THE HIV SEROPOSITIVITY IN CHILDREN WITH SICKLE CELL ANAEMIA AT KENYATTA NATIONAL HOSPITAL

BY

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A DISSERTATION SUBMITTED IN PART FULLFILMENT FOR THE DEGREE OF

MASTER OF MEDICINE

OF THE UNIVERSITY OF NAIROBI

(1988)
DECLARATION

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

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### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>Haemoglobin A</td>
</tr>
<tr>
<td>AIDS</td>
<td>Acquired Immuno Deficiency Syndrome</td>
</tr>
<tr>
<td>AS</td>
<td>Sickle cell trait</td>
</tr>
<tr>
<td>Conf.</td>
<td>Confidence</td>
</tr>
<tr>
<td>DF</td>
<td>Degree of Freedom</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme Linked Immuno Absorbent Assay</td>
</tr>
<tr>
<td>Hb</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immuno Deficiency Virus</td>
</tr>
<tr>
<td>KEMRI</td>
<td>Kenya Medical Research Institute</td>
</tr>
<tr>
<td>MCH</td>
<td>Mean Corpuscular Haemoglobin</td>
</tr>
<tr>
<td>MCV</td>
<td>Mean Corpuscular Volume</td>
</tr>
<tr>
<td>MRC</td>
<td>Medical Research Centre</td>
</tr>
<tr>
<td>RBC</td>
<td>Red Blood Cell</td>
</tr>
<tr>
<td>SCA</td>
<td>Sickle Cell Anaemia</td>
</tr>
<tr>
<td>SCD</td>
<td>Sickle Cell Disease</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SE</td>
<td>Standard Error</td>
</tr>
<tr>
<td>SF</td>
<td>Foetal Haemoglobin</td>
</tr>
<tr>
<td>T4</td>
<td>T helper lymphocyte</td>
</tr>
<tr>
<td>T8</td>
<td>T suppressor lymphocyte</td>
</tr>
<tr>
<td>WBC</td>
<td>White Blood Cell</td>
</tr>
</tbody>
</table>
A total of 429 children comprising of 198 previously transfused sicklers and 231 non transfused control children were recruited for the study. The transfused sicklers had received transfusions at least 6 months prior to the study. They all had a transfusion between 1982 and 1987. The control group comprised of 106 children with sickle cell disease and 125 children with Hb AA. All the children were tested for HIV using Wellcozyme ELISA test in duplicate. In this study, all the children were ELISA negative and hence the Western Blots were not carried out. All the HIV serological analysis were carried out at KEMRI - MRC which is the main National research centre for HIV studies in Nairobi.

The results indicated that none of the transfused sicklers were HIV Seropositive. Similarly, none of the control children were HIV seropositive.

From this study, it can be concluded that at the moment the sicklers seen at Kenyatta National Hospital, though subjected to repeated blood transfusions are not at a greater risk of being HIV seropositive than other non transfused children without any other HIV associated risk factors. However, as the blood is associated with other hazards such as Hepatitis B and as the natural history of HIV is still not completely understood, liberal transfusions should be discouraged.
INTRODUCTION

Sickle cell anaemia is an inherited disorder of haemoglobin. The usual mode of inheritance is autosomal recessive. The defect is in the beta chain of haemoglobin where glutamic acid is replaced by valine in position 6 of the amino acid chain \((1)\). The abnormality leads to a haemoglobin molecule which has reduced solubility, and in a state of reduced oxygen tension in the blood, the haemoglobin forms tactoids and hence deformities of red blood cell to a sickled form. The sickled red blood cells are unable to pass through the thin capillary vessels and they are removed from circulation in the spleen and at other reticulo endothelial sites. In severe hypoxia a heterozygous person may behave in a similar manner to a homozygous patient \((1)\).

The initial presentation of sickle cell crisis is usually about 6 months of age \((1)\). Before this age, an infant is protected from haemolytic crises by the high level of foetal haemoglobin, which is synthesized from 2 weeks of embryonic life \((1)\). Foetal haemoglobin level decreases as the infant grows older. However, in some cases there is persistence of foetal haemoglobin into childhood \((1)\).

A child with sickle cell anaemia may present with any of the following crises: haemolytic, thrombotic, megaloblastic, sequestration and aplastic \((1)\).
Any of the crises may be precipitated by hypoxia or infections (1). Children with sickle cell disease are more prone to both bacterial and malarial infections than non sickle cell disease children. This is because they have defective cellular and humoral immunities (1).

The sickle cell trait is prevalent in Kenya. The disease causes high morbidity and mortality in the infants and children (1). The distribution of the trait is not uniform in Kenya. Among the highly prevalent areas are the Western region e.g. (Luo, Luhy) and Coastal regions (Taveta, Digos, Nyika) where the trait is present in over 20% of the population. The trait is not found in the Central and Rift Valley regions (2).

Apparently, the same regions with high prevalence of sickle cell anaemia are the same regions with high malaria endemicity (2). This means that the children suffer from chronic haemolysis caused by sickle cell anaemia and/or malarial infections (2).

Frequent haemolysis from sickle cell anaemia and frequent malaria infection lead to repeated hospitalization, multiple blood transfusion and injections (2). Repeated transfusions have been associated with transmission of the HIV (3,4,5). In developing countries where facilities and resources are poor, proper blood screening and sterilisation of needles may be inadequate as they are reused several times without adequate sterilisation. This is likely to lead to transmission of virus such as HIV (6,7).
The first reported case of AIDS was in the USA in June 1981. Retrospective studies of sera collected before 1981 indicate virus isolation in specimens as far back as 1978. The first reported cases were all from homosexual and bisexual men (6). Later, the disease was noticed to be transmitted through the heterosexual route (6,14,15). In 38 cases reviewed by Piot et al (15) in 1983, all the patients had contacted AIDS through the heterosexual route. They concluded that blood transfusion does not seem to play a significant role in the transmission of AIDS in Africa. The first reported case of AIDS in Kenya was in 1984 (16). Since then many cases of AIDS have been diagnosed (17,18). A study of HIV infection in Nairobi prostitutes showed high seropositivity. The control population in that study was only 2% seropositive (17).

Blood transfusion has already been shown to play a role in HIV transmission (3,4,5). The first few cases of blood related HIV transmission were noticed in 1982 in haemophiliacs receiving commercially prepared factor VIII concentrates (19). Since then, a high percentage of haemophiliacs have become HIV positive. However, only a small percentage has developed clinical AIDS (19). Haemophiliacs treated with only heat-treated factor VIII concentrates show very low rates of seroconversion (6,19,20, 21). A study done locally, indicated HIV seropositivity in 26% of Haemophiliacs (22). Other studies have shown HIV transmission with whole blood transfusions (5). Napier (5) reported 26 cases of HIV infection through blood
transfusion in Britain in June 1987. Mann et al (23) in 1987 also reported higher HIV seropositivity in children with sickle cell anaemia with previous blood transfusion than non transfused patients. HIV seropositivity was reported to be related to the number of transfusions (23). In 1984, Izzia et al (24) reported a case of AIDS in Zaire in a 19 year old man who had sickle cell anaemia and had had multiple blood transfusions. They concluded that sicklers are a high risk group for AIDS (24).

Sicklers, like their counterpart haemophiliacs, have been shown to be at risk of acquiring AIDS through blood transfusions (23,24). Already controlled measures are being applied to reduce transmission of HIV to haemophiliacs. No studies on the relationship of blood transfusion and HIV seropositivity in sicklers have been published in this country. This study was therefore deemed necessary. Also, should there ever be any remedy for HIV, this risk group once identified could benefit from early treatment. Identification of the risk groups is necessary so that alerted health workers can apply appropriate precautions. With this in mind, the author was prompted to look into the seropositivity of HIV in children with sickle cell anaemia who have had previous blood transfusion at Kenyatta National Hospital.
AIM OF THE STUDY

To determine the prevalence of HIV seropositivity in children with sickle cell anaemia who have been transfused.

OBJECTIVES

1. To determine the HIV serological status of transfused sicklers and controls of both non-transfused sicklers and non-transfused normal children.

2. To find out whether frequent blood transfusions in sicklers have exposed them to an increased risk of transfusion-related HIV infection.
The study was carried out at two different time intervals between June 1987 and September 1988 for a total period of 9 months. The first recruitment of the children for the study was between June 1987 and December 1987. A follow-up re-evaluation study of the previously recruited children with an inclusion of new patients was carried out between June 1988 and September 1988. During the re-evaluation period, fresh specimens of blood were drawn from the same children for HIV analysis.

STUDY AREA
The sicklers were recruited from the paediatric haematology clinic and the paediatric wards. The control non sicklers were siblings accompanying the sicklers to the clinic and children attending the paediatric demonstration clinic.

SAMPLE SIZE
Approximately 300-400 children with sickle cell anaemia are being followed up at the paediatric clinics and wards at Kenyatta National Hospital. Two thirds of these children have had previous blood transfusions. The author performed the two assessments between June 1987 and September 1988. The first assessment was between July 1987 and December 1987. The final assessment was between June 1988 and September 1988. A total of 429 children were assessed comprising of 198 transfused sicklers, 106 non transfused sicklers and 125 non transfused normal children. Sixty five of the previously assessed transfused sicklers were rechecked during the second recruitment period by taking fresh blood samples for HIV analysis.
INCLUSION AND EXCLUSION CRITERIA

INCLUSION

(i) The age range of both the studied children and the controls was 1-12 years.

(ii) The transfused sicklers had to have had a transfusion at least 6 months prior to recruitment and between 1982 and 1987.

(iii) An attempt was made to match the studied children and the controls with respect of age and geographical origin of the parents. For the purpose of this study, the home district was regarded as the geographical origin as opposed to residential status.

EXCLUSION

(i) All non sicklers who had had a previous blood transfusion.

(ii) All children with severe systemic disorders such as pneumonia, meningitis, neoplasms, protein calorie malnutrition and already diagnosed to have HIV infection.

(iii) All sicklers with only one blood transfusion received less than 6 months prior to this study.
Reviews of the recruited children was carried out at the paediatric haematology clinic 1 week after enrollment. Treatment was also offered at the time of review in instances where a treatable medical condition was detected during the investigation.

ETHICAL CONSIDERATIONS

A written consent was obtained from Kenyatta National Hospital ethical committee. Also a verbal consent was given by the parents or guardians of the children.

DATA AND SPECIMEN COLLECTION

The author attended the haematology clinic every Monday morning. The first 5 sicklers to arrive at the clinic and met the inclusion criteria were recruited for the study. Similarly, all the sicklers who were admitted in the paediatric wards during this study period were included during a routine daily visit to the wards. The non sicklers were recruited from the paediatric demonstration unit every Friday morning. The first 5 children to arrive at the clinic and matched the sicklers for age, sex and if their parents were coming mainly from Western part of Kenya were entered for the study. A few normal children were recruited from siblings accompanying the sicklers to the clinic.
A general medical examination was carried out in all the children and recorded on a questionnaire which is shown in Appendix 1.

Using a sterile procedure, 5 mls of blood was collected from a peripheral vein. Two mls of the blood was put in one sequestrene bottle and sent to the Haematology department of Kenyatta National Hospital for analysis of haemogram, malaria parasites and Hb electrophoresis. three mls was taken to the paediatric department where it was centrifuged and the serum stored at -70 degrees centigrade and was later transferred to KEMRI for HIV analysis. All the sera collected were taken to KEMRI within one week of collection.

The outline of the investigations carried out on the samples of blood collected are shown below.

a) Haemogram and Peripheral blood film

The haemogram was carried out using a coulter counter model 'S'IV Plus.

The differential white blood cells counts and malarial parasites were done manually by staining a thin blood film with May Grunward Giemsa stain and the film was reported using light microscope as outlined in "Practical Haematology" by Dacie et al (25).
This was carried out using paper electrophoresis as outlined by Dacie et al (25).

c) HIV serologic tests using ELISA and Western Blot Techniques

i) ELISA test. Using Wellcozyme anti-HIV Kit.

Microwells are coated with chemically inactivated antigen from HIV grown in culture. The antigen is captured specifically into purified anti-HIV previously immobilized in the wells. Test samples or control sera are incubated in the wells with anti-HIV chemically conjugated to the enzyme horse radish peroxidase (the conjugate). Competition of binding to the immobilized antigen occurs between antibodies to HIV in the sample of control serum and the conjugate; a specimen containing antibody to HIV will block the binding of the conjugate while specimens not containing antibody to HIV will allow binding of conjugate to occur. After thorough washing of the wells to remove the samples and excess conjugate the enzyme remaining bound to the wells is visualised using 3, 3′, 5, 5′-tetramethyl benzidine and hydrogen peroxide to give a yellow colour after termination of enzymic reaction with sulphuric acid. The amount of conjugate, and hence the colour, remaining in the wells is inversely related to the concentration of antibody to HIV in the sample.
There were no ELISA positive HIV children in this study and hence the Western Blots were not carried out.

**DATA ANALYSIS**

The data obtained was analysed using analysis of variance, unpaired t-test and chi square. There were 65 children whose blood samples were analysed for HIV at two different time intervals. However, their HIV status remained negative. Thus, during the final data analysis they were entered once for the study.
RESULTS

A total of 429 children comprising of 198 children with sickle cell anaemia who had had previous blood transfusions, 106 children with sickle cell anaemia who had had no previous blood transfusions and 125 non sicklers who had not had previous blood transfusions were recruited into the study.
Table 1 shows distribution of children according to their origin in Kenya. The table indicates that 219 out of 304 (72%) of children seen at Kenyatta National Hospital with sickle cell disease come from Nyanza province, 78 out of 304 (26%) from Western province and 7 out of 304 (2%) from the rest of the country. There was no significant difference in the distribution of children by home area district in the three groups of children.

TABLE 1

<table>
<thead>
<tr>
<th>Column</th>
<th>District</th>
<th>SCA</th>
<th>Transfused</th>
<th>A</th>
<th>SCA non</th>
<th>Transfused</th>
<th>B</th>
<th>Hb AA non</th>
<th>Transfused</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIYA</td>
<td>70</td>
<td>36</td>
<td>51</td>
<td>157</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KISUMU</td>
<td>51</td>
<td>19</td>
<td>20</td>
<td>90</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOUTH NYANZA</td>
<td>28</td>
<td>15</td>
<td>17</td>
<td>60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUSIA</td>
<td>20</td>
<td>19</td>
<td>9</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KAKAMEGA</td>
<td>14</td>
<td>13</td>
<td>19</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUNGOMA</td>
<td>10</td>
<td>2</td>
<td>6</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OTHERS</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>198</td>
<td>106</td>
<td>125</td>
<td>429</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chi Square 3.05  DF 2  Significance 0.22
Table 2 shows that over 92% of the parents were married and the couples were staying together. There was no appreciable difference between the study and the control children.

<table>
<thead>
<tr>
<th>MARITAL STATUS</th>
<th>SCA with Transfusions</th>
<th>A SCA Non Transfused</th>
<th>B Hb AA non Transfused</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Married</td>
<td>95.9</td>
<td>88.2</td>
<td>96.0</td>
<td>92.2</td>
</tr>
<tr>
<td>Single</td>
<td>0</td>
<td>3.9</td>
<td>2.0</td>
<td>1.9</td>
</tr>
<tr>
<td>Divorced</td>
<td>1.0</td>
<td>3.8</td>
<td>0</td>
<td>0.9</td>
</tr>
<tr>
<td>Widowed</td>
<td>3.1</td>
<td>3.9</td>
<td>2.0</td>
<td>2.8</td>
</tr>
<tr>
<td>Total</td>
<td>46.1</td>
<td>24.7</td>
<td>23.2</td>
<td>100</td>
</tr>
</tbody>
</table>
The table shows that though the males were slightly more than the females, the ratio of M:F was 1.07:1 in all the groups of children in the study.

**TABLE 3**

<table>
<thead>
<tr>
<th>Gender</th>
<th>SCA with Transfusions</th>
<th>CONTROLS</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SCA with no transfusion</td>
<td>Hb AA non transfused</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>98</td>
<td>49</td>
<td>61</td>
<td>208</td>
</tr>
<tr>
<td>Males</td>
<td>100</td>
<td>57</td>
<td>64</td>
<td>221</td>
</tr>
<tr>
<td>Total</td>
<td>198</td>
<td>106</td>
<td>125</td>
<td>429</td>
</tr>
</tbody>
</table>

**HOSPITAL ADMISSIONS**

The transfused sicklers had 2.9 hospital admissions per child, while the non transfused sicklers had 1.2 admissions per child. The non sicklers control group had not had any hospital admissions.
THE PATTERN OF BLOOD TRANSFUSIONS IN THE TRANSFUSED SICKLERS

From the table below it can be seen that the range of transfusions in the study population ranged from 1 - 13. The average number of transfusions per child is 2.4. The majority of the transfusions had occurred between 1982 and 1987.

<table>
<thead>
<tr>
<th>Number of transfusions</th>
<th>Number of Children</th>
<th>Total Number of transfusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>2</td>
<td>49</td>
<td>98</td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td>99</td>
</tr>
<tr>
<td>4</td>
<td>22</td>
<td>88</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>≥ 6</td>
<td>10</td>
<td>94</td>
</tr>
<tr>
<td>Total</td>
<td>198</td>
<td>479</td>
</tr>
</tbody>
</table>

Average number of transfusions = 2.4

Range of transfusions = 1 - 13 times.
Table 5 stresses the well known fact that children with sickle cell anaemia have a significantly lower haemoglobin level than other normal children. Among the sicklers, there is no significant difference in the haemoglobin levels whether the children are transfused or not. The low haemoglobin levels in the sicklers is attributed to chronic haemolyses. Although the significance of carrying out haemoglobin levels in sicklers may not be very apparent at the moment, in future studies, if there will be an increase in HIV in sicklers, the haemoglobin levels are likely to decrease further as a result of combined haemolyses from repeated infections and also due to autoimmune anaemia and thrombocytopenia which have been shown to be features of HIV.

<table>
<thead>
<tr>
<th>Group</th>
<th>Count</th>
<th>Mean</th>
<th>SD</th>
<th>SE</th>
<th>95% Conf. for Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCA Transfused</td>
<td>198</td>
<td>7.43</td>
<td>1.80</td>
<td>0.13</td>
<td>7.17 - 7.69</td>
</tr>
<tr>
<td>SCA Non Transfused</td>
<td>106</td>
<td>7.84</td>
<td>2.12</td>
<td>0.21</td>
<td>7.42 - 8.26</td>
</tr>
<tr>
<td>Non SCA Transfused</td>
<td>125</td>
<td>10.72</td>
<td>2.39</td>
<td>0.22</td>
<td>10.28 - 11.16</td>
</tr>
<tr>
<td>Total</td>
<td>429</td>
<td>8.45</td>
<td>2.54</td>
<td>0.12</td>
<td>8.21 - 8.79</td>
</tr>
</tbody>
</table>

P Value $\leq 0.01$
THE AVERAGE TOTAL WBC IN THE 3 GROUPS OF CHILDREN (CORRECTED FOR NORMOBLASTS).

Table 6 shows that the children with sickle cell anaemia had a higher average total white blood cell counts even when corrected for normoblasts than non sicklers. Among the sicklers, the transfused group had a slightly higher average total white blood cells than the non transfused group. This was not statistically significant. The increased number of white blood cells observed in the sicklers is due to the marked bone marrow hyperplasia which is a feature of the condition.

TABLE 6

<table>
<thead>
<tr>
<th>Group</th>
<th>Count</th>
<th>Mean</th>
<th>SD</th>
<th>SE</th>
<th>95% Conf. for Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCA Transfused</td>
<td>198</td>
<td>20.27</td>
<td>9.94</td>
<td>0.70</td>
<td>18.87 - 2.67</td>
</tr>
<tr>
<td>SCA Non Transfused</td>
<td>106</td>
<td>17.53</td>
<td>10.08</td>
<td>1.01</td>
<td>15.51 - 19.53</td>
</tr>
<tr>
<td>Non SCA Non Transfused</td>
<td>125</td>
<td>8.47</td>
<td>2.26</td>
<td>0.27</td>
<td>7.93 - 9.01</td>
</tr>
<tr>
<td>Total</td>
<td>429</td>
<td>16.31</td>
<td>9.76</td>
<td>0.48</td>
<td>15.35 - 17.27</td>
</tr>
</tbody>
</table>

P Value ≤ 0.01
THE AVERAGE DIFFERENTIAL NEUTROPHILS, LYMPHOCYTES, EOSINOPHILS, MONOCYTES AND BASOPHILS COUNT IN PERCENTAGES AS RELATED TO THE TOTAL NUMBER OF WHITE BLOOD CELL COUNTS.

There was no statistically significant difference observed as regards neutrophils, lymphocytes, eosinophils, monocytes and basophils counts in the 3 groups of the children in the study.

MALARIA PARASITES

Malaria parasites were reported in 10% of the 429 children. The children with malaria parasites in their peripheral film comprised of 10 previously transfused sicklers, 8 non transfused sicklers and 18 non sicklers.

HIV SEROPOSITIVITY AS CONFIRMED BY WESTERN BLOT

All the transfused sicklers and the control non transfused children were HIV seronegative on duplicate wellcozyme ELISA technique. It was thus not necessary to carry out Western Blot Method as there were no ELISA positive sera.
DISCUSSION

The majority of children with sickle cell disease come from Western part of the country. As this is a very common problem in this part of the country, sometimes children presenting with signs and symptoms resembling sickle cell anaemia have been diagnosed and treated for sickle cell anaemia without a confirmatory Hb electrophoresis. In the pediatrics general haematology clinic there is no proper register of sicklers and their records are lumped together with files of children with other haematological disorders. In many of these children's files, Hb electrophoresis results were found missing. It was therefore found necessary to perform Hb electrophoresis in most of the recruited children. A few of the children who had been followed in the clinic as sicklers were found not to have sickle cell disease. Another observation which was made during the study was that the majority of the sicklers who are being followed at the clinic are not residing in Nairobi. Thus most of them travel long distances to attend the clinic in Nairobi.
Multiple blood transfusion has been shown to be a route of HIV transmission (3,4,5). The first blood product associated with an AIDS case was in a haemophiliac patient in 1982 (19).

Further studies in the haemophiliacs revealed that in severe form of haemophilia, over 75% of them have developed HIV antibodies (8,19,20,21,22). Children living in the same environment as haemophiliacs have been shown to be HIV negative if there are no other associated factors (26). Observations from this study indicate that it is very unusual for the virus to be spread horizontally (26). An on-going study on haemophiliacs at Kenyatta National Hospital by Dr. Kitonyi et al (22) indicates that 26% of haemophiliac A are HIV positive as confirmed by the Western Blot. In the general population in Kenya, the HIV seropositivity is less than 1%; the high HIV seropositivity in haemophiliacs has been attributed to transfusions with factor VIII concentrates especially from paid up donors (22).
Whole blood transfusion related HIV have now been shown to occur. In 1986, Church et al (4) reported 36 cases of AIDS related to whole blood transfusion in children. 24 out of these children were below the age of 24 months. However, although all those children had received transfusions, the HIV seropositivity of parents which was crucial in determining whether some of them may have acquired HIV through vertical transmission has not been elaborated. The incubation period between transfusion and HIV antibodies appearance in the recipient blood has been estimated to be 6 weeks to 2 months (5). However the incubation period of AIDS from transfusion could be as long as 5 years (5,13). A study at Mana Yemo Hospital in Zaire by Mann et al (23) reported a higher HIV seropositivity in children with sickle cell disease and previously transfused than other children in the hospital without sickle cell disease. Overall 17% of SCD children with previous transfusion were HIV positive. Compared to 11% of other hospitalised children and only 1% of healthy children (23,27). HIV seropositivity was associated with an increased number of transfusions (23,27).

Izzia et al (24) also identified sicklers as a high risk group by reporting a case of HIV in a 19 year old male sickler who had had multiple blood transfusions. However they ignored the fact that this was a mature man and could also have got HIV through heterosexual route which is the main route of infection in Africa (15). This makes their conclusion misleading.
In this study, the transfused sicklers and the controls did not differ significantly in their home of origin, sex distribution of children and the marital status of their parents. The haematological investigations underlined the already known fact that the sicklers have lower haemoglobin levels and higher total white blood cells than the normal Hb AA children. There was no statistical significant difference observed in the percentage differential white blood cells counts in the study and the two controls. Unlike HIV infected children who may have pancytopenia (4) the sicklers had a high number of white blood cells.

In this study, malaria parasites were reported in 10% of the children. However, there were no HIV positive cases identified. Hence the correlation between malaria parasites and HIV as previously reported by Wendler et al (14) in 1986 on seroepidemiology of HIV in Africa could not be established. This study collaborates earlier findings by Greenbert et al (29) which failed to establish any correlation between HIV antibodies and presence of malaria parasites in blood.
The previously transfused sicklers had the highest rate of hospitalization followed by the non transfused sicklers. The majority of non sicklers had not had any hospital admission. This implies that, transfused children had a higher morbidity rate requiring hospital admissions than the non transfused children. If the rate of blood transfusion transmitted HIV is related to the number of transfusions, the sicklers would have been expected to be at higher risk of HIV than the controls of the non transfused children. This was not found to be true in the study. Some of the explanations of the findings would be that, unlike in Zaire where HIV seroprevalence in the general population is extremely high (23,27), in Kenya, the prevalence of HIV is still low (14,31). Therefore, the risk of transfusing a child with infected blood is still quite low. The sicklers have also been shown to have low haemoglobin levels. Any of the condition that would depress their haemoglobin level further is likely to make them have more blood transfusions and admissions. AIDS has already been shown to cause autoimmune related anaemia and thrombocytopenia (4). Before one can establish that the blood transfusions have led to the increased HIV in the sicklers, this issue should be explored. This can only be done by following up the sicklers who have not yet been transfused and have been shown to be HIV negative. So far, the evidence from this study indicates that transfusions have not led to increased risk of HIV in the sicklers.
The other possible reasons for the difference in HIV seropositivity in the sicklers in this study and high rate in Zaire is that unlike Zaire where the sicklers had a high average rate of transfusion (5.8 per child transfused) (23,27), in the studied transfused sicklers here, the transfusion rate was low (2.4 per child transfused). This means that the sicklers in Zaire were from higher HIV donor population and also from a higher transfusion rate per child than Kenya. Low transfusion HIV in Kenya could also be due to the fact that there are no paid up donor as blood donor services still depend on volunteers and relative donors (31). It is also a well known fact that prostitutes and other high risk HIV population are unlikely to donate blood frequently unless there is a monetary gain. The volunteer donors are likely to be at a lower risk of having HIV than the paid up donors. The future of HIV rates in the sickler will depend on the number of HIV infected people in the population and the effectiveness of blood screening for HIV. A bulletin published by the Kenya National AIDS Control Programme indicates that by 1981 the HIV seropositivity in Nairobi prostitutes was 4%. By 1985 the percentage had increased to 59% (31). In 1981 the report indicates that there were 0% infection rate in pregnant women. This rate had increased to 2% in 1985 (31). By 1987 the overall HIV rate in the blood donors was 1.7%(31).
With the increasing number of HIV positive people in the population, blood donors who are HIV positive are also likely to increase (31). This may lead to appearance of cases of transfusion related HIV. Sicklers as one of the group of children requiring frequent blood transfusion are likely to be affected. But as it is at the moment, the sicklers should not be isolated as a high risk group. With increased coverage facilities for screening of all the blood donated for HIV, transfusion-related HIV will be minimised.

Since 1987, most blood banks in Kenya have started screening blood for HIV. This has been intensified in 1988 (31). This will help to filter any detected HIV contaminated blood. It may also help to halt the epidemic of transfusion HIV.

Unfortunately, a few cases of transfusion HIV have been reported in patients who have been transfused with HIV negative donors blood who have later seroconverted (32). Similarly, false positive HIV serologic tests have been reported (33,34,35). This is likely to complicate the complete prevention of HIV by blood transfusion. It is therefore advisable to transfuse a patient only when it is completely necessary.

Finally, although this study has shown no transfusion-related HIV exposure in our sicklers, this situation is likely to change in future with the increase in HIV in the general population. It is even likely to be complicated further by the rise in the number of vertically transmitted HIV (36,37,38). The number of HIV infected expectant mothers has been shown to be on the increase (31).
So far the complete natural history of HIV has not unfolded. To minimise the rise in the number of HIV infection in the children, the current screening of blood and campaigns on HIV should be sustained and enhanced.
CONCLUSIONS

1. The average number of transfusions from birth of 2.4 per sickler seen at Kenyatta National Hospital was far much lower than that of 5.8 per sickler reported at Mama Yemo in Zaire. Whether the low transfusion rates among our sicklers was contributing to the very low HIV seroprevalence and the high rate of transfusions of sicklers in Zaire was responsible for high HIV seroprevalence could not be established.

2. All the sicklers and normal children in this study were seronegative to the ELISA HIV test. This means that at the moment, the sicklers at Kenyatta National Hospital, although they have had frequent blood transfusions, have not been at a higher risk of being HIV seropositive than other normal children.
RECOMMENDATIONS

1. HIV in general population has been shown to be increasing and blood donors with HIV contaminated blood are likely to increase leading to increased transfusion-related HIV. There is also the incubation period before seroconversion to HIV. It is difficult to tell whether the seronegative children will seroconvert later. A follow-up study on the sicklers is necessary to re-evaluate whether they will remain negative.

2. Since at the moment blood transfusion has not been shown to be a very important route of HIV infection in our sicklers, it is necessary and important to explore other routes of HIV infection for any sickler who presents with a positive HIV serology and has had blood transfusion, before associating the HIV to the transfusion as this could be an incidental finding and not necessary as a result of the blood transfusion.

3. The government policy of establishing HIV screening facilities at provincial and district levels is very important and should be sustained. This will help to filter most of HIV contaminated blood and also provide a relatively safe blood transfusions for all patients and especially sicklers who may require more frequent transfusions.
4. It is very important for sicklers and all those who may require frequent transfusions that the National Health Education programme geared towards all aspects of the HIV disease should be sustained and enhanced so that all the high risk positive HIV individuals are discouraged from donating blood in order to minimise transfusion related HIV.

5. As the natural history of HIV has not completely unfolded, unnecessary blood transfusions to the sicklers and any other patients should be discouraged. Health workers should continue taking all the necessary precautions while dealing with any potential HIV risk groups.
ACKNOWLEDGEMENTS

I would like to express my deep appreciation to the following:-

1. My three supervisors G.W. Kitonyi, D.W.M. Kinuthia and J.S. Meme for their guidance during the study period and also for reading the manuscript and making the necessary corrections.

2. All those children and their parents who agreed to participate in the study.

3. Kenya Medical Research Institute and especially Susan Oogo and David Libondo for carrying out the HIV serology analysis on all the children recruited.

4. Personnel working at Haematology Department - Kenyatta National Hospital for analysis of the blood for full haemogram, malarial parasites and Hb electrophoresis. Special thanks to F. Waigwa, B. Kahinga who analysed Hb electrophoresis in all the children.

5. A Khadudu, J. Kioni and T. Mbithi from the paediatric department - University of Nairobi, for assistance in centrifuging the specimen for HIV analysis.

6. Miss Bibiana M. Kamau of B.A.T Kenya Limited for her excellent secretarial work.

7. Mr. J. N. Kiragu of B.A.T Kenya Limited for proof reading the manuscript.

8. Last but not least, all those individuals who have contributed to this study in one way or another, in form of suggestions, criticism and materials.
REFERENCES

1. Song, J.
Pathology of sickle cell disease.

2. Foy, H. and Kendall, A.G.
Health and Disease in Kenya.

3. Perkins, A.H., Samson, S., Garner, J.,
Echenberg, D., Allen, J.R., Cowan, M. and Alevy, J.
Risk of AIDS for recipient of blood components from
Donors who subsequently developed AIDS

4. Meerstadt, D. and Brueton, M.J.
Paediatric AIDS.

5. Napier, J.A.
AIDS and blood transfusion.

6. Acquired Immune Deficiency Syndrome —
an assessment of the present situation
in the world. Memorandum from a WHO meeting.

7. Geddes, A.M.M.
Risk of AIDS to health care workers.

8. Youle, M.
AIDS series - current treatment and future prospects.

9. Merz, B.
HIV vaccine approved for clinical trials (News).

10. Ezzel, C.
Trials of vaccine against AIDS to begin in humans (News).

11. Newmark, P.
U.K. edges slowly towards first trials of an AIDS vaccine
(News)

12. Jones, P., Hamilton, P.J., Bird, C.,
Fearns, M., Oxley, A., Tedder, R.,
Cheingsoneg-Popov, R. and Codd, A.
AIDS and haemophilia; morbidity and mortality
in a well defined population.
13. Hilgartner, M.W.
AIDS in the transfused patient.

14. Wendler, I, Scheider, J., Gras, B.,
Fleming, A.F., Hunsmann, G. and Schmit, Z.H.
Seroepidemiology of human immunodeficiency
virus in Africa.

15. Piot, P., Quinn, T.C., Taelman, H.,
Acquired Immunodeficiency Syndrome in a heterosexual
population in Zaire.

16. Obel A.O.K., Shariff S.K., McLigeyo, S.O.,
Gitonga E, Shah M.V. and Gitau W.
Acquired Immunodeficiency Syndrome in an African.

17. Kreiss, J.K., Koech, D., Plummer F.A.,
Holmes, K.K., Lightforte, M., Piot, P.,
Ronald, A.R., Ndinya-Achola, J.O., Da Costa,
L.J.D., Roberts, F., Ngugi, N.N.
and Quinn, T.C.
AIDS virus infection in Nairobi prostitutes

18. Biggar, R.J., Johnson, B.K., Osler, D.,
Sarin, P.S., Ochiong, D., Tukei, P., Nsanze, H.,
Alexander, S., Bodner, A.J., Siongok, T.A.,
Gallo, C.R. and Blattner, W.A.
Regional variation in prevalence of antibody against
human T. Lymphotrophic virus type I and III in Kenya.

19. Aledort, L.M.
The current status of transfusion therapy.

20. Goedert, J.J., Sarnagadharan, M.G.,
Eyster, M.E., Weiss, S.H., Bodner, A.J.,
Gallo, R.C. and Blattner, W.A.
Antibodies reactive with Human T. Cell
leukaemia viruses in the serum of haemophiliacs
receiving factor VIII concentrate.

21. Lilleyman, J.L.
Haemophilia, Blood transfusion, and the AIDS virus.

22. Kitonyi, G.W., Bowry, T. and Kasili, E.G.
HIV studies in Kenyan patients with congenital bleeding
disorders.
Proceedings from the 11th International Conference on AIDS,
Gally, P.N., Kayember, K., Mann, J.M., Ryder, R.W., Mbasa, M., Francis, H., et al.

HIV infection in African children with sickle cell anaemia.


Izza, K.W., Lepira, B., Kayembe, K., Odio, W.,

Acquired Immune deficiency (AIDS) and sickle cell anaemia.


Dacie, J.V. and Lewis, S.M.


Berther, A., Fauchet, R., Genefet, N., Pommerenil, Chamaret, S., Fonlupt, J., Bueguen, M., Ruffault, A.

Transmissibility of HIV in haemophiliac and non hemophiliac children living in a private school in France


HIV seroprevalence in paediatric patients 2 - 14 years of age at Mama Yemo Hospital, Kinsasha, Zaire.


ELISA HTLV retrovirus antibody reactivity associated with malaria and immune complexes in healthy Africans.


Evaluation of serological cross reactivity between antibodies to Plasmodium and HTLV - III/LAV.


Natural history of human immunodeficiency virus infection in Zaire.


The National AIDS Control Programme of Kenya

A five year plan for AIDS control 1987-1991

A bulletin from the Ministry of Health, Nairobi, Kenya.
32. Espanol, T., Garcia-Armui, R., Bofill, A., Suna, J. and Bertan, J.M.
Hypogammaglobinaemia and negative HIV antibodies in AIDS.

33. Thorpe, R., Bird, C., Carret, A.J., Minor, P.D.,
Schild, G.C. and Thomas, D.P.
False - positive immuno blot results with antibodies
against human immunodeficiency virus.

34. Vanderpoel, C.L., Reesink, H.W., Telie, TH., Husman, M
and Miedema, F.
Blood donations reactive for HIV in Western Blot,
but non infective in culture and recipients of blood.

35. Michail-Marianov, V., Tzivaras, A., Piperi-Lowes, L.,
Kattamis, C., Ladasv and Papadakov, R.
False - positive HTLV III antibody tests in
multitransfused patients with thalassaemia.

36. Meerstadt, D and Brueton, M.J.
AIDS Series - Paediatric AIDS

37. Lifson, A.R. and Rogers, M.F.
Vertical Transmission of human immunodeficiency virus.

38. Sprecher, S., Soumenkoft, G., Puissant F. and
Degueldre, M.
Vertical transmission of HIV in a 15 week foetus.
## APPENDIX 1

### QUESTIONNAIRE FOR SCD WITH MULTIPLE TRANFUSION IN RELATIONSHIP TO HIV SEROPOSITIVITY STUDY

<table>
<thead>
<tr>
<th>COLUMN</th>
<th>VARIABLES</th>
<th>CODES</th>
<th>INTERPRETATION OF CODE</th>
</tr>
</thead>
</table>
| 1      | Group     |       | 0) SCD Transfused  
1) SCD Non Transfused  
2) Normal children without transfusion |
| 2 - 4  | Individual Patients Number |       |                        |
| 5      | Recruitment |       | 0) Paediatric ward  
1) Paediatric Haematology clinic,  
2) Paediatric demonstration clinic |
| 6 - 8  | Age to nearest 0.5 years |       |                        |
| 9      | Sex       |       | 0) Female  
1) Male |
| 10     | Home District |       | 0) Siaya  
1) Kisumu  
2) South Nyanza  
3) Busia  
4) Kakamega  
5) Bungoma  
6) Others |
| 11     | Parents marital status |       | 0) Married  
1) Not married  
2) Divorced  
3) Widowed |
| 12     | Hb Electrophoresis |       | 0) AA  
1) AS  
2) SS  
3) SF |
<p>| 13     | Number of admissions to hospital |       |                        |
| 14     | Number of transfusions |       |                        |</p>
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<tbody>
<tr>
<td>15 - 17</td>
<td>Hb to the nearest 0.1 gm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 - 20</td>
<td>WBC to nearest 0.1</td>
<td></td>
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</tr>
<tr>
<td>21 - 22</td>
<td>% Lymphocytes count</td>
<td></td>
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</tr>
<tr>
<td>23 - 24</td>
<td>% Neutrophils</td>
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<tr>
<td>25</td>
<td>% Monocytes</td>
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<td></td>
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<tr>
<td>26 - 27</td>
<td>% Eosinophils</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>% Basophils</td>
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</tr>
<tr>
<td>29</td>
<td>Malarial Parasites</td>
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<tr>
<td>30</td>
<td>Wellcozyme Elisa Test</td>
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<tr>
<td>31</td>
<td>Organon W Blot</td>
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<tr>
<td>0) Absent</td>
<td>1) Present</td>
<td>2) Not done</td>
<td></td>
</tr>
<tr>
<td>0) Negative</td>
<td>1) Positive</td>
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<tr>
<td>0) Negative</td>
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