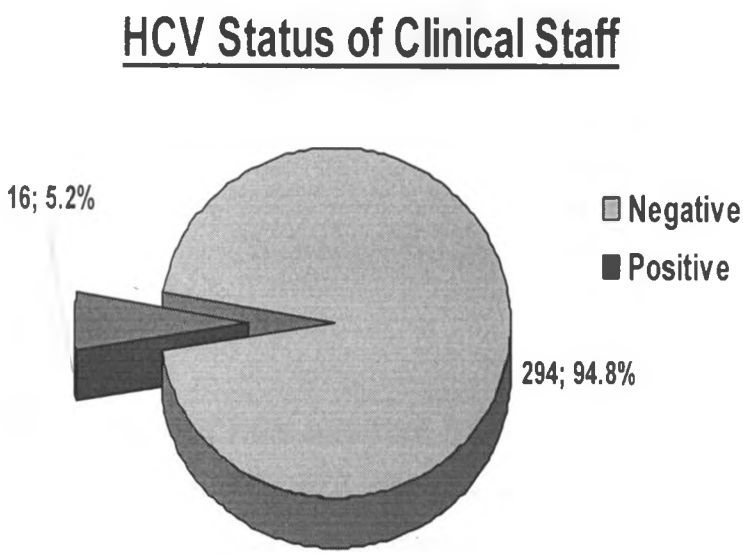
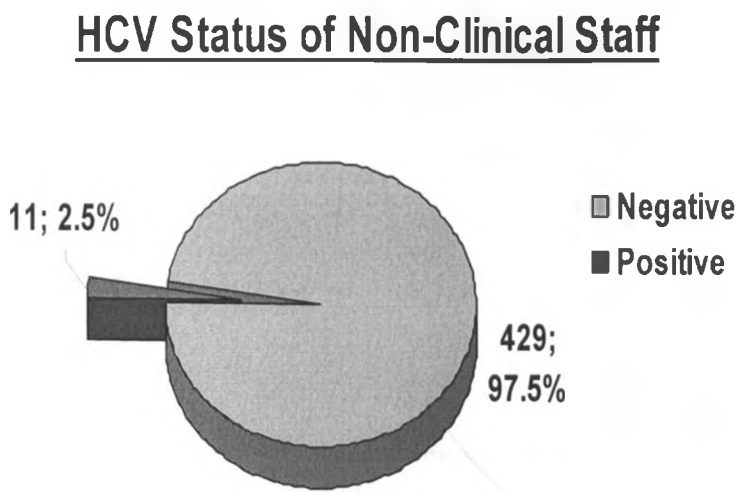


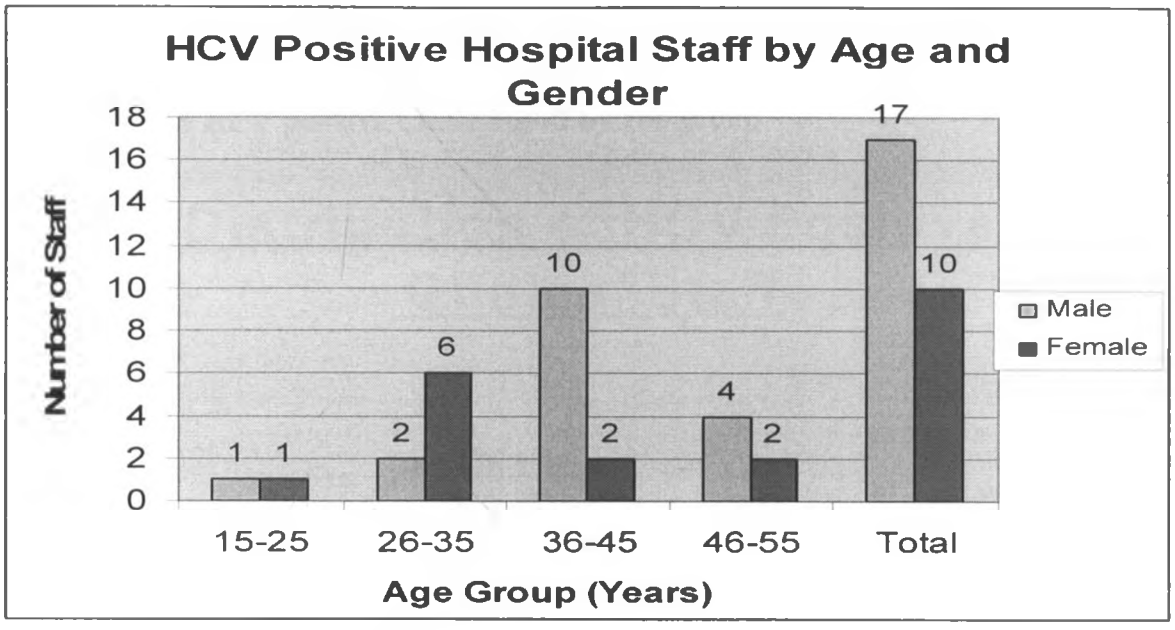
Figure II.7: Prevalence of HCV among all non-clinical staff



Distribution of HCV positive hospital staff by age and gender

The 27 persons with HCV had an age range of 21 to 53 years and a mean of 37 years, as shown below. The distribution of HCV positive hospital staff by age and gender was as shown in the histogram below (Figure II.8) There were more males than females affected (63% vs. 37%).

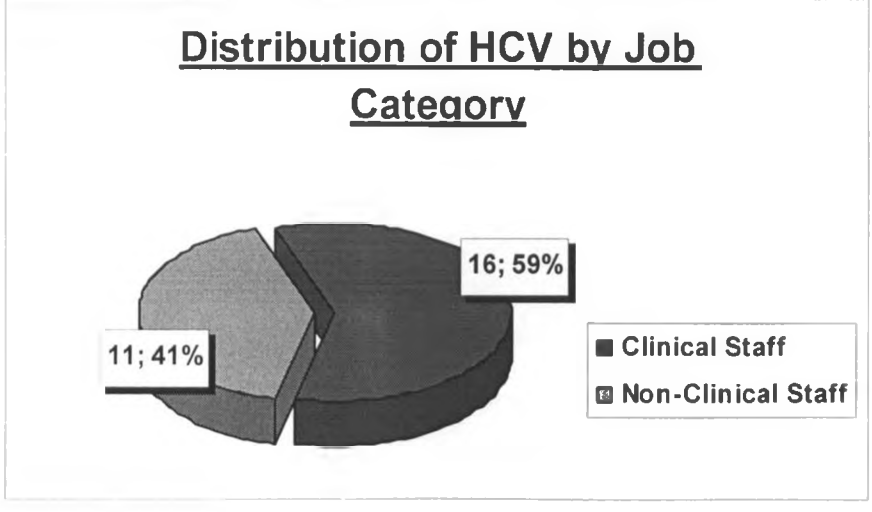
Figure II.8: Distribution of HCV positive hospital staff by age and gender



HCV positive staff by Job Category

There were more HCV positive subjects among the category of clinical hospital staff than the non-clinical staff. (59.26% compared with 40.74% non-clinical staff). The prevalence of HCV infection among the clinical staff was 5.16% while in the non-clinical staff it was 2.5%.

Figure II.9: HCV positive staff by Job Category



HCV positive staff by specific job group

The distribution of the HCV positive staff by job group is as shown below (Figures II.10 and II.11). This followed the distribution of the job groups. All the groups were represented and there were no significant differences between them.

Figure II.10: HCV positive Clinical staff by Job group

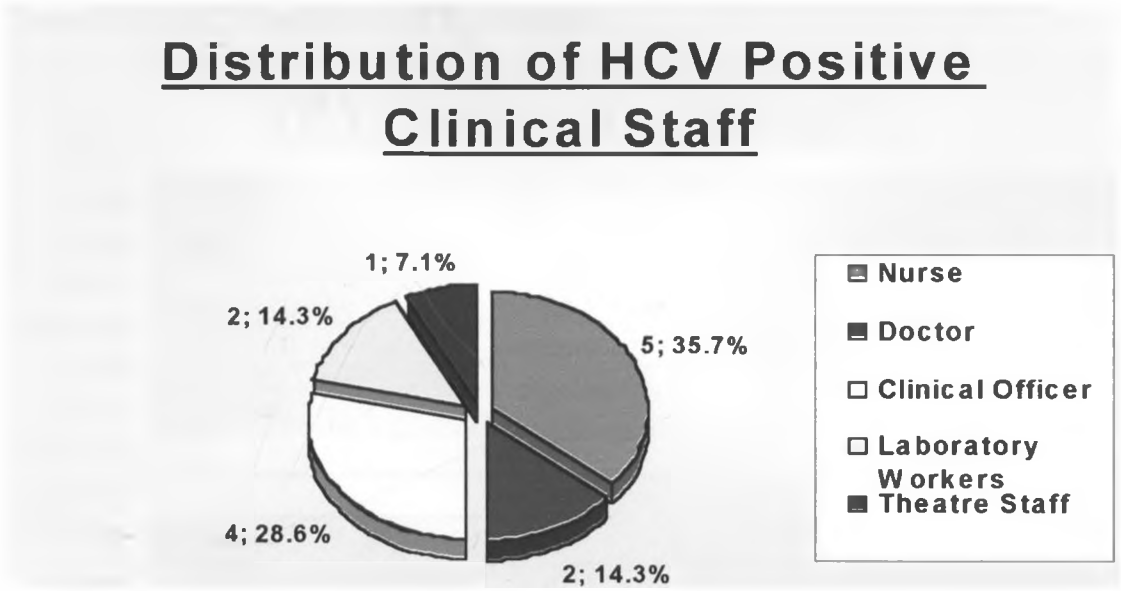
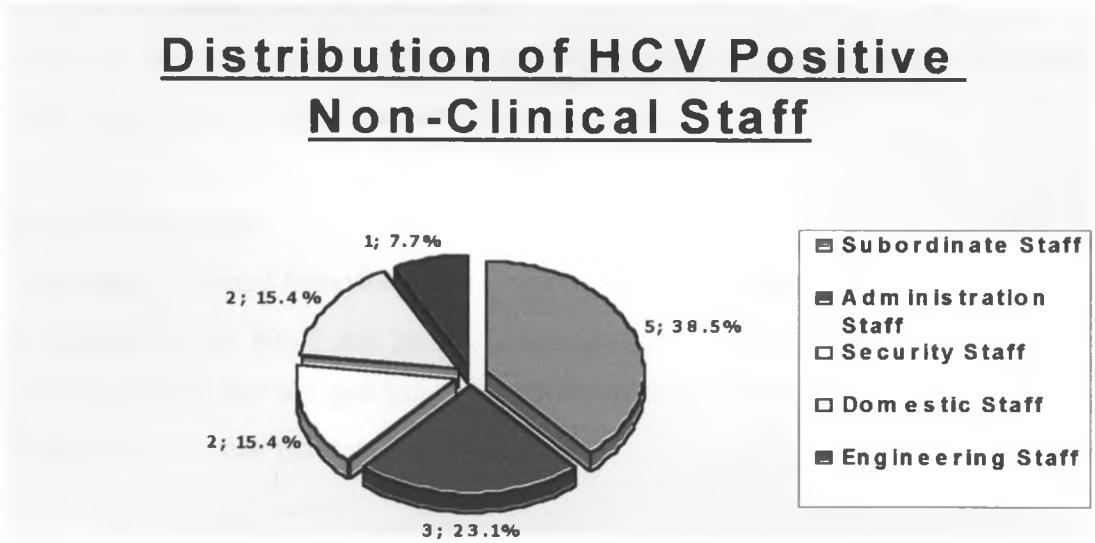


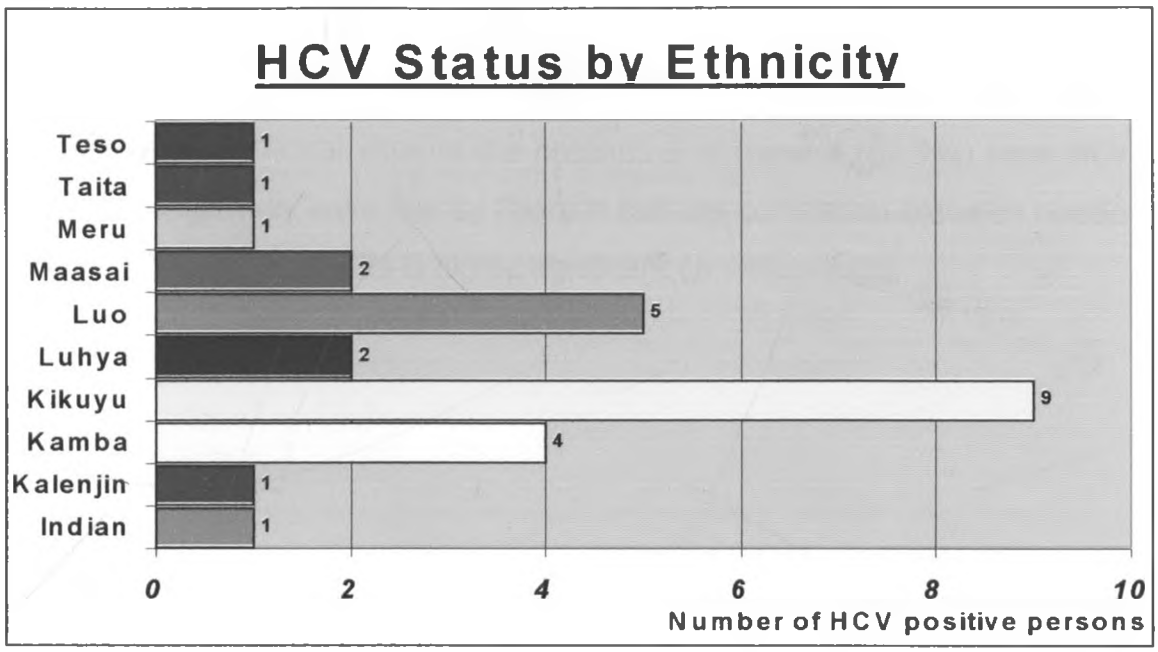
Figure II.11: HCV positive non-clinical staff by Job Group



HCV positive staff by ethnicity

The HCV positive subjects were from cross-section of ethnic backgrounds. (Figure II.12) This distribution follows the population structure of the region and the employment structure of the hospital by ethnicity.

Figure II.12: HCV positive staff by ethnicity



Risk factors among the hospital staff

The risk factors assessed included history of sexual promiscuity, blood transfusion, needle prick injury, tattoos and intravenous drug use.

Sexual promiscuity

Promiscuity in sexual behaviour was assessed using the questionnaire but was apparently not present in this study sub group. Promiscuity was defined as having more than one sexual partner in the last previous year. None of the respondents admitted to having had more than one sexual partner.

Blood transfusion.

None of the subjects gave history of having had a blood transfusion.

HCV positive staff by needle prick injury

Of all the hospital staff 8 admitted to having hard a needle prick injury while in the course of their clinical work in the hospital. 5 of these 8 (62.5%) were HCV positive. Although they were few by Pearson test the correlation between needle prick and HCV positive results is highly significant (*p value=0.01*).

III. WARD INPATIENTS

A total of 989 patients were screened for the study. 5 were excluded due to lack of complete data, 4 because they gave past history of jaundice and 3 because of failure to give consent. 977 patients were recruited in the study.

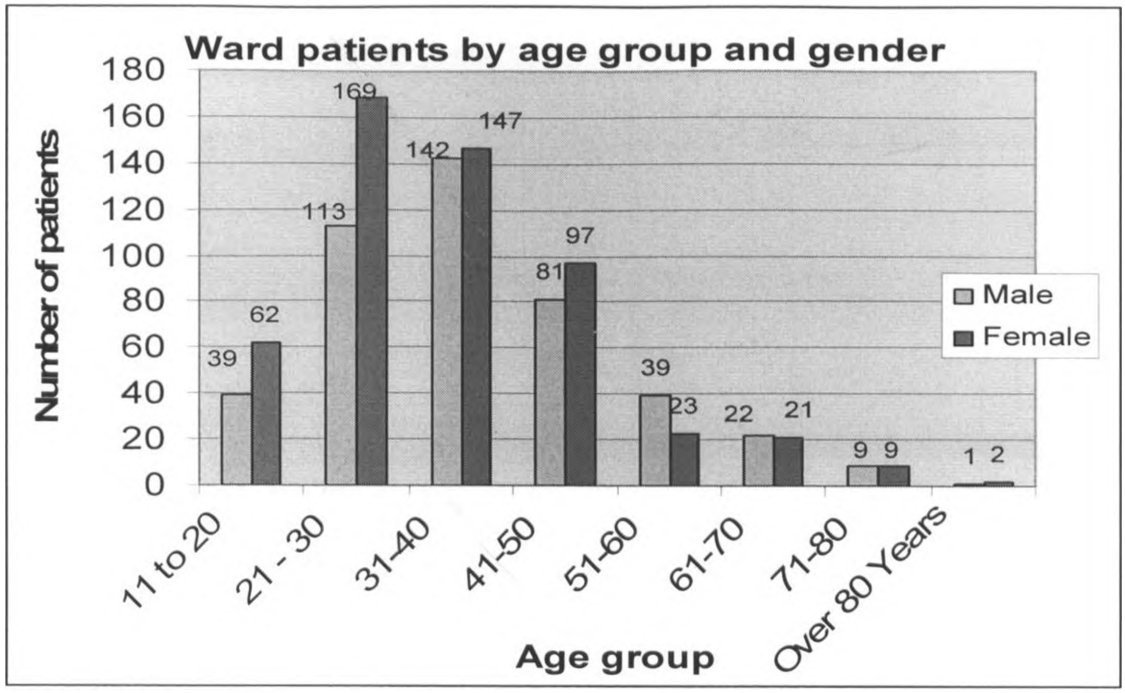
Ward inpatients by age and gender

The age range of the patients recruited was from 13 to 88 years with a mean of 36.34 years. There were more females in the study than males (531; 54.35% vs 446; 45.65%). (Table II.1)

Table III.1 Ward inpatients by age and gender

		SEX		Total
		Male (%)	Female (%)	
Age Group	11-20 Years	39(38.6)	62(61.4)	101
	21-30 Years	113(40)	169(60)	282
	31-40 Years	142(49)	147(51)	289
	41-50 Years	81(45.5)	97(54.5)	178
	51-60 Years	39(63)	23(37)	62
	61-70 Years	22(51)	21(49)	43
	71-80 Years	9(50)	9(50)	18
	81 and Above	1(25)	3(75)	4
	Total	446(45.6)	531(54.4)	977

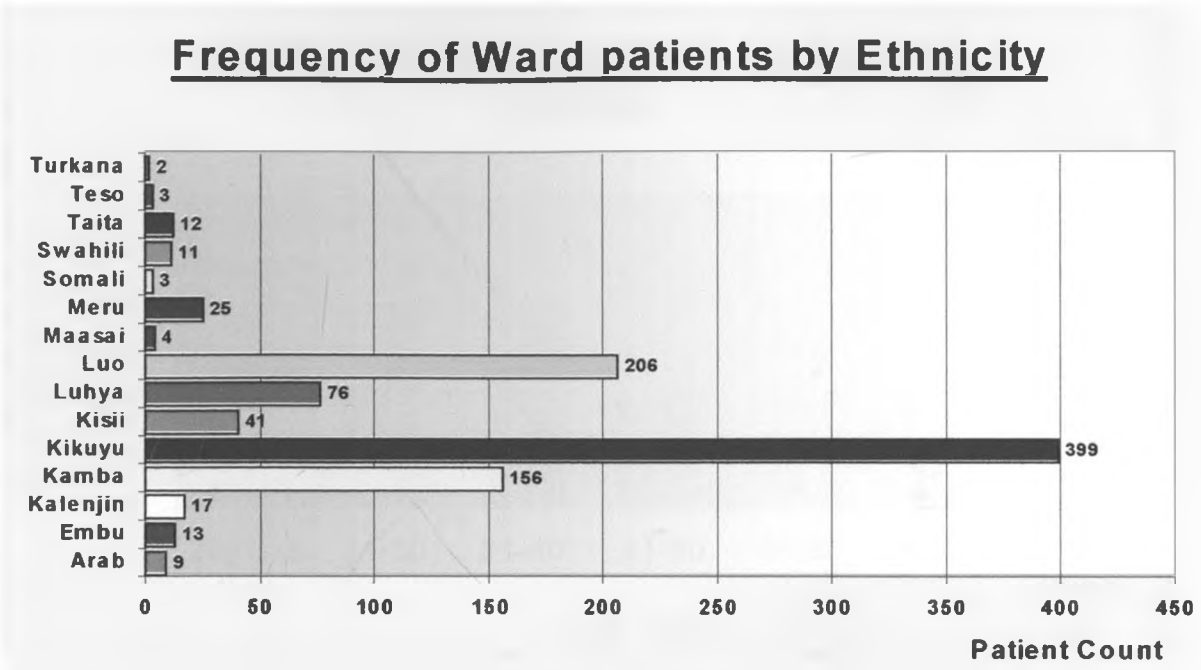
Figure III.1: Ward inpatients by age and gender



Ward inpatients by ethnicity

The patients recruited in the study represented a cross section of ethnic group as present in the community. The most were Kikuyu (39.8%) followed by Luo (20.7%).19 ethnic groups were represented in the study.

Figure III.2: Ward inpatients by ethnicity



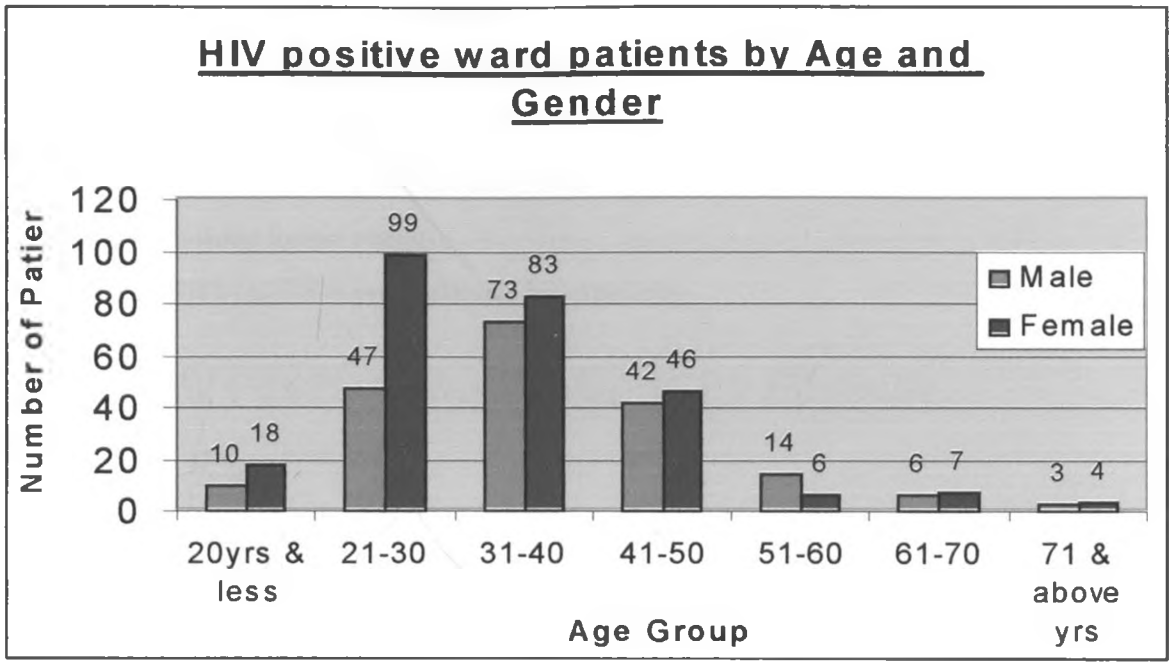
HIV/AIDS WARD PATIENTS

460 patients who were HIV positive and had at least one AIDS defining illness were screened for the study. Two failed to give consent and were therefore excluded. The remaining 458 patients were recruited into the study.

HIV/AIDS ward patients by age and gender

Most of these were also female (263 (57.4%) vs 195 (42.6%) These were aged between 16 and 71 years of age. The mean age of these subjects was 33years.

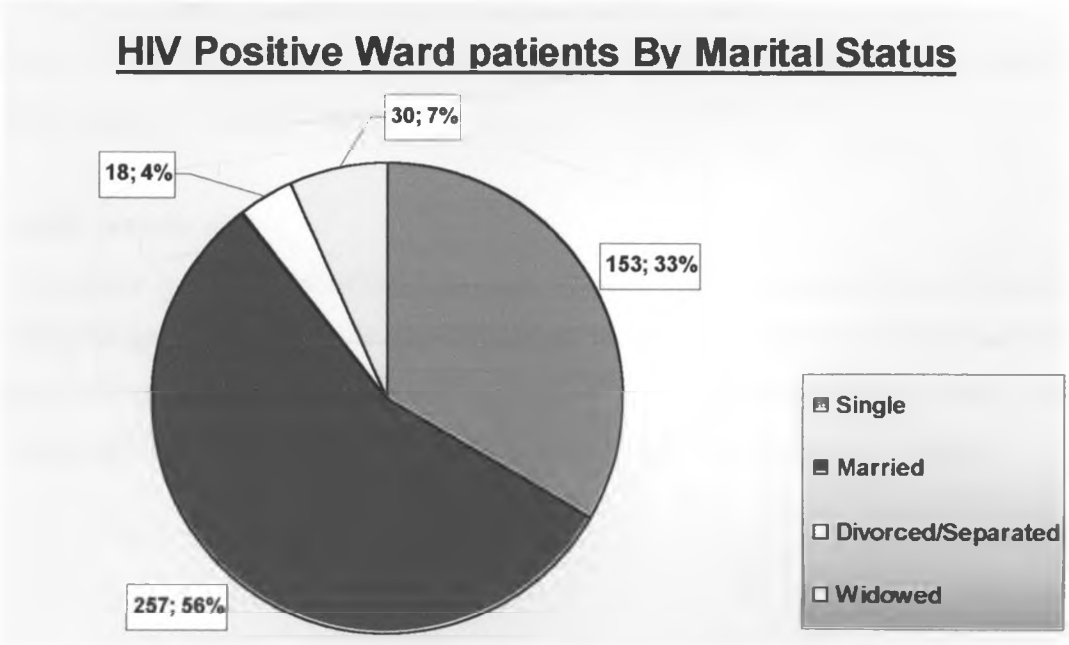
Figure III.3: HIV/AIDS ward patients by age and gender



HIV positive ward patients by marital status

The majority of the patients with HIV infection were married (257; 56.1%) 153 (33.4%) were single.

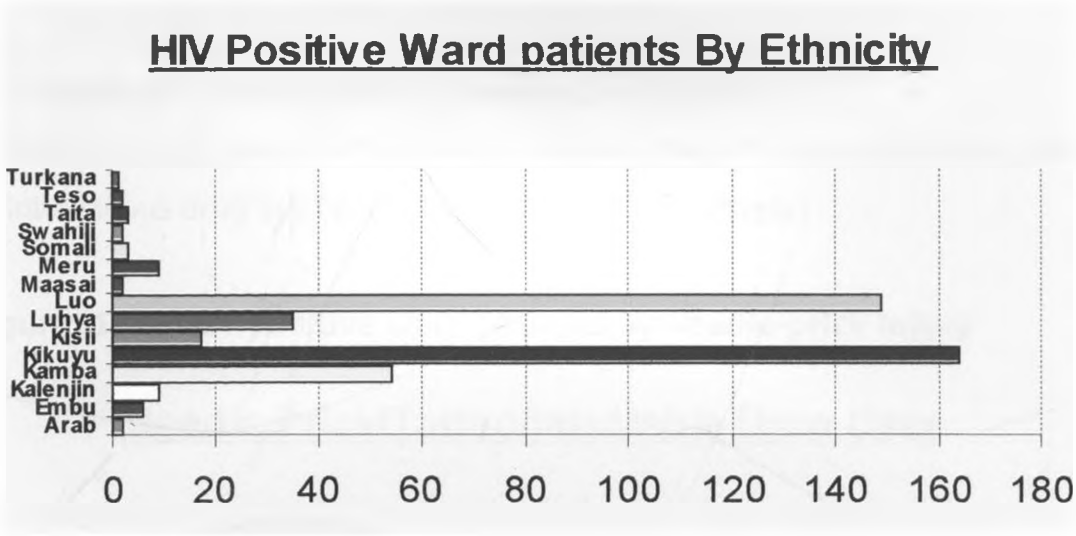
Figure III.4: HIV/AIDS ward patients by marital status



HIV positive ward patients by ethnicity

The distribution of HIV/AIDS patients in this study by ethnicity closely follows the demographics of the region in which this study was done. Of note there was an overrepresentaton of patients from the Luo community (32.5%) in comparison to the rest. In the total ward patient population they made up a total of 20.7%. This has been shown in other studies done in this region.

Figure III.5: HIV/AIDS ward patients by ethnicity



HIV positive ward patients by risk factors for HCV/HIV

The risk factors assessed included history of blood transfusion, needle prick injury, tattoos and intravenous drug use.

Sexual promiscuity

Promiscuity in sexual behaviour was also assessed but was apparently not present in this study sub group. Promiscuity was defined as having more than one sexual partner in the last previous year. None of the respondents admitted to having had more than one sexual partner, with 35.5% of the subjects subscribing to having had no sexual partner.

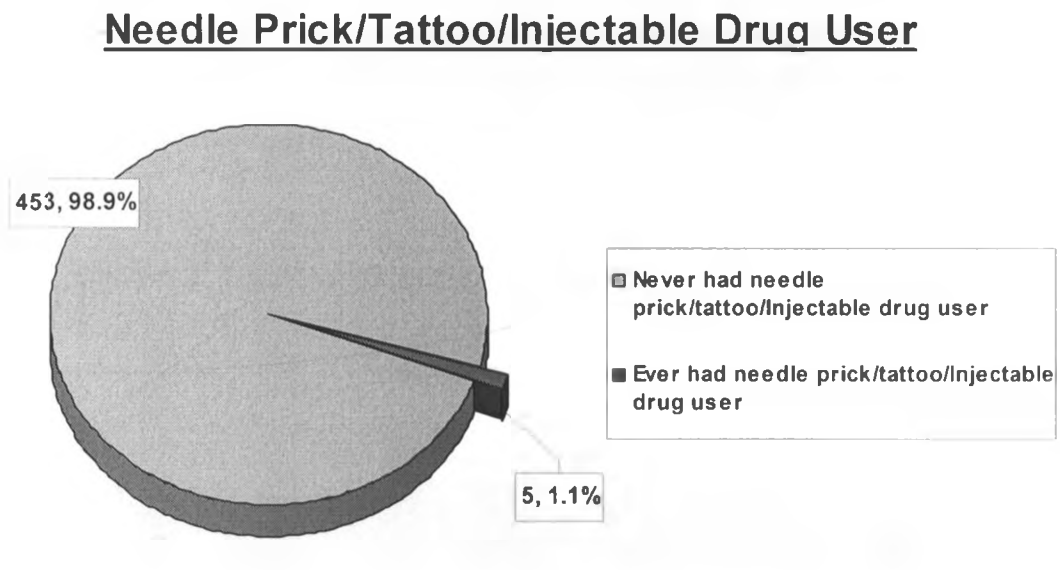
Blood transfusion.

Only one (0.2%) of the HIV/AIDS patient gave history of having had a blood transfusion.

HIV positive ward patients by needle-prick injury

In total 5 people gave positive history of having had a needle prick injury, tattoo or intravenous drug use (2, 2 and 1 person(s) respectively)

Figure III.6: HIV positive ward patients by needle-prick injury



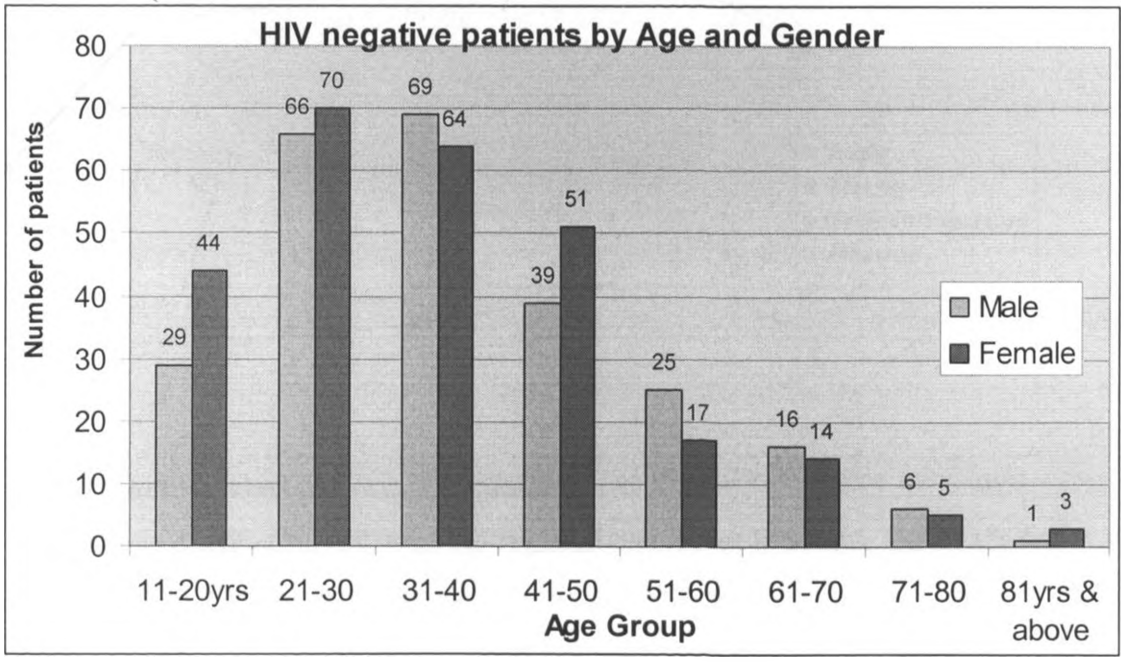
HIV NEGATIVE WARD PATIENTS

529 HIV negative were screened, matched to the HIV/AIDS patients by cohort of admission and age (5-year margin) as closely as was possible. 11 patients were excluded – 6 gave history of jaundice, 3 failed to give consent and 2 had incomplete or contrasting data. 518 patients were recruited into the study.

HIV negative ward patients by age and gender

The distribution of the HIV negative patients was as shown in figure III.7. It followed the normal distribution and was similar to that of the HIV/AIDS patients, with an age range of 14 to 88 years and a mean of 35 years. There were more females (267; 51.54%) than males (251; 48.45%). (Figure III.7)

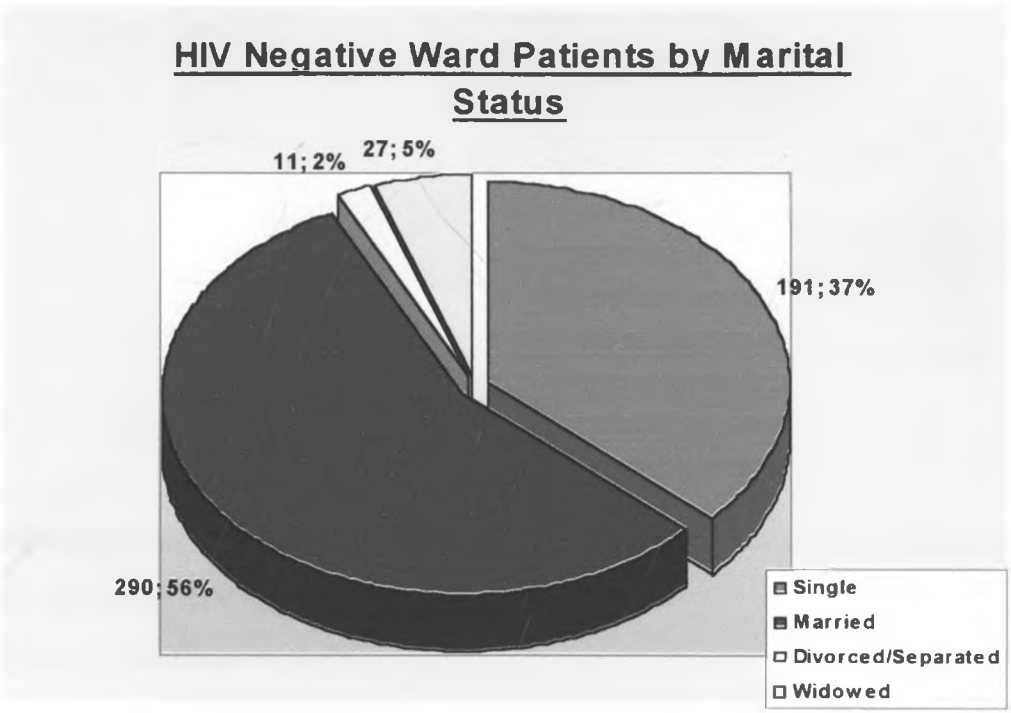
Figure III.7: HIV negative ward patients by age and gender



HIV negative ward patients by marital status

By marital status, the distribution of the HIV negative patients was similar to that of the HIV/AIDS patients. The majority (56%) were married.

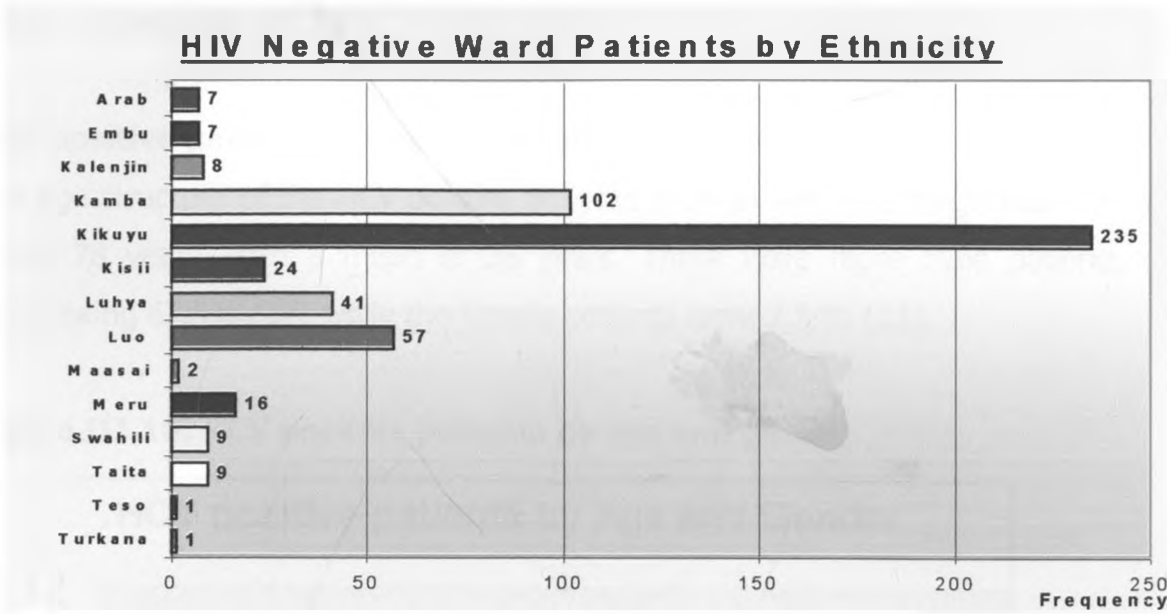
Figure III.8: HIV negative ward patients by marital status



HIV negative ward patients by ethnicity

The ethnic distribution followed the regional one, but it is notable the as alluded to earlier (figure III.5), the Luo ethnic group was underrepresented here.

Figure III.9: HIV negative ward patients by ethnicity



PREVALENCE OF HCV AMONG HIV NEGATIVE WARD PATIENTS

23 of the 518 HIV negative patients had HCV

This is a prevalence of 4.4%

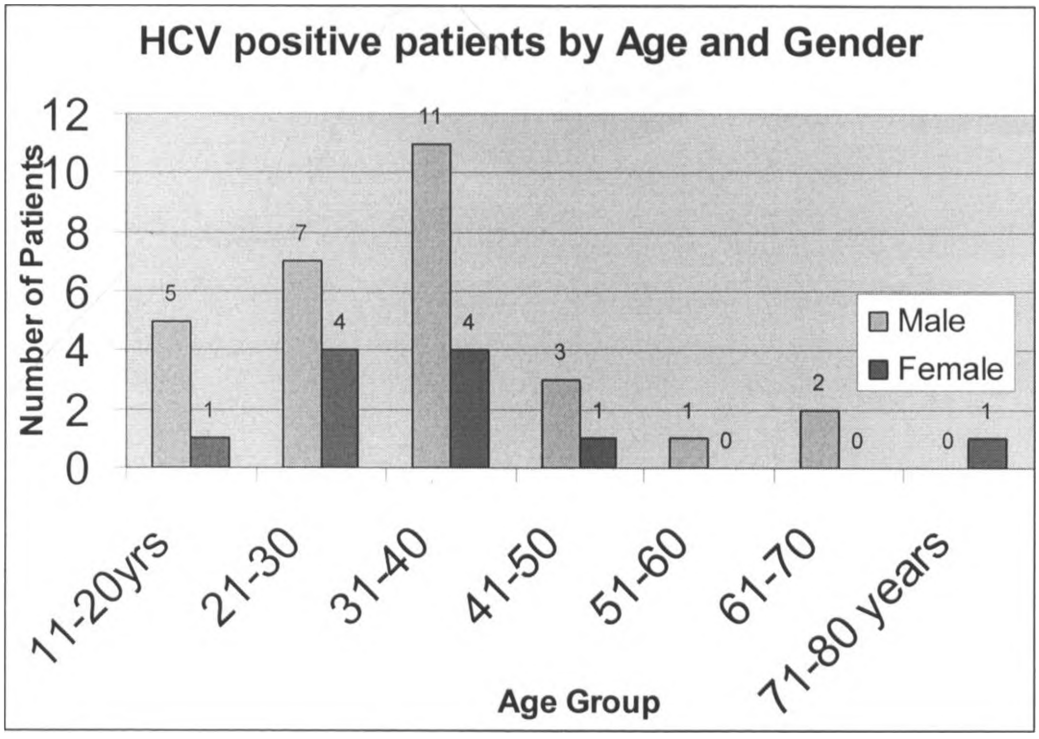
HCV POSITIVE WARD PATIENTS

The total number of patients who tested positive for HCV infection was 41. This gives a prevalence of 4.19%.

HCV positive patients by age and gender

The age structure of the HCV positive patients is as shown. The range was from 14 to 78 years, with a mean of 35 years. There were more male patients, constituting 67.5% (27) while the female patients were 2.5 % (13).

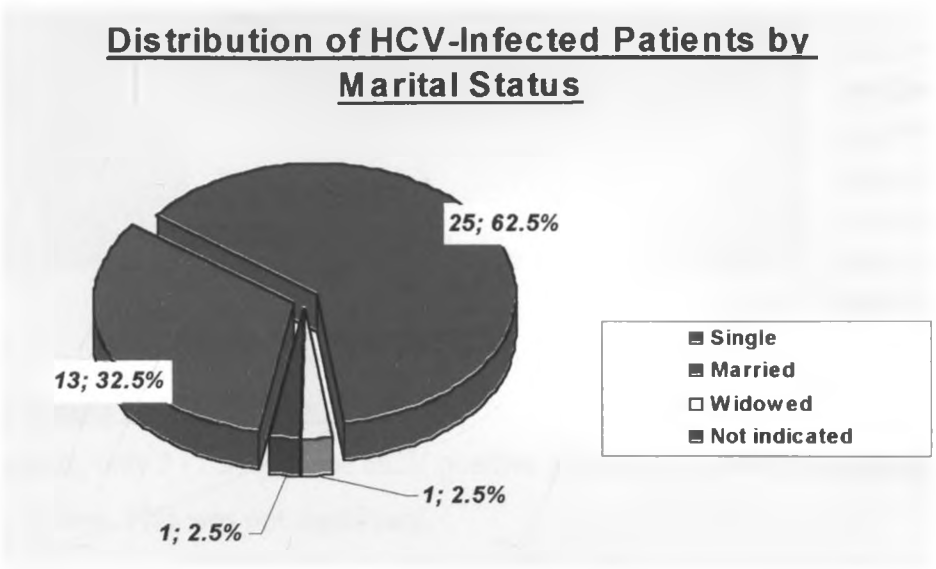
Figure III.10: HCV positive patients by age and gender



HCV positive patients by marital status

The majority (62.5%) of these patients were married. 32.5% were single while only one patient was widowed.

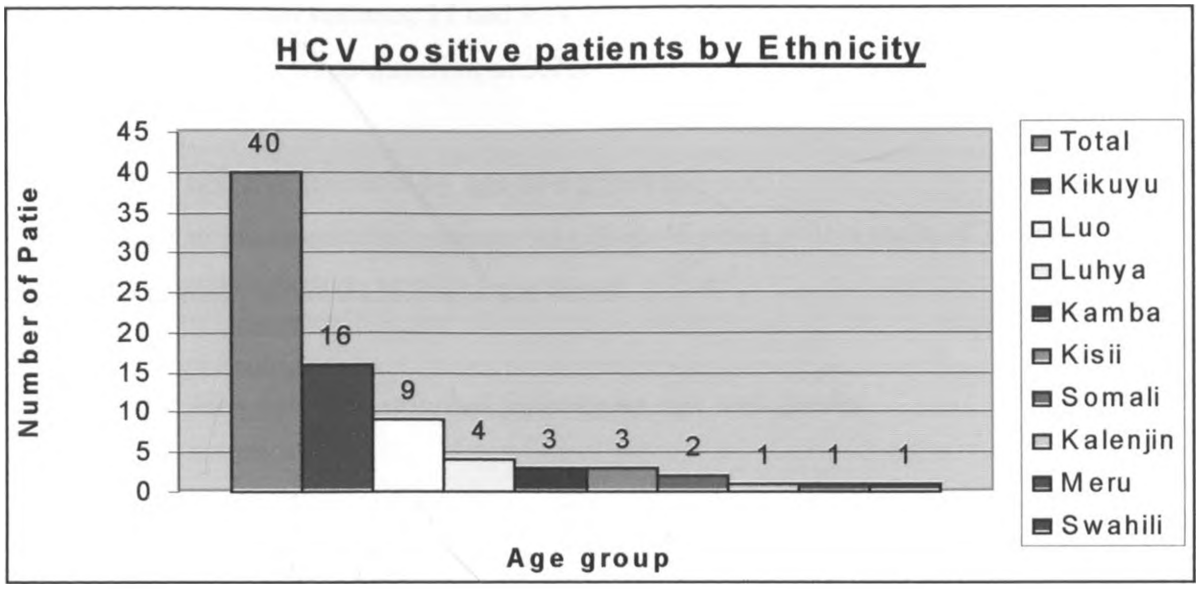
Figure III.11: HCV positive patients by Marital status



HCV positive patients by ethnicity

The distribution of the HCV positive patients followed the regional distribution and there were no significant differences among the various groups. 10 ethnic groups were represented, the most populous one being Kikuyu. (figure III.12)

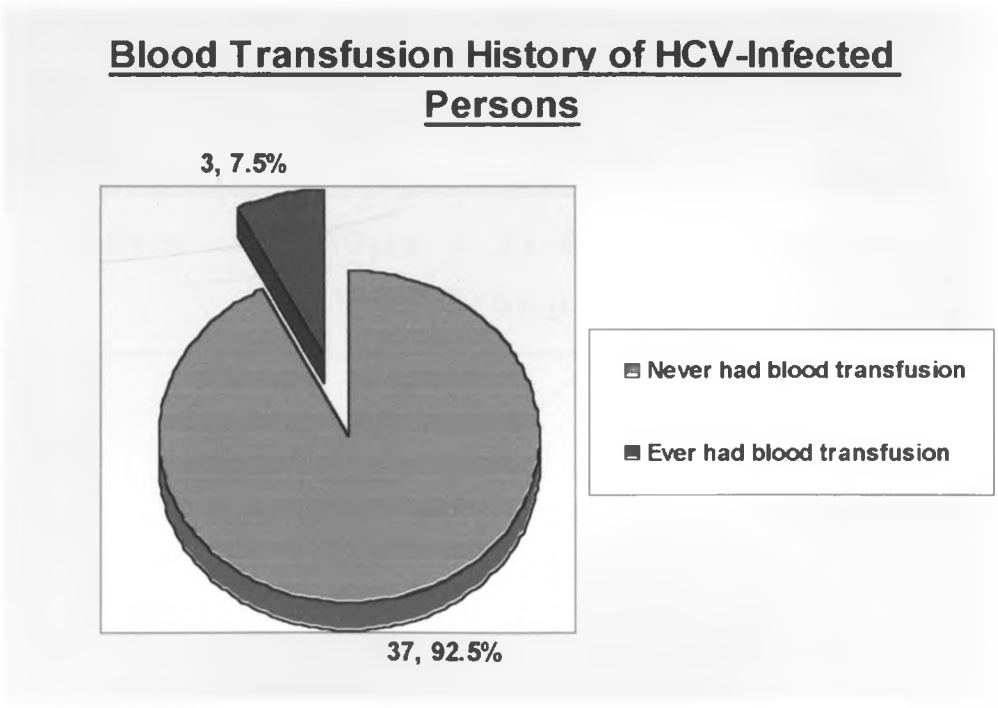
Figure III.12: HCV positive patients by ethnicity



HCV positive patients by risk factors for HCV

Of all the risk factors, only 3 (7.5%) of the HCV positive patients gave history of having had a blood transfusion. This was not significant.

Figure III.13: HCV positive patients by risk factors for HCV



HIV/HCV CO-INFECTED PATIENTS

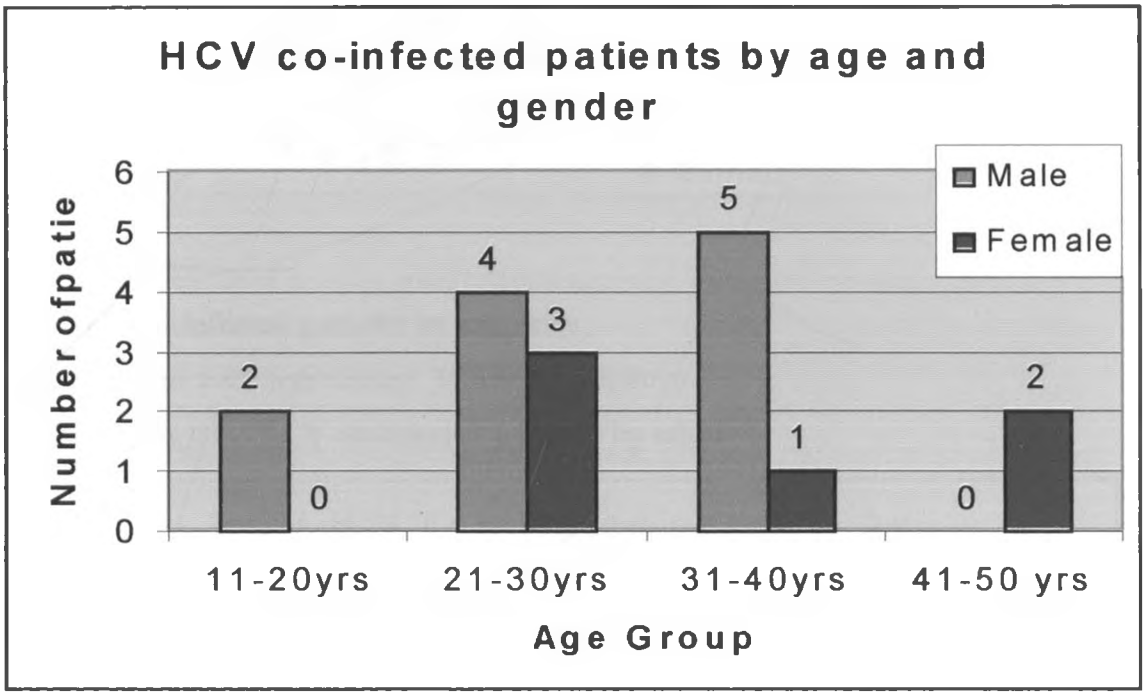
Among 518 AIDS ward patients, 17 had HIV/HCV co-infection

This gave a prevalence of co-infection of 3.7%

HIV/HCV co-infected patients by age and gender

The age range of the co-infected patients was 18 to 44 years, with a mean of 31 years. 11 (64.7%) were males while 6 (35.3%) were female.

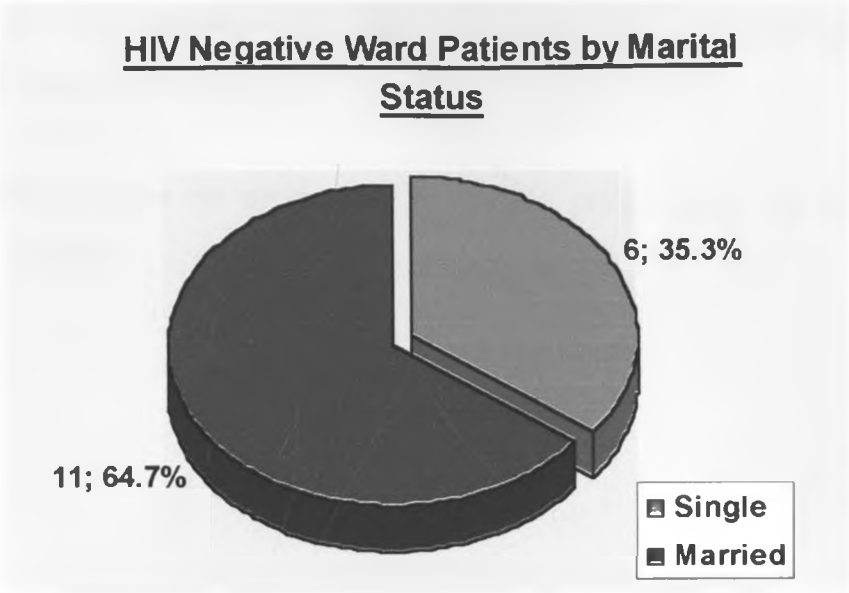
Figure III.14: HIV/HCV co-infected patients by age and gender



HIV/HCV co-infected patients by marital status

Majority of the co-infected patients were married. (figure III.5)

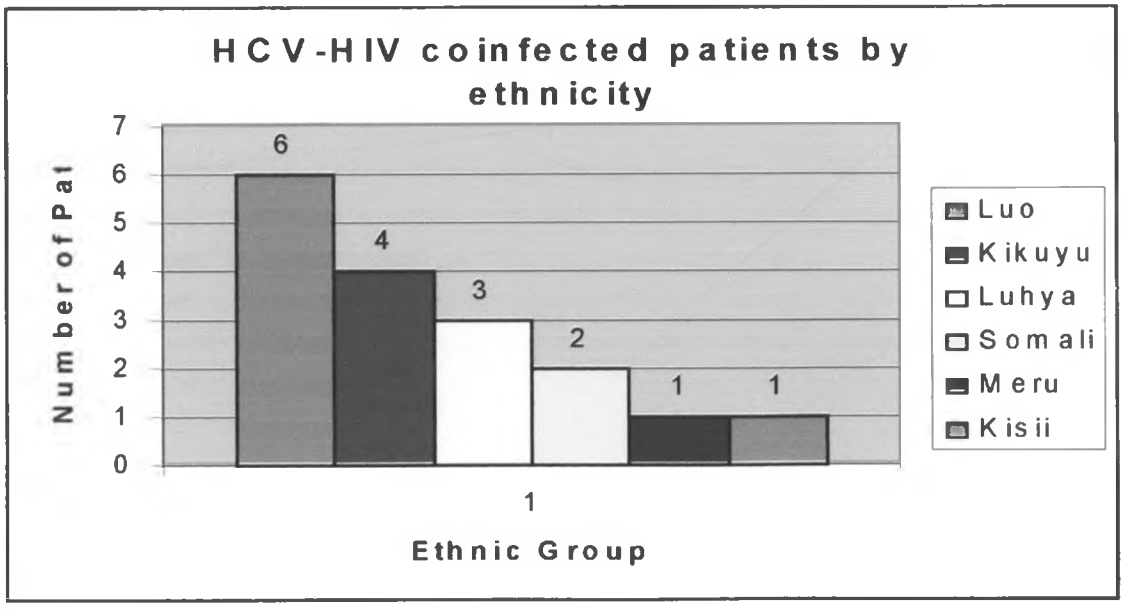
Figure III.15: HIV/HCV co-infected patients by marital status



HIV/HCV co-infected patients by ethnicity

6 ethnic groups were represented. 35.3% were Kikuyu.

Figure III.16: HIV/HCV co-infected patients by ethnicity



HIV/HCV co-infected patients by risk factors for HCV infection

Only one of the co-infected patients had had a blood transfusion. This was a 21 year old haemophiliac young man who had received more than 10 units of blood/blood products.

There were no incidences of needle prick, tattoo or IVDU in this group of patients.

IV. VCT CLIENTS

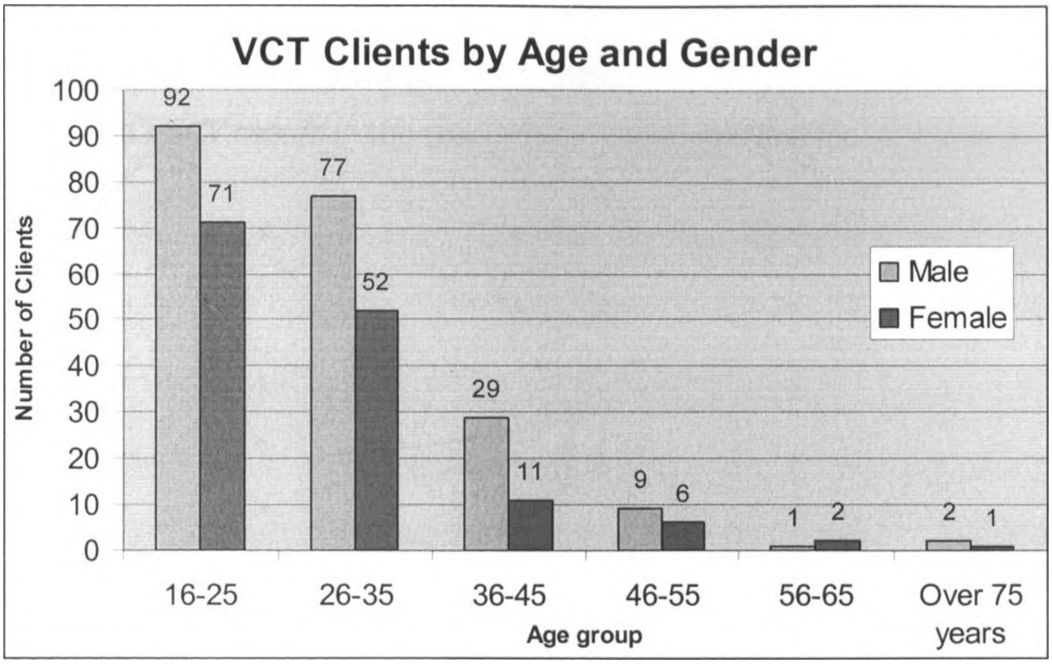
Clients were recruited from the KNH HIV-VCT in a consecutive manner. Of 365 clients screened, 13 were excluded; 5 gave a positive history of jaundice and 8 failed to give consent. Consequently 353 clients were excluded

HIV-VCT clients by age and gender

The range of the clients recruited in the study was from 16 to 78 years with a mean of 30 years.

Most of the clients were male 210 (49.49%) versus 143 (40.594%) female clients.

Figure IV.1 HIV-VCT clients by age and gender



HIV-VCT clients by risk factors for HCV infection

None of the VCT clients had had a blood transfusion

2 (0.6%) of the clients had used intravenous drugs with sharing of needles. Both were HIV positive.

69 (19.5%) of the clients seen admitted to having had more than one sexual partners over the last one year. 5 (7.2%) of these were HIV positive

Prevalence of HIV infection among VCT clients

Of the 353 VCT clients seen in this study, 33 were positive for HIV infection.

This gives a prevalence of 9.3%

This prevalence of HIV infection is similar to that seen in the VCT for the months preceding this study.

Prevalence of HCV Among VCTClients

Of the 353 VCT clients in the study, none tested positive for HCV infection.

STUDY LIMITATION

Due to financial constraints, it was not possible to determine the HCV genotypes of all the patients who tested positive for HCV. This would have answered the question on the genotype present locally more conclusively.

DISCUSSION

This is the first study done in this region on the prevalence of HCV in fairly large, broad sub-groups of the population. Previous studies on HCV focused on small groups of individuals and clinical groups who were thought to have a specific risk factor for HCV such as patients on maintenance dialysis. Results of these studies, as expected had varying results as seen below.

Prevalence Rates of HCV infections in different Kenyan populations (%)

Population	HCV
Patients with HCC (KNH)	9.7 (18)
Patients on maintenance dialysis (KNH)	6.1 (19)
Patients with SCD (KNH)	16.1 (20)
Patients with acute icteric hepatitis (KNH)	7.1 (21)

What is the HCV prevalence in the general population? This study was carried out in an attempt to answer this question. Four sub-populations were studied. The one with the highest prevalence was the clinical hospital staff with a prevalence of 5.2%. The prevalence among the HIV/AIDS and HIV negative in-patients was similar. The non-clinical hospital staff had a lower prevalence of 2.5%. Which of these sub-populations best represents the general population? Probably the non-clinical hospital staff or the HIV negative patients are representative. Whatever group one chooses the prevalence of HCV seems to be rising compared to estimations given earlier of between 0.2 and 0.9% (10, 11)

The prevalence among the blood donors is low at 0.79%. However, this is a pre-screened and therefore low-risk group. Previous studies revealed a prevalence of 0.9% among blood donors, but these were not a pre-screened group (12).

As much as the prevalence of HCV and HIV in this group is low it is important to note that had this blood not been screened for the two infections, over a period of six months, 49 and 66 patients would have been infected with HCV and HIV respectively, and, unfortunately, iatrogenically.

HCV prevalence among clinical hospital staff was significantly greater than that among non-clinical hospital staff (5.2% *versus* 2.5%). This brings up the possibility that clinical work is a risk factor for HCV acquisition. In this group of staff, only five admitted to having had a needle prick injury while in the course of clinical work. This number was too small to allow for subgroup analysis. The subjects relied on recall to respond to the questionnaire and this might have led to underreporting of such accidents. Nevertheless, it is necessary to study further and understand the reasons for this higher prevalence among clinical hospital staff. This may impact on the protection clinicians accord themselves during their work and their response to a needle prick injury. Another issue that arises here is the possibility of clinician to patient transmission of HCV.

There was no difference in HCV prevalence among HIV/AIDS and HIV negative patients. This is different from studies done elsewhere in Europe and America, in which the HCV prevalence among HIV infected patients is much higher giving a high rate of co-infection with HIV and HCV. Studies done in N. America and Europe found the prevalence of co-infection to be high, ranging from 15 to 30%. The number is much higher in patients infected through IVDU and haemophiliacs who have had multiple blood transfusions than those who acquired the infection through sexual practices (36, 37).

This difference could be explained by the fact that the main mode of HIV transmission is different. While here it remains heterosexual sex, in the West it is mainly by homosexual contact and IVDU both of which are more efficient methods of HCV transmission.

For these same reasons, our co-infection rates remain low.

In this study, the HCV genotypes of 5 persons were determined. These 5 were selected randomly. All were genotype 4. This corroborates with the experience of most of the gatroenterologists in this region in their clinical work. No other studies have been done in this region as far is this is concerned. It would have been possible to comment on this more conclusively had the genotypes of all those who tested positive been determined as envisaged earlier. This was not possible immediately.

CONCLUSIONS

The table below is a summary of the prevalence of HCV, HIV and HCV/HIV co-infection in the subgroups studied.

Table V.1: Prevalence of HCV, HIV HCV/HIV Co-Infection (%)

SUB-GROUP	HCV	HIV	HIV/HCV Co-infection
Blood Donors	0.79	1.07	0.02
Clinical hospital Staff	5.2	-	-
Non-clinical hospital staff	2.5	-	-
HIV/AIDS patients	3.7	100	3.7
HIV Negative patients	4.4	-	-
VCT Clients	0	9.3	0

1. The prevalence of HCV/HIV co-infection among blood donors in the NBTSC was very low, at 0.02%. The prevalence of HCV and HIV in this group was 0.79% and 1.07% respectively.
2. The prevalence of HCV infection among KNH staff was 3.6% with a significantly higher prevalence among the clinical staff of 5.2%. The prevalence among the non-clinical staff was 2.5%.
3. Among HIV/AIDS medical in-patients, the prevalence of HCV was 3.7% while in the HIV negative group of patients, it was 4.4%. The prevalence of co-infection with HCV and HIV was 3.7%
4. While the HIV prevalence among the KNH HIV-VCT attendees was 9.3%, none of the clients tested positive for HCV.
5. The determination of genotypes for 5 patients was done. All were genotype 4
6. The incidence of risk factors in the persons with HCV and/or HIV infection(s) was low.

RECOMMENDATIONS

1. Routine screening for HCV should be done for all blood donated.
2. Follow-up of the trend of HCV prevalence in hospital staff should be done and possible unique risk factors established.
3. Screening for HCV alongside HIV and HBV for hospital staff should be done following needle prick injury.
4. Studies on HCV prevalence in high risk groups such as intravenous drug users should be done.
5. One of the objectives in this study was not achieved fully as explained above (page 58). This could be done in another study and more people screened in order to conclusively answer the question on the HCV genotypes in our population.

DECLARATION

This study was carried out with financial backing from Roche Pharma. The principal investigator handled all the data and its analysis and the company named did not handle any of the data at any time during or after the study.

APPENDICES

Appendix I: QUESTIONNAIRE

Demographics

Hospital No_____ Study No _____

Age (years) _____ Ethnicity _____

Sex _____ Marital status _____

Occupation _____ Residence _____

Job Group_____

History:

1	Have you received a blood transfusion in the last one year?	Yes	No
2	How many sexual partners have you had in the last one year?	≤1	>1
3	Have you ever injected yourself with a needle and /or drug?	Yes	No
4	Have you ever suffered from jaundice (yellowness of eyes and mucous membranes)?	Yes	No
5	Have you been vaccinated for Hepatitis?	Yes	No
6	Have you undergone traditional circumcision, ear piercing or tatooing?	Yes	No
7	Have you ever had an accidental needle-prick injury in the course of your work? (For hospital Staff)	Yes	No

Appendix II: DATA COLLECTION FORM (Blood donors)

Study No	
NBTSC No.	
Age	
Sex	
Date of Collection	
Collection Center	
Occupation	
HIV Status	
HCV Status	

Appendix III HCV ANTIBODY ASSAY

PRINCIPLE

The INNOTEST HCV AB test is an enzyme immunoassy for the detection of antibodies to HCV. Microplates 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 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 1:10 dilution of the test serum is made by adding diluent to serum in the well and mixing.
2. The wells are incubated at 37° C for 60 minutes (Ab capture)
3. Each well is washed 6 times with wash solution.
4. Conjugate (containing enzyme labeled anti-human IgG) is added to each well, mixed and incubated at 37° 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.
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 2.75 cut-off value respectively.

Appendix IV: Consent Form for those ≥18 years

PREVALENCE OF HCV, GENOTYPIC SUBTYPES AND HIV CO-INFECTION AMONG VARIOUS BROAD SUBGROUPS OF THE KENYAN POPULATION

CONSENT FORM

I,_____ do voluntarily agree to take part in the above named study. The nature of the study has been explained to me and will involve filling in a standard questionnaire, undergoing a physical examination and having blood samples taken from me for Hepatitis C markers. While the results will remain the confidential property of the investigator, significant findings that may influence further management of my condition may be available to me.

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

Name: _____

Signature: _____

Date: _____

Witness: _____

Appendix V: Consent Form for those <18 years

PREVALENCE OF HCV, GENOTYPIC SUBTYPES AND HIV CO-INFECTION AMONG VARIOUS BROAD SUBGROUPS OF THE KENYAN POPULATION

CONSENT FORM

I,_____do voluntarily agree that my charge _____(name) can take part in the above named study. The nature of the study has been explained to me and will involve filling in a standard questionnaire, undergoing a physical examination and having a blood sample taken from him/her, to be tested for Hepatitis C markers. While the results will remain the confidential property of the investigator, significant findings that may influence further management of his/her condition may be available to me.

I also understand that he/she is free to withdraw from the study at any time without forfeiting any medical benefits due to him/her.

Name:_____

Signature:_____

Date:_____

Witness:_____

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