SEROPREVALENCE OF HEPATITIS C VIRUS ANTIBODY IN TRANSFUSED CHILDREN AT KENYATTA NATIONAL HOSPITAL.

A DESSERTATION PRESENTED IN PART FULFILMENT FOR THE DEGREE OF MASTER OF MEDICINE (PAEDIATRICS) OF THE UNIVERSITY OF NAIROBI.

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DECLARATION.

I declare that this dissertation is my original work and has not been published elsewhere or presented for a degree in any other university.

Signature---------- Date---31/10/2002---

This dissertation has been submitted to the university of Nairobi with our approval as university lectures,

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DEDICATION.

This book is dedicated to my children, Pauline and Darius, and to my husband Jackton.

ACKNOWLEDGEMENTS.

1 I am grateful to my supervisors, Dr Ngacha and Prof. Ogutu for their support and guidance.

2 I am also grateful to Mr. Joshua Oluyali, Mr. Duncan Marigi and Lucy Njoka for facilitating the typing and printing of this work.

3 My family members have been a source of encouragement at all times. May God bless you all.

4 I am grateful to my lecturers and the entire staff at the Department of paediatrics for their support.

5 Janet Musia's contribution in data analysis was highly appreciated.

6 Dr Rose Kamenwa's words of inspiration will ever be appreciated.

7 Dr Dalton Wamalwa , Dr Phelgona Otieno and Dr Lucy Ng'ang'a's contributions were highly appreciated. God bless you.

LIST OF ABBREVIATIONS.

HCV	Hepatitis C Virus.
Anti-HCV	Antibody against hepatitis C virus.
ALT	Alanine aminotransferase.
RNA	Ribonucleic acid.
HBsAG	Hepatitis B surface antigen.
PCR	Polymerase chain reaction.
RIBA	Recombinant Immunoblot Assay.
HIV	Human immunodeficiency virus.
WHO	World health organization.
D.R.C	Democratic republic of Congo
NAD	Nicotinamide adenine dinucleotide.
NADH	Nicotinamide adenine dinucleotide (reduced form.)
LDH	Lactic dehydrogenase.
D.N.A	Deoxyribonucleic acid.
cD.N.A	Copy deoxyribonucleic acid.
RT-PCR	Reverse transcription polymerase chain reaction.
FFP	Fresh frozen plasma.
C.I	Confidence interval.
R.R	Relative risk
O.R	Odd's ratio
D.F	Degree of freedom
KNH	Kenyatta national hospital

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6.ABSRACT

Objective. To determine the seroprevalence of HCV antibody in children with history of exposure to blood/blood products at KNH.

Study design. A cross sectional survey was carried out in an attempt to describe the prevalence of HCV antibody in children with history of exposure to blood/blood products at KNH.

Setting. The general paediatric wards, paediatric oncology ward, haematology clinic, paediatric filter clinic and paediatric outpatient clinics of KNH.

Study period. June 2001 to August 2001

Study subjects. A total of 90 children were identified from the various study areas within Kenyatta national hospital as follows, 10 cases (11%) from hematology clinic, 22 cases (24%) from paediatric oncology ward, 57 cases (63%) from the general paediatric wards and 1 case (1%) from paediatric filter clinic. The youngest child was 2 years 1 month old while the oldest child was 12 years old, the median age was six and half years. Blood samples were taken from the 90 children for screening for HCV antibody using second generation ELISA method.

Results. Among the 90 children tested for HCV antibody, 25 children (27.8%) tested positive for anti-HCV.

There was no significant sex difference in the number of study cases who tested positive for HCV antibody.

We did not demonstrate an increasing trend in HCV positivity with an increase in the number of exposures to blood/blood products.

Children with history of exposure to blood products (FFP, Platelet concentrates) did not have an increased risk of testing positive for HCV antibody than those exposed to whole blood/packed cells.

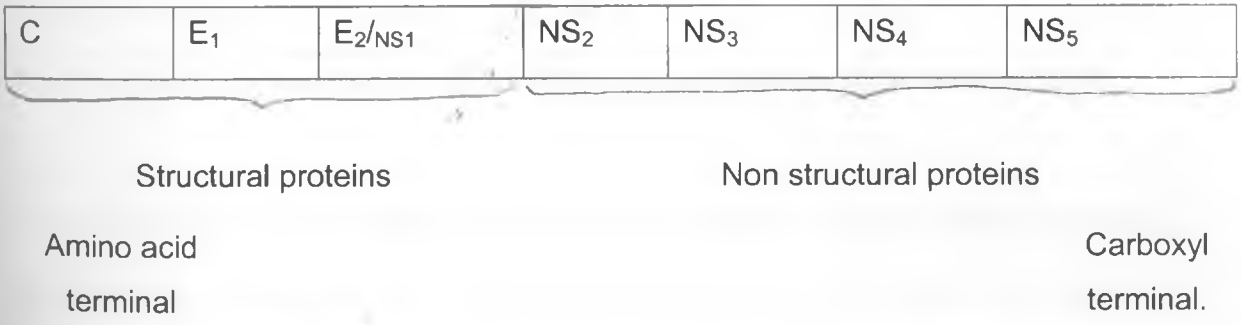
Conclusions. The prevalence of HCV antibody in children with history of exposure to blood/blood products at KNH was 27.8% (95% C.I= 18.5 – 37.0).

INTRODUCTION AND LITERATURE REVIEW

HCV was first described as a major cause of post transfusion non-A non-B viral hepatitis in 1975 (1) and isolated in 1989 (2). It is an RNA virus closely related to the family of flaviviridae accounting for 80-90% of community acquired non-A non-B viral hepatitis (3,4). It is a linear single stranded lipid enveloped RNA virus with a genome of 94 nucleotides (5,6). It has an amino acid terminal, a carboxyl group terminal and multiple regions of genome representing multiple viral proteins namely C1, E1, E2/NS1, NS2, NS3, NS4, and NS5 protein antigens (3,6) (Fig.1). The exact physical appearance is not known because it has not been visualized under an electron microscope.

The HCV genome contains a single large open reading frame that follows a relatively lengthy 5' non-translated region of approximately 342 bases. This is thought to play a critical role in controlling virus translation and may be important in the pathogenesis of HCV infection.

Figure 1. Hepatitis C viral genome.



Epidemiology of HCV infection

WHO estimates 170 million people i.e. 3% of the world's population to be infected with HCV (7).

Table 1.

HCV: estimated prevalence rates and number infected by W.H.O. region as of June 1999 (8).

W.H.O REGION.	TOTAL POPULATION (millions).	PREVALENCE RATE (%).	INFECTED POPULATION. (Millions).	NO. OF COUNTRIES WITH NO AVAILUABLE DATA
AFRICA	602	5.3	31.9	12
AMERICA	785	1.7	13.1	7
EASTERN MEDITERRANEAN	446	4.6	21.3	5
EUROPE	858	1.03	8.9	19
SOUTH EAST ASIA	1500	2.15	32.5	3
WEST PACIFIC	1600	3.9	62.2	11

Source: Global prevalence update. Weekly epidemiological record, 1999.

Prevalence rates vary widely among different countries. Among African countries, the highest prevalence rate was recorded in Egypt (18%), while no case was reported from Zambia (table 2). The figures are average estimates as different population groups were studied and methods of data collection and interpretation varied from country to country.

Table 2. Average prevalence rates for HCV antibody in the adult general population for some African countries (8).

COUNTRY.	PREVALENCE RATE (%).	COUNTRY.	PREVALENCE RATE (%).
Kenya	0.2-0.9	Central Africa.	4.5
Egypt	18	D.R.C	6.4
Burundi	11	Nigeria	1.4
Rwanda	17	Uganda	1.2
Cameroon	12.5	Ethiopia	0.8
Zimbabwe	7.7	Tanzania	0.7
South African blacks	0.75	Zambia	0.0
South African whites	0.16	Ghana	2.8

Source: Global prevalence update, weekly epidemiological record, 1999.

Comparable figures include 0.5-1.4% in North America (9), 0.05% in Canada (8), 0.6% in Argentina (8), 1.1% in France (8), 0.1% in Germany (8), 0.02% in the United Kingdom (8), 1.8% in India (8), 1.2-1.5% in Japan (8) and 2% in Russia (8).

Information on the prevalence rates of HCV antibody in the paediatric general population is scarce.

HCV and blood transfusions.

Majority of HCV infections are identified in children with repeated exposure to blood and its products (10,11,12). A study conducted in India on 75 children aged between 2 and 13 years who had been transfused more than one unit of blood found 21% to be anti-HCV positive, 15% had history of post transfusion hepatitis, 57% of whom had HCV antibody (10). Studies in the United States of America found out that transfusions were responsible for 5-10% of cases of symptomatic non-A non-B hepatitis seen annually (13). A Taiwanese study on post transfusion HCV infection in children who underwent open-heart surgery found the prevalence rate of HCV antibody to be 4-5% (14).

The screening of blood and its products for HCV antibody has significantly reduced the prevalence of post transfusion HCV infection. A Taiwanese study carried out to assess the impact of screening blood for HCV virus antibody on post transfusion hepatitis found 7 recipients out of 80 subjects who received unscreened blood to be positive for anti-HCV and HCV-RNA i.e. 8.8%, and none of the 112 recipients of screened blood tested positive for anti-HCV or HCV-RNA. Second generation ELISA method was used to screen the blood (15). An American study on 75 leukemia survivors who had 25 or more blood donor exposures found 9 to be anti-HCV positive i.e. 12%. All were infected before HCV screening tests were implemented. The mean HCV-RNA level in this population was noted to be higher than their anti-HCV positive immunocompetent controls. Liver biopsies showed moderate portal inflammation and 50% had evidence of bridging fibrosis (16). A Kenyan study on patients with nephrological problems, patients with chronic liver

disease and volunteer blood donors found a prevalence of 0.9% in blood donors, 2.6% in chronic liver disease, and 6.3% in those who underwent renal transplant (17). The higher prevalence in those who underwent renal transplant was attributed to multiple transfusions.

An Indian study of 39 patients with thalassaemia major who received more than one unit of unscreened blood transfusions and followed up serologically for a period of 3 years (1993-1995) observed a gradual increase in the number of patients who tested positive for anti-HCV. An increase in the number of blood units transfused per case per year was also observed (18)

Other studies have equally demonstrated high prevalence rates of HCV antibody in those at risk of multiple blood transfusions. Prevalence rates in children with thalassaemia ranges between 40-60% (18,19,20) and haemophilia up to 98% (21). In those undergoing hemodialysis, prevalence rates vary from country to country with rates of 7-85% in South Africa (5,22) and 8-36% in Spain (23).

In neonates, prevalence is particularly high in those transfused with blood from infected donors (24). Perinatal transmission occurs in approximately 5% of cases (25), the risk being higher in those mothers with higher titres of HCV-RNA and infected with HIV (26). The presence of HCV antibody in the newborn may imply passive transfer of these antibodies from the mothers. A repeat test after one year is recommended (24).

In children with chronic HCV infection, perinatal blood transfusion has been found to be the most common source of viral infection (10,11).

Other possible modes of transmission of HCV in the general population include

intravenous drug abuse and contaminated injection devices. Sexual, household and nosocomial transmission may occur but rarely (27,28).

Pathogenesis of HCV infection

Infection in most individuals tends to become persistent due to inability of the immune system to mount an effective antiviral response and clear the virus during the heightened phase.

Immunocompetent infected individuals develop antibodies reactive with the core protein (C) and several non-structural proteins including NS3 and NS4. Immunodeficient infected individuals may not produce antibodies at all.

The role of cell-mediated immunity in chronic HCV infection is still being explored. Cytotoxic CD8+ T-lymphocytes have been identified in the livers of chronically infected patients and several T-cell epitopes have been defined, but their relative contribution to immunity and disease pathogenesis is uncertain.

The frequency with which persistent HCV infection progresses to serious liver disease is still poorly documented. Liver biopsy results range from chronic persistent or lobular hepatitis or chronic active hepatitis to liver cirrhosis.

The overall long term mortality in patients who develop post transfusion HCV infection and those who do not is the same, but there is evidence of slight increase in mortality due to liver disease in patients with post transfusion HCV infection i.e. 3.3% vs. 1.5% (29).

HCV has a very high spontaneous nucleotide substitution rate. Infection may persist following emergence of mutant viruses that escape the host's immune system (3,30,31). The viraemia can be demonstrated by detection of HCV-RNA (3,5,32).

Effects of HCV infection

Acute HCV infection is relatively mild with non-specific signs and symptoms appearing in approximately 20-30% of cases while 70-80% of cases develop persistent viral infection (9,33). HCV-RNA is detected in serum within one to three weeks of contact and viraemia appears to be maximal at the onset of hepatitis with elevation of serum ALT levels occurring soon after in 50% of cases.

Antibodies may not appear until 6-12 weeks after the onset of hepatitis. A small proportion of individuals clear the infection during the acute phase and antibody response may be limited.

The interval between transfusion and seroconversion ranges between 10 to 39 weeks with an average of 22 weeks. After presentation with hepatitis, the mean interval to seroconversion ranges between 4 to 32 weeks with an average of 15 weeks (34).

Different studies have found HCV to be a common cause of chronic liver disease with 67% of post transfusion HCV infection and 33% of sporadic community acquired cases becoming chronic (34,37-39). Chronic elevation of serum ALT occurs in 80% of cases. Patients with persistent elevation of ALT levels who test positive for anti-HCV are presumed to have chronic HCV infection but other causes of chronic hepatitis should be considered hence the need for liver biopsy.

Most patients with chronic HCV infection have levels of antibodies that are detectable by 2nd generation ELISA method. Viral RNA is also detectable by PCR in plasma or serum of these patients.

An association between chronic HCV infection and liver cirrhosis has been documented. A Spanish study of 306 patients with chronic HCV infection who were followed up for a period of 95 months demonstrated liver cirrhosis in 83 cases (27%) and most of these were over 45 years of age (41).

Various studies have documented an association between chronic HCV infection, and hepatocellular carcinoma (42). Events leading to the development of the hepatocellular carcinoma are not yet well understood but it has been hypothesized that inflammatory cells, particularly monocytes and macrophages that are present in the liver generate free hydroxyl radicals that are capable of damaging cellular DNA and may be the proximate cause of malignant transformation.

HCV detection.

The expression and synthesis of specific viral proteins using recombinant technology has led to the development of clinically useful diagnostic tests based on the detection of serum antibody to these viral antigens (2). Commercially available diagnostic tests for HCV detect antibodies to recombinant viral antigens and are most useful in screening blood donors who are chronic HCV carriers as well as diagnosing chronic infection. Majority of immunocompetent individuals ultimately develop antibodies that persist over a long period of time (6,43). Under certain circumstances where seroconversion may not occur such as in acute

infection and immunosuppression, the antibody tests cannot be relied on for diagnosis due to a high false negativity rate.

In the ELISA method, an indicator antigen is conjugated to an enzyme, the presence of which is detected by adding a substrate that is converted to a colored product by the action of the enzyme.

First generation ELISA method detects antibody to a single HCV antigen i.e 100, a 363 amino acid fusion polypeptide representing part of the NS4 region of the HCV genome. It was found to have a sensitivity of 80-90% (34) with false positive tests being found in patients with autoimmune diseases, in older stored serum samples and sera collected from the tropics. The test was also noted to have a poor positive predictive value when applied to low risk population such as blood donors. This led to the development of second generation ELISA (15).

Second generation ELISA detects antibody to the core protein and non-structural HCV protein NS3 (2,44). Its sensitivity is approximated to be 97-100%. Screening blood donors in Japan between 1989 and 1991 using first generation ELISA method reduced the incidence of post transfusion hepatitis from 9.6% (216/2,240) to 3.7% (24/655). With the introduction of second generation ELISA in 1992 a further decrease to 0.9% (3/326) was observed (15).

Third and fourth generation ELISA kits that detect antibody to the core protein, NS3 and NS4 proteins are still under evaluation.

Other useful tests that may be done to confirm the presence of HCV antibody or HCV-RNA, include RIBA, PCR, and branched chain cDNA hybridization.

STUDY JUSTIFICATION AND UTILITY.

1. At KNH, blood is routinely screened for HIV, Hepatitis B virus and syphilis but not HCV.
2. Transfusion of blood and its products is the major mode of transmission of HCV in areas where screening is not routinely done. Prevalence of post transfusion HCV antibody is relatively low in countries where donor blood is routinely screened for anti – HCV, e.g., United States and England.
3. Results of this study will form part of the basis for recommendation for routine screening of donor blood for HCV antibody at KNH

SPECIFIC OBJECTIVE.

To determine the prevalence of HCV antibody in children with previous exposure to blood /blood products at KNH.

MATERIALS AND METHODS.

Study design.

A cross sectional survey was carried out in an attempt to describe the prevalence of HCV antibody in children exposed to blood/blood products.

Study site.

This study was carried out at the general paediatric wards, paediatric oncology ward, hematology clinic, paediatric filter clinic and the general paediatric outpatient clinic of KNH.

KNH is located in Nairobi, Kenya's capital city. It is the national referral hospital for both district and provincial hospitals in the country. It also serves as the primary health facility for most people who reside in Nairobi. It is the teaching hospital for the university of Nairobi medical school.

The hospital has five general paediatric wards where all children who require in patient care are admitted. The paediatric oncology ward is a special unit of the hospital that takes care of children with confirmed diagnosis of paediatric malignant conditions. It has a capacity of 32 beds. Children with various hematological conditions are followed up at the hematology clinic. It is run by specialist hematologists on Mondays between 8.00 am and 1200 noon. Approximately 50 children go through this clinic weekly. The paediatric filter clinic aims at identifying children who require inpatient care from those who require outpatient care. It is a paramedical clinic run by registered clinical officers with further training in paediatrics. The general paediatric outpatient clinic is run on Thursdays between 2.00pm and 4.00pm. Approximately 150 children go through this clinic weekly.

Study population.

Any child aged between age 2 years and 12 years who met the inclusion criteria was recruited into this study.

Inclusion criteria

1. Any child aged 2 years to 12 years with history of transfusion with whole blood or blood products (packed cells, FFP, platelet concentrates).
2. The child received the transfusion in Kenya.

Exclusion criteria.

1 Transfusion within the past three months

2 Lack of consent.

3 Inability to verify history of previous exposure to blood/blood products.

Case selection.

The investigator visited the study areas mentioned above and enquired about history of transfusion from the parent/guardian. For those in the outpatient clinics, (hematology clinic, paediatric out patient clinic and paediatric filter clinic), the files or discharge summaries were reviewed to confirm history of transfusion.

In-patients (general paediatric wards, and paediatric oncology ward) had their files in their respective wards that had details of admissions. Incase of a lost file, most of the parents/guardians had discharge summaries of their children. The doctor's order to transfuse the patient was checked including the group and cross match cards to confirm that blood was cross-matched for that patient. The nurse's observation charts to confirm that the child received the blood/blood product were also checked.

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All the children who met the inclusion criteria were recruited into the study. The investigator explained the rationale and benefits of the study to the parent or guardian and subsequently obtained an informed written consent. The patient's history of illness was taken and physical examination done. The total number and details of transfusion were noted and details recorded in a study questionnaire.

The weights of the children were taken and expressed as a percentage of their expected weight for age according to Wellcome trust classification (45).

After the interview 1ml of blood was taken from a peripheral vein under aseptic precautions. The sample was forwarded to the immunology laboratory for HCV screening using second generation ELISA method as per the manufacture's instructions, details of which are described below.

HCV assay

Serum was separated and stored at 2^{0}C. The storage was done until the desired sample size was obtained. Two wells for positive controls (from the kit), two wells for negative control (from the kit), and two additional known positives (from a local hospital-Nairobi hospital), were set up for quality control.

Fifty microlitres of specimen diluent was dispensed to the wells. Five microliters of specimen sample was added to each well and incubated for 30 minutes at 37^{O} C. Each well was washed five times with a diluent's wash buffer.

One drop of enzyme conjugate was added to each well, mixed gently and incubated for 20 minutes at 37^{O}C. The wells were then washed five times as before.

A substrate solution was added, mixed gently and incubated for 10 minutes at 37 degrees centigrade.

One drop of stop solution was added to stop the color reaction. Optical density at 450 was read with an EIA reader.

ELISA test result interpretation

The manufacturer's instructions on the HCV assay kit were followed in identifying the positives and negatives.

The optical density of a blank well was taken to correct for all optical density readings from the wells.

The cut off value was calculated as follows:

Average of negative controls+0.15

Negative control (1)----------0.046

Negative control (2)----------0.044

Average of the negative controls =0.045.

Cut off value =0.045+0.15.

=0.195

Any reading above or equal to the cut off value was considered positive, while that below was considered negative.

Sample size

With an anticipated prevalence of 6.3% in the high risk group as per the Kenyan study (17) and a level of significance of 0.05 and degree of freedom of 0.05, the sample size was calculated using the formula below,

$$N = \frac{Z^2_{(1-\alpha/2)} .p\ (1-P)}{d^2}$$

Where

N Is the minimum sample size

P Is the anticipated prevalence of HCV antibody in transfused children

α Is the significance level

d Is the degree of precision

N=90.

Study period

June 2001- August 2001

Ethical considerations.

Approval to carry out this study was granted by Kenyatta national hospital ethical committee. An informed consent was sought from the parent/guardian and confidentiality maintained. Results of the various individuals were put in their files for future reference after obtaining a verbal consent from the parent/guardian.

Data analysis.

Data obtained was entered into the statistical package for social sciences (spss), version 10.05. The proportion of children with exposure to blood/blood products who tested positive for HCV antibody was calculated.

An evaluation of the influence of the number of exposures to blood/blood products was done through stratified analysis.

The role of potential confounders for anti-HCV positivity was explored using Odds ratios to compare the proportion of children with and those without the confounders who tested positive for HCV antibody.

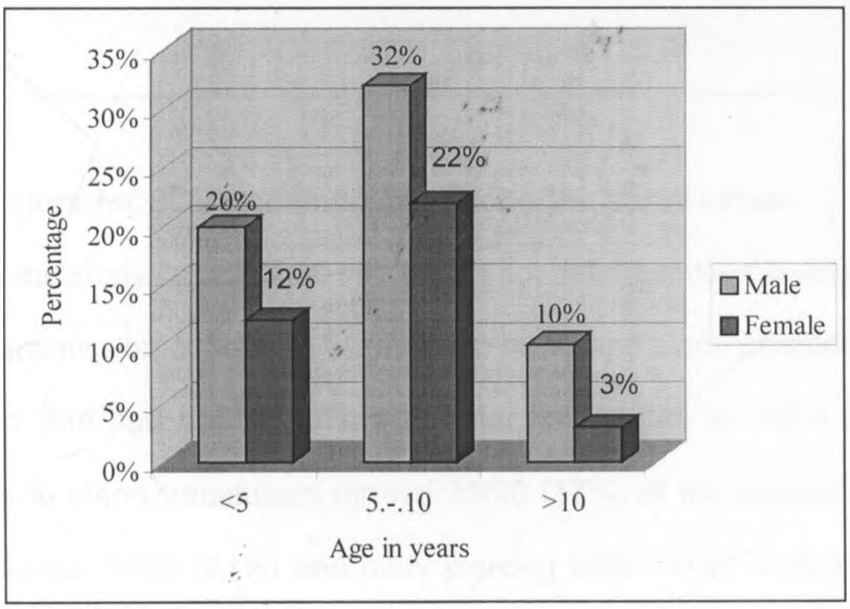
RESULTS.

A total of 90 children from the various study areas within KNH, [10 cases (11%) from hematology clinic, 22 cases (24%) from paediatric oncology ward, 57 cases (63%) from the general paediatric wards and 1 case (1%) from paediatric filter clinic] were tested. The age range was two years one month to twelve years old with a median age of six and half years. Thirty-four of the ninety cases were females (38%) while fifty-six cases were males (62%) giving a male to female ratio of 1.6:1. The graph below shows their age and sex distribution.

Age and sex distribution of the study cases.

More than half of the study cases 54% (49/90) were in the age group 5-10 years as shown in fig.2.

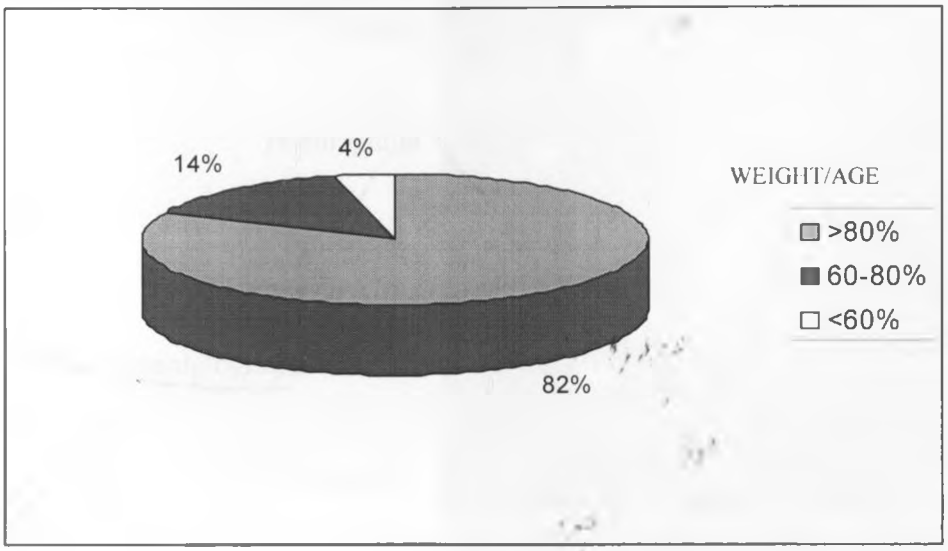
Figure 2. Age and sex distribution of the study cases.



Nutritional status (weight/age) of the study cases.

Using the welcome trust classification for nutritional status, appendix 3, most of the study cases 74/90 (82%) were of normal nutritional status. 12/90 (14%) were underweight while 4/90 (4%) of the children were marasmic as shown in fig 3. None of the study cases had kwashiorkor or marasmic kwashiorkor.

Figure 3. Nutritional status (weight/age) of the study cases.



Risk factors for HCV transmission among the study cases.

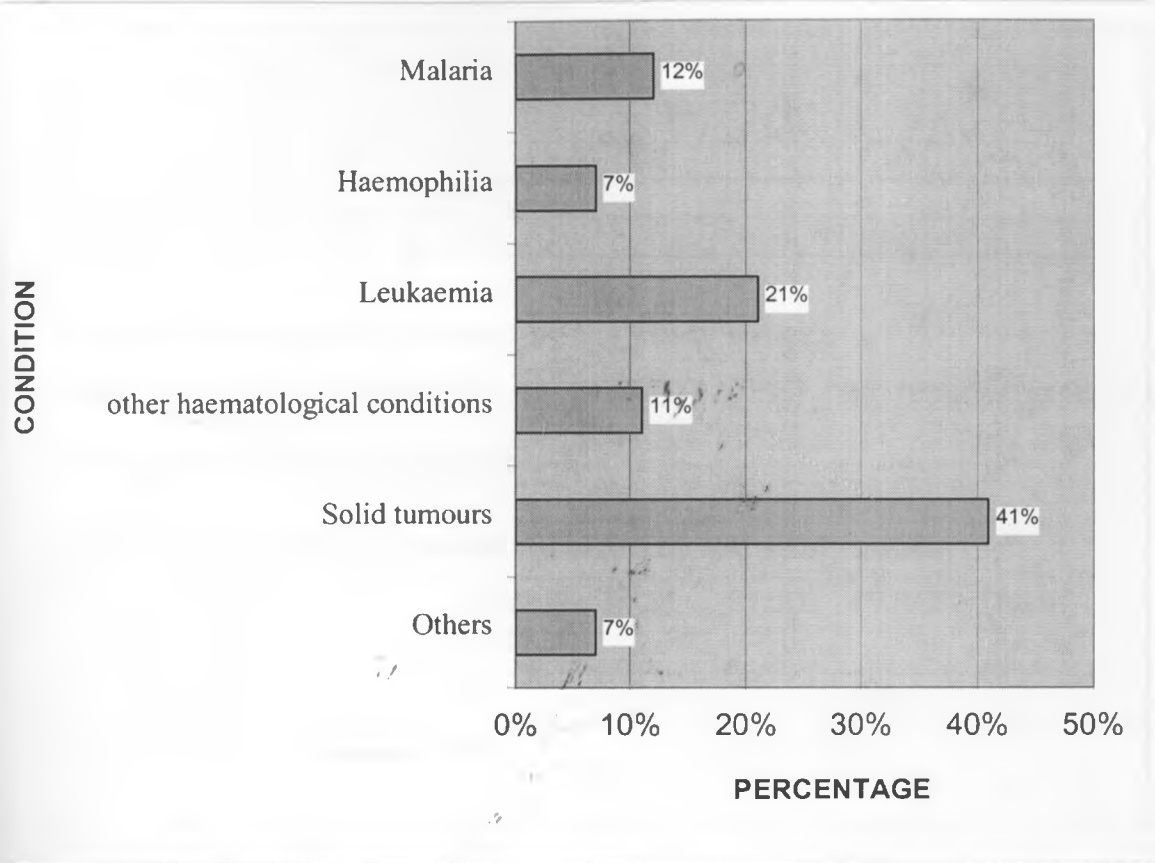
Most of the study cases 57/90 (64%) had not been exposed to any other mode of HCV transmission other than exposure to blood and blood products.

Children who had been exposed to other risk factors for HCV transmission in addition to blood transfusion formed 33/90 (37%) of the population. Sharing of razor blades 19/90 (21%) and body piercing 9/90 (10%) were found to be the commonest.

Underlying conditions that necessitated transfusion of the study cases.

More than half of the study cases, 56/90 (62%) had malignancy (solid tumors and leukaemia) as the underlying condition that necessitated transfusion as shown in figure 5.

Figure 5. Conditions that necessitated transfusion of the study cases.



Other hematological conditions refer to aplastic anaemia, spherocytosis, iron deficiency anaemia, sickle cell disease and idiopathic thrombocytopenic purpura, while others refer to chronic renal failure, Gaucher's disease and transfusion secondary to acute blood loss during surgery.

Number of exposures to blood/blood product of the study cases.

The number of children with one exposure and those with multiple exposures to blood/blood products was the same 45/90 (50%) as shown in table 3. The median number of exposure was two (range 1 to 14).

Table 3. Numbers of exposures to blood/blood products of the study cases.

NUMBER OF BLOOD UNITS RECEIVED	NUMBER OF STUDY CASES	PERCENTAGE
1	45	50
2	19	21
3	5	6
4	5	6
5	6	7
6	2	2
>7	8	9
TOTAL	90	100

Blood/blood product received by the study cases.

More than half of the study cases 62/90 (69%) had received whole blood transfusion only as shown in table 4.

Table 4. Blood/blood product received by the study cases.

BLOOD/BLOOD PRODUCT	NUMBER OF STUDY CASES	PERCENTAGE
Whole blood	62	69
Whole blood + packed cells	7	8
Whole blood + FFP	2	2
Packed cells	14	16
FFP	2	2
Packed cells + FFP	1	1
Whole blood + platelet concentrates	1	1
Platelet concentrates	1	1
TOTAL	90	100

Prevalence of HCV antibody in the study population.

Among the 90 children who were tested for HCV antibody, 25 of these children tested positive giving a prevalence rate of 27.8% (95% C.I= 18.5 – 37.0). The median age for those who tested positive was six and half years. The male to female ratio was 1.3:1, whereby 14/25 (56%) were males and 11/25 (44%) were females.

Sex distribution of anti HCV positive study cases.

Fourteen out of fifty six (25%) of males compared to eleven out of thirty three (32%) of females tested positive for HCV antibody. O.R = 0.7 (95% C.I= 0.25 – 1.97) as shown in table 5.

Table 5. Sex distribution and anti-HCV status of study cases.

	HCV POSITIVE CASES	HCV NEGATIVE CASES
MALE (n=56)	14 (25)	42 (75)
FEMALE (n=34)	11(32)	23 (68)

P=0.45 O.R=0.70 (95% C.I=0.25-1.97) 1DF

Nutritional status (weight/age) of anti-HCV positive study cases.

Twenty-one out of seventy four (29%) of the children who were well nourished (expected weight/age of >80%) compared to a quarter (25%) of the malnourished children (expected weight/age of <80%) tested positive for HCV antibody.

O.R=0.84 (95% C.I = 0.02 - 3.28).

Number of exposures to blood/blood product of anti-HCV positive study cases

Fourteen out of forty five (31%) children with history of multiple exposures to blood/blood product compared to eleven out of forty five (24%) of those with history of one exposure tested positive for HCV antibody.

O.R=0.7 (95% C.I.= 0.26 – 1.99).

Blood/blood product received by anti-HCV positive study cases

Most of the study cases who tested positive for HCV antibody had received whole blood transfusion 23/25 (92%) as shown in table 6a.

One out of four (25%) of the children who received blood products compared to twenty-four out of eighty five (28%) of those who received whole blood/packed cells tested positive for HCV antibody. O.R=0.64 (95% C.I= 0.03 – 6.61) as shown in table 6b.

Table 6a. Blood/blood product received by anti-HCV positive study cases

BLOOD/BLOOD PRODUCT	ANTI-HCV POSITIVE CASES	ANTI HCV NEGATIVE CASES
WHOLE BLOOD (n=62)	22(35)	40(65)
WHOLE BLOOD + PACKED CELLS (n=7)	1(14)	6(86)
WHOLE BLOOD + FFP (n=2)	0(0)	2(100)
PACKED CELLS (n=14)	1(7)	13(93)
OTHERS (n=5)	1(20)	4(80)

Others refer to platelet concentrates and FFP.

Table6b. Whole blood/blood products and anti-HCV positivity of study cases

	ANTI-HCV POSITIVE CASES	ANTI-HCV NEGATIVE CASES
BLOOD PRODUCTS (n=5)	1(20)	4(80)
WHOLE BLOOD/PACKED CELLS (n=85)	24(28)	61(72)

O.R= 0.64 (95% C.I. 0.03 – 6.61) FISHERS EXACT TEST=0.6.

Blood products refer to FFP and platelet concentrates

Anti-HCV status of study cases exposed to other modes of HCV transmission

Approximately one third of the study cases who tested positive for anti-HCV 8/25 (32%) had been exposed to other risk factors for HCV transmission in addition to blood transfusion.

Eight out of the thirty three (24%)of those children exposed to other modes of HCV transmission in addition to history of exposure to blood/blood products as compared to seventeen out of fifty seven (30%) of those with history of exposure to blood/blood products alone tested positive for HCV antibody. O.R=O.75 (95% C.I= 0.25 – 3.21) as shown in table 7.

Table 7. Anti-HCV status of study cases exposed to other modes of HCV transmission

	ANTI-HCV POSITIVE CASES	ANTI-HCV NEGATIVE CASES
TRANSFUSION PLUS OTHER MODES OF HCV TRANSMISSION (n=33)	8(24)	25(76)
TRANSFUSION ALONE (n=57)	17(30)	40(70)

O.R=0.75 (95% C.I= 0.25-3.21) P=0.57.

DISCUSSION.

Results of this study show that HCV antibody is common in children presenting at KNH with history of exposure to blood/blood products as depicted by a prevalence rate of 27.8% (25/90). This was a cross sectional prevalence study aimed at providing a reliable source of data for further studies on HCV and hence advocate for timely interventions. This is quite significant as it implies that approximately one in every three children presenting at KNH with history of exposure to blood/ blood product may test positive for anti-HCV.

Information on HCV infection in children in developing countries is scarce. Results of this study are comparable to those of Juneja et al who found the prevalence rate of anti-HCV to be 21% in children with more than one exposure to blood/blood products. That study included only those with more than one exposure who did not have any other risk factors for HCV transmission. Thomas et al (46) however found the prevalence of anti-HCV in children who underwent cardiac surgery before HCV antibody screening of donor blood was implemented in Germany to be 14.6% after excluding those with other risk factors for HCV transmission. The differences noted could be due to the fact that our study included those children exposed to other modes of HCV transmission in addition to blood transfusion.

Although our study had more males 56/90 (62%) than females 34/90 (38%) with a male to female ratio of 1.6:1, males did not have an increased risk of testing positive for anti-HCV than the females O.R=0.7 (95% C.I=0.25-1.97). In their study on the natural history of HCV infection in America, Alter M J et al (9) found

out that the risk of testing anti-HCV positive for males and females was also the same.

Patients with malignancy have reduced immunity secondary to the disease process and cytotoxic therapy and hence may not be able to mount an effective immune response to the invading virus. In our study, children with malignancy (solid tumours and leukaemia) as the underlying condition that necessitated transfusion were found to have a higher prevalence of HCV antibody 16/25 (64%) than those without malignancy. This was however not significant and is probably due to a sampling bias since more than half of the study cases 59/90 (62%) had malignancy as the underlying condition that necessitated their transfusion.

Repeated exposures to blood/blood products has been documented in other studies to be associated with an increase in the number of study cases who test positive for anti-HCV. Choudhry et al (18), in their study on 39 children with thalassemia major demonstrated an increase in the number of children who tested positive for HCV antibody with an increase in the number of exposures to blood/blood product per cases per year. The median number of transfusion in that study was eight (range 5 to 20). We did not demonstrate a similar trend in our study probably because of a lower median number of exposures to blood/blood products, (median = 2, range 1 to 14) and few numbers of children who had received more than two transfusions.

Recipients of blood products have been known to have a higher risk of testing positive for anti-HCV because of exposure to different donors per session of

transfusion. Trocei C.L et al (21) found that children with haemophilia who received fresh frozen plasma before heat treatment of blood products was introduced, were more likely to test positive for HCV antibody than those who received whole blood. We did not demonstrate this in our study probably due to small numbers.

Various studies have documented transmission of HCV by other modes other than blood transfusion. Bartoli F. et al (47) found out that 7 out of 77 children (9%) exposed to percutenous modes of transmission of HCV in addition to transfusion tested positive for HCV antibody. In our study children who had been exposed to other risk factors for HCV transmission in addition to transfusion 8/25 (32%) did not however have an increased risk of testing positive for HCV antibody than those exposed to blood/blood product alone O.R =0.7 (95% C.I= 0.25 - 3.21). This observation could be attributed to the difference in the number of study cases recruited into the two studies.

One of the factors associated with a false negative ELISA test result is immunosuppression. We feel that the prevalence rate of 27.8% found in this study may be an underestimate. More than half of the study cases who tested positive for HCV antibody 16/25 (64%) had malignancy as the underlying condition that necessitated transfusion. Although these children did not have an increased risk of testing positive for anti-HCV, some children with HCV infection may have been missed by the ELISA method due to their inability to mount an effective immune response as a result of immunosuppression secondary to malignancy and cytotoxic therapy. Paul M. et al (16) found a prevalence of 12%

(9/75) in leukaemia survivors with 25 or more exposures to donor blood using second generation ELISA method but Albert et al (50), in their 10-year follow up study of 119 leukaemia survivors observed that it was only HCV-RNA that correctly identified HCV infection in this group of patients as antibodies became detectable only after chemotherapy was withdrawn.

Study cases with severe malnutrition i.e. weight/age <60% were relatively very few 4/90 (4%). Like those children with malignancy, this group may give false negative ELISA test results, as they may not be able to mount an effective immune response in response to HCV infection. Although half of them tested positive for HCV antibody 2/4 (50%) a bigger sample size will be required to make valid conclusions.

Our study was designed to determine the prevalence of HCV antibody in children with history of exposure to blood/blood products and not the association between various factors and HCV positivity. We cannot therefore make definite valid conclusions as to whether associations do exist in this population. Different studies with appropriate study designs will have to be carried out to make these conclusions.

This study however shows that HCV antibody is common in children with history of exposure to blood/blood products through transfusion. The author recommends routine screening of donor blood to prevent further transmission of HCV antibody and complications associated with HCV infection.

CONCLUSION.

The prevalence of HCV antibody in transfused children at KNH was 27.8%.

RECOMMENDATION.

We recommend routine screening of blood for HCV antibody before transfusion.

Appendix 1,

Questionnaire

Name.....

Case number.....

OP/IP number----------

1. Age

Completed years ☐

Completed months ☐

2. Sex

(a) Male ☐

(b) Female ☐

3. Weight (Kg). ☐

4 Number of exposures to blood/blood product ☐

5. Details of transfusion

Date of Transfusion	Whole blood.	Blood products e.g., FFP, Platelet concentrates.	Underlying condition-necessitating transfusion	Number of exposures
1)				
2)				
3)				
4)				
5)				
6)				
7)				
8)				
9)				
10)				

NB. If the underlying condition necessitating transfusion is malignancy, please specify.

6. Has the patient been injected in the past using recycled needles?

a) Yes ☐

b) No ☐

c) Don't know ☐

7. If yes approximately how many times in the past 1 year.

(a) Less than 5 times ☐

(b) 5-10 times ☐

(C) More than 10 times ☐

8. Has the patient ever undergone any traditional therapeutic rites necessitating use of unsterilized sharp objects e.g. circumcision?

(a) Yes ☐

(b) No ☐

(c) Don't know ☐

If yes, specify.----------

9. Has the patient ever undergone any of the following? (Percutenous modes of transmission), please tick where applicable. ☐

(a) Tattooing. ☐

(b) Body piecing. ☐

(c) Acupuncture. ☐

(d) Sharing of razor blades for cutting hair. ☐

(e) Sharing of toothbrushes ☐

(f) Others, Specify---------- ☐

10. Hepatitis C antibody

(a) Absent ☐

(b) Present ☐

Appendix 2. Consent.

Investigator; Dr Nyabiage L.O.

Postgraduate student,

Department of Paediatrics and Child health,

University of Nairobi.

This is a research activity aimed at finding out the presence of antibody against HCV in serum of children who have been exposed to blood/blood products in the past.

Results of this study will form a basis for recommendation of screening blood before transfusion. Parents are advised to seek treatment for their children early to avoid multiple blood transfusions where possible.

1mls of blood will be taken from a peripheral vein using a sterile needle and a sterile syringe. There will be a little pain and minimal bleeding from the puncture site. This blood will be taken to the laboratory for HCV screening. The weight of the child will also be taken. The investigator will ask some questions of which the parent/guardian will be expected to respond to appropriately. He/she may choose not to answer the questions or refuse the child to be included in this study.

The investigator will bear the cost of this study. Data obtained will be confidential and submitted to the relevant authorities on request.

Signature of the investigator----------

Date ----------

Parent/guardian's statement.

The study described above has been explained to me. I voluntarily consent to participate in this activity. I have had an opportunity to ask questions. I understand that the investigator will answer future questions I may have about this research.

Signature of the parent/guardian----------

Date----------

Appendix 3

Wellcome trust classification (45)

EXPECTED WEIGHT FOR AGE	OEDEMA ABSENT	OEDEMA PRESENT
60% - 80%	Underweight	Kwashiorkor
<60%	Marasmus	Marasmic kwashiorkor

Children whose expected weight for age is more than 80% to 100% are considered to be normal.

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