A CONTROLLED STUDY OF THE ROLE OF BLOOD TRANSFUSION IN CAUSING HEPATITIS IN THE PAEDIATRIC POPULATION IN KENYA.

By

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DECLARATION

I declare that this work was entirely mine (except where stated) the study was carried out by myself and that I have not submitted it to any other University.

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I declare that this work was carried out by the student and has been submitted to the University with my approval.

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SUMMARY

263 children from Luhya and Luo communities in Nairobi, were studied for Serum SGPT levels and HBV markers, to compare transfused groups with non transfused groups with SCD and those having Hb AA. Sickle cell tract (AS) children were also employed as controls.

Among the transfused groups, the prevalances of asymptomatic liver damage was found to be significantly higher (37%) than that of the non transfused children (10%). ($p<0.05$). The majority of hepatitis B surface antigen positive children (over 60%) in the whole study had elevated SGPT levels. This association was more evident in the transfused groups. These findings are contrary to the known fact that majority of adult surface antigen positive carriers have normal LFTs.

The infectious pool for HBV was found to be higher in transfused than non transfused children, whether they are sicklers or not.

The development of sterile immunity to HBV infection in both sicklers and non sicklers occurs far less frequently in transfused group as compared to non transfused controls.

CONTENTS

LIST OF TABLES

INTRODUCTION

Blood transfusion is a common procedure in medical and surgical practice. However, the use of blood or its products carries certain risks. Chronic liver disease is a well known long term complication.

Ndinya-Achola et al (1) in their study of some of the infectious hazards of blood transfusion in Nairobi placed viral hepatitis second only to Malaria, with 3.7% and 27% risks respectively. Bowry et al (2), have estimated 10-14% of volunteer blood donors to be definitely infectious with hepatitis B Virus (HBV) to the non-immune. This figure rises to 37%, if high titres of core Antibody is acceptable as a marker of active infection. The marked differences in two reports from same country are attributed mainly to use of more sensitive lab assays in the latter study.

Sang (3), reviewed liver pathology in 30 sickle cell patients seen over 10 years, and reported macronodular Cirrhosis in 40% of them. He blamed serum hepatitis transmitted by blood transfusions which these patients had received for his findings. But Lesi et al (4), using complement fixation tests, reported HBsAg prevalence of 4.5% among Nigerian sickle cell patients, most of whom had been transfused. This was not significantly different from that of the general population, (5%). They concluded that blood transfusion was not important in transmitting HBV infection among sickle cell patients. They postulated that other factors such as mosquitoes and close personal contact, which were equally present in the general population, were more important.

Kujwalire et al (5), in Dar-es-Salaam using CIEP method reported the prevalence of HBV surface antibody in adults with sickle cell trait to be significantly higher than that of normal haemoglobin individuals.

A high incidence of hepatitis and chronic liver disease has also been reported in haemophiliacs, another group of patients who frequently receive blood and its components (19).

In developed countries, post transfusion hepatitis, develops in approximately 7% of recipients despite the routine screening of donor for HBV employing third generation tests (6).

Post transfusion viral hepatitis is caused mainly by two agents: Hepatitis B Virus and non A, non B Virus. HBV can be diagnosed by Serological tests, while non A non B has no specific serological tests. In developed countries the use of third generation serological methods to screen donor blood, has greatly reduced (but not eliminated) post transfusion hepatitis B. It now accounts for 10-15% of cases; non A non B constitutes the remainder. Non A non B is usually asymptomatic, but often runs a prolonged course with persistent or intermittent elevation of SGPT levels.

In Kenya the CIEP method is routinely used to screen donor blood for HBV. Although it is cheap, it is relatively insensitive.

* Counterimmune Electrophresis

It allows many infected donors to escape recognition and hence its exclusion. It is therefore important to study the true incidence of post transfusion hepatitis locally, especially in Paediatric population where the non-immune pool is likely to be higher than in adults.

Developing countries have high carrier rates of HBV even in absence of blood transfusion (7,8). A high incidence of chronic liver disease in Kenyan children has been reported by Bowry and Cameron (9). Viral hepatitis is one of the proven causes of acute and chronic liver disease. It has also a strong association with primary hepatocellular carcinoma. (5,10,11,15)

In Kenya sickle cell disease occurs predominantly in tribes from the Western and Coastal regions. Sicklers tend to be chronically ill and frequently require blood transfusion to correct anaemia. Blood transfusion is an important route for HBV transmission, especially in U.S.A., Europe, and Australia, but not the only one. Infection may also be acquired through the skin, the oral route and close personal contact as may occur in crowded conditions (7,8). The role of blood transfusion in causing hepatitis has not been evaluated before in Kenya. It was to fulfill the need for such, that this study was organised.

AIMS AND OBJECTIVES

This study aimed at investigating:

The safety of blood transfusion in the paediatric population, including sickle cell patients in causing liver cell damage and HBV transmission.

MATERIALS AND METHODS

PATIENTS

263 children of Luo and Luhya communities living in Nairobi were selected for the study. Children from the same community were selected to eliminate genetic and environmental variations. They satisfied the following criteria:

1) They were all over seven months old, since maternal IgG persists for up to six months which could confuse the picture (12).

2) They did not have any medical problems associated with increased HBV secretion, such as malignancy, Down's Syndrome, Insulin dependant Diabetes, or Immuno-Suppressive therapy (13,14,15).

3) They were not on drugs known to elevate SGPT, such as phenothiazines, barbiturates, Epamutin , or cytotoxic drugs. They had no history of anaesthesia and they were well nourished.

4) Those transfused had received blood three months or earlier. This is the average incubation period of HBV (13,14,15,16).

On contact with the patient, history was taken, and physical examination performed and recorded (Appendix I). All the children with haemoglobin AA regardless of transfusion status and AS were clinically within normal limits. The 59 SS transfused and the 48 SS non transfused children had some or all of the following signs:- hand and foot syndrome, bone tenderness, jaundice, anaemia, fever abdominal tenderness and hepatosplenomegaly.

LABORATORY METHODS

Investigations relevant to patient management were carried out where necessary. Otherwise for the purpose of this study 8 mls of blood were drawn and distributed as below:

1. 2 mls in a squestrene bottle for Hb electrophresis. This was gently shaken to prevent clotting.
2. 4 mls in a Universal Sterile container for HBV serology.
3. 2 mls in a plain sterile bottle for SGPT.

Hb Electrophresis was done in Haematology Laboratories, Department of Human Pathology, by the Cellulose Agar Paper Electrophresis (CAPE) on all children whose haemoglobin genotype was unknown.

Serology for HBV markers: Sera was obtained by Centrifuging whole blood at 1500 RPM for 5 minutes. The serum was removed and alliquated. 2 mls were stored at $-2^{o}C$ and 1 ml at $4^{o}C$ till testing time. The tests were carried out in Immunology Laboratories Department of Human Pathology. HBsAg was tested for on Sera from 263 children by the Passive Haemagglutivation method (PHA) using Wellcome's Hepatest Kit (Appendix II). HB core antibody and HB surface antibody were tested for on a representative sample from each control group, chosen by random sampling numbers, as the test is extremely expensive.

The standard Radioimmunoassay (RAI) methods described in Appendix II, USAB and CORAB respectively, of Abbot Laboratories were used.

SGPT:

was done in Department of Paediatrics Laboratory on Sera from all 263 children. . The serum was prepared by centrifuging whole blood at 1500 RPM for 5 minutes.

Specimens were examined with the naked eye and discarded if found haemolysed. Where delay was inevitable serum was stored in the dark for a maximum of 48 hours. SGPT was estimated by the Reitman - Frankel method using Instruction sheets for Manual Assays "77 of Boheiringer Manheim - Diagnostica Kits. Although other LFT's were useful in excluding other diseases, only SGPT results were presented in this study because:

a) Bilirubin especially the indirect is usually high in sickle cell disease and sometimes the trait because of haemolysis (13,14,15).

b) Alkaline phosphates, besides liver disease, are also elevated in bone disease, pancreatic disease, and growing children.

c) SGPT is the earliest enzyme to rise in viral hepatitis and is more specific for liver cell damage than SGOT.

Out of 280 children who were recruited for the study, 17 were dropped because they did not satisfy some of the criteria mentioned earlier.

The 263 who were left in the study were categorized as below:

- 59 children with sickle cell disease and previously transfused SS(T).
- 48 with sickle cell disease without previous transfusion SS (NT).
- 57 children with sickle cell trait, not previously transfused AS (NT)
- 27 healthy children with normal haemoglobin and previously transfused AA(T).
- 72 healthy normal haemoglobin children without previous transfusion AA (NT).

42 of the 57 sickle cell trait children were siblings of known sickle cell patients, while 18 of the 72 AA(NT) children were siblings of known sickle cell patients. Only one of the 27 AA (T) children was a sibling of a known sickle cell patient. The age distribution of all children is shown in Table 1.

RESULTS

TABLE 1: Age distribution of 263 children according to haemoglobin genotype and transfusion status

Patient Groups / Age Groups	SS (T)	SS (NT)	AA (T)	AA (NT)	AS (NT)
months - 2 Years	(14%) 8	(29%) 14	(30%) 8	(30%) 23	(24%) 14
- 4 years	(15%) 9	(21%) 10	(22%) 6	(24%) 17	(18%) 10
-6 years	(15%) 9	(14%) 7	(11%) 3	(14%) 10	(14%) 8
-8 years	(12%) 7	(13%) 6	(11%) 3	(4%) 3	(10%) 6
-10 years	(22%) 13	(10%) 5	(15%) 4	(8%) 6	(17%) 10
0-12 years	(12%) 7	(9%) 4	(11%) 3	(10%) 7	(10%) 5
2-14 years	(10%) 6	(4%) 2	- -	(8%) 6	(7%) 4
tal	59	48	27	72	57

ver Functions Tests

T results and HBsAg performed on all 263 children in the study are shown Table 2. There were no relationships between age, SGPT levels, or number of nsfusions.

TABLE 2: SGPT Results of 263 Children

	Elevated SGPT		Normal SGPT		Total Number Tested
SS (T)	(37%)	22	(63%)	37	59
SS (NT)	(15%)	7	(85%)	41	48
AA (T)	(37%)	10	(63%)	17	27
AA (NT)	(6%)	4	(94%)	68	72
AS (NT)	(7%)	4	(93%)	53	57

1. SS (T) Vs SS (NT) x^2 = 12.6 $p < .05$ difference significant
2. AA (T) AA (NT) x^2 = 28.5 $p < .05$ difference significant
3. SS (T) Vs AA (T) x^2 = 0 $p > .05$ difference not significant
4. AA (NT)Vs SS (NT) x^2 = 4.1 $p < .05$ difference significant
5. SS (NT) Vs AS (NT)x^2 = 3.3 $p > .05$ difference not significant
6. AA (NT) Vs AS (NT)x^2 = 0.1 $p > .05$ difference not significant

Both transfused groups of children had a significantly higher prevalence of SGPT elevation than their non transfused counterparts. ($p < .05$).

HBs Ag Results

Results of surface Antibody detection are shown in Table 3.

TABLE 3: HBsAg Results in the 263 Children

	HBsAg+	Total Number Tested
SS (T)	(10%) 6	59
SS (NT)	(8%) 4	48
AA (T)	(15%) 4	27
AA (NT)	(6%) 4	72
AS (NT)	(2%) 1	57

Comparison of different Groups

1. SS (T) Vs SS (NT): x^2 = 0.24 $p > 0.05$ difference not significant
2. AA (T) Vs AA (NT): x^2 = 4.30 $p < 0.05$ Significant difference
3. SS (T) Vs AA (T) : x^2 = 2.20 $p > 0.05$ difference not significant
4. SS (NT)Vs AA (NT): x^2 = 0.20 $p > 0.05$ difference not significant
5. SS (NT)Vs AS (NT): x^2 = 3.60 $p > 0.05$ difference not significant
6. AA (NT)Vs AS (NT): x^2 = 2.00 $p > 0.05$ difference not significant

The HBsAg positive cases ranged from 2-12 years old. The only statistically significant differnce in HBsAg rates was that between AA transfused children and AA non transfused children. There were no statistically significent differences between other groups.

(see footnotes to Table 3)

HBc Ab results (Table 4)

Of the 40 SS(T) children tested, 10(25%) were positive for the core antibody, 6 had high titres.

In the SS(NT) group there were no HBcAb positive. 6(35%) of the 17AA transfused children were positive for the core antibody; 2 of them with high titres.

In the AA(NT) group, only 2(7%), of the 30 children tested were positive for the core antibody; both had low titres.

Of the 31 sickle cell trait tested, 7(23%) were positive for the core antibody; 3 of them with high titres.

There were statistically significant differences in prevalences of core antibody between SS(T) and SS(NT); and between AA(T) and AA(NT) respectively, the transfused groups having higher prevalences than their non transfused counterparts ($p < .05$).

Results of Surface Antibody Detection

Results of HBsAb detection are shown in Table 4.

15(38%) out of the 40 SS(T) children tested were HBs positive.

7 of the positive cases had high titres. 11(55%) out of the 20SS(NT) children tested were positive for the surface antibody.

5 of the 11 positive had high titres.

In the AA(T) group, 8(47%) out of the 17 children tested were positive for the surface antibody. 3 had high titres. In the AA(NT) group, 8(27%) out of the 30 children tested were positive for the surface antibody. 2 of them had high antibody titres. 11(35%) out of the 31 sickle cell trait children tested were positive for the surface antibody. 7 of them had high titres of the antibody. There were statistically significant differences in surface antibody rates between the transfused groups and non transfused groups: SS(T) and SS(NT), AA(T) and AA(NT), respectively ($p < .05$). However, comparisons of AS with other groups did not show statistically significant differences.

TABLE 4: Interrelationship between the HBV serological markers in [illegible]

HBsAg+ HBsAb- HBcAb-	HBsAg+ HBsAb+ HBcAb-	HBsAg+ HBsAb- HBcAb+	HBsAg+ HBsAb+ HBcAb+	HBsAg- HBsAb- HBcAb+	HBsAg- HBsAb+ HBcAb+	HBsAg- HBsAb+ HBcAb-	HBsAg- HBsAb- HBcAb-	Total Tested
(7.5%)3	- -	(5%)2	- -	- -	(18%) 7	(18%) 7	(53%)21	40
- -	- -	- -	- -	- -	- -	(55%) 11	(45%) 8	20
- -	(6%) 1	(18%) 3	(6%) 1	(6 %) 1	(12%) 2	(29%) 5	(35%) 6	17
(3%)1	- -	- -	- -	(3%) 1	(3%) 1	(23%) 7	(70%) 21	30
- -	- -	- -	(3%) 1	(3%) 1	(16%) 5	(16%) 5	(61%) 19	31

RELATIONSHIP BETWEEN SGPT AND HBsAg.

TABLE 5 : Relationship between SGPT and HBsAg.

SGPT/HBsAg / Groups	Elevated SGPT	Elevated SGPT HBsAg+	Elevated SGPT HBsAg-	All HBsAg+	HBsAg+ Normal SGPT	Total No Tested.
SS (T)	(37%)22	(9%)5	(29%)17	(10%)6	(2%) 1	59
SS (NT)	(15%)7	(2%)1	(13%)6	(8%) 4	(6%) 3	46
AA (T)	(37%)10	(11%)3	(26%)7	(15%)4	(4%) 1	27
AA(NT)	(6%) 4	(3%)2	(3%)2	(6%)4	(3%) 2	72
AS(NT)	(7%) 4	(2%)1	(5%)3	(2%)1	- -	57

In the SS (T) Group there were 22 (37%) children with elevated SGPT levels, 5 of whom were also HBsAg positive.

In the SS (NT) Group there were 7 (15%) children with elevated SGPT levels, one of whom was also HBsAg positive.

In the AA (T) Group there were 10 (37%) children with elevated SGPT levels, 3 of these were also HBsAg positive.

Relationship of SGPT values with HBV markies (Table 5,6)

In the AA(NT) Group, there were 4 (6%) cases with elevated SGPT levels, 2 of whom were also HBsAg positive.

Among the sickle cell trait Group, there were 4 (7%) children with elevated SGPT levels. Only one was HBsAg positive; the other 3 were HBsAg negative.

There was a statistically significant difference in percentages of children who had elevated SGPT with positive HBsAg, between transfused and non transfused sicklers ($p < .05$). Similarly, a significant difference was also found between transfused and non transfused AA children.

SGPT elevation associated with core antibody also showed similar pattern in relation to both transfused and non transfused groups. The relationship between the serological profiles, and SGPT elevation is also shown in Table 6:

a) Neither core antibody nor surface antibody as sole markers associated with SGPT elevation showed any statistically significant difference between any of the transfused groups of children and their non-transfused counterparts.

b) But the two antibodies present together in association with raised SGPT levels, revealed statistically significant differences between

   i Transfused sicklers and non transfused sicklers ($p < .05$)

   ii Transfused AA children and their non transfused counterparts ($p < .05$)

c) Only the two transfused groups showed SGPT elevation without any serological markers (both 18%). The differences between them and their non transfused counterparts were statistically significant. ($p < .05$)

TABLE 8 : Relationship between individual Serological Markers and SGPT

	SS(T)	SS(NT)	AA(T)	AA(NT)	ASNT
HBsAg+ Elevated SGPT	4	-	12	-	3
HBsAg+ Normal SGPT	3	-	6	3	-
HBsAg- HBsAb- HBsAb+ Elevated SGPT	-	-	(6%)1	-	(3%) 1
HBsAg- HBsAb- HBcAb+ Normal SGPT	- -	- -	- -	(3%)1	- -
HBsAg- HBsAb+ HBcAb- Elevated SGPT	(3%)1	(10%)2	- -	- -	- -
HBsAg- HBsAb+ HBcAb+ Normal SGPT	(15%)6	(45%)9	(29%)5	(23%)7	(16%)5
HBsAg- HBsAb+ HBcAb+ Elevated SGPT	(8%)3	- -	(12%)2	(3%)1	(3%)1
HBsAg- HBsAb+ HBcAb+ Normal SGPT	(13%)3	- -	- -	- -	(13%)4
HBsAg- HBsAb- HBcAb- Elevated SGPT	(28%)11	- -	(29%)5	- -	- -
HBsAg- HBsAb- HBcAb- Normal SGPT	(40%)16	(45%)9	(24%)4	(67%)20	(25%)9
Total no. of Cases tested	40	20	17	30	31

DISCUSSION

Hepatitis as judged by liver enzymes was found in the transfused groups more frequently than the non transfused groups of both SCD and normal Haemoglobin. The commonest known causes of post transfusion hepatitis are HBV and non A non B Virus. The dynamics of HBV markers in acute hepatitis are outlined in appendix 111. The difference in HBsAg among the transfused sicklers (Table 3), did not differ significantly from that of sicklers who had not been transfused, while that between the transfused and non transfused AA children was significant. It is therefore, conclusive that transfusion is infectious predominantly to AA children and not sickle cell children. However, the results of core and surface Antibodies in Tables 4 and 6, clearly reflect that HBV infection follows a very different course in the transfused groups against the non transfused controls.

It is generally believed that the findings of HBsAb as a sole marker, means that one has in the past encountered the hepatitis B virus and developed a state of sterile immunity against reinfection. The numbers and percentages of children who developed sterile immunity are shown in Table 7.

When development of sterile immunity rate is calculated in relations to exposure of HBV, 34% of SS(T), 100%SS (NT), 45% of AA(T) and 75% of AA(NT) were shown to develop that state. The comparison of transfused with non transfused groups in both cases show that exposure in transfused groups reduce their chances of developing sterile immunity.

TABLE 7: Children who developed sterile immunity

Markers / Groups	HBsAb as Sole Marker	No. Exposed to HBV	Total No. Tested
SS(T)	(35%) 7	20	40
SS(NT)	(100%) 11	11	20
AA(T)	(45%) 5	11	17
AA(NT)	(70%) 5	11	10
AS(NT)	(42%) 5	12	31

1. SS(T) Vs SS(NT) = $p.<.05$ difference statistically significant
2. AA(T) Vs AA(NT) = $p.<.05$ difference significant
3. SS(T) Vs AA(T) = $p.>.05$ difference not significant
4. SS(NT) Vs AA(NT) = $p.>.05$ difference not significant
5. SSNT Vs AS(NT) = $p.>.05$ difference not significant
6. AANT Vs ASNT = $p.>.05$ difference not significant

It has been suggested that HBsAb is but one indicator of prior exposure to HBV and only a small proportion of those exposed become chronic carriers (17) of the virus.

The absence of development of sterile immunity in these groups may be attributed to the route and dose of the virus encountered by the transfused group. Intravenous exposure and high doses may stimulate a T suppressor cell response preferentially rather than T helper cell response.

It may also delay development of sterile immunity. Infection through other routes like skin or gut as is likely to have occurred in the non transfused groups, seemed to stimulate development of sterile immunity in majority of the exposed children, (AA and SS non transfused).

SCD is known to be clinically mild in patients with high Fetal Haemoglobin levels. They have lower incidence of anaemia, autosplenectomy and infections. Although fetal haemoglobin studies were not done, it was felt that those sicklers who were not transfused had a more mild form of the disease than those who were transfused. This may alter immunocompetence resulting in better recovery. It may also serve to explain why non transfused SS children had lower prevalence of HBsAg and higher prevalence of HBsAb than the transfused SS, when the two otherwise share the same invironment.

Children without Serological Markers:

Comparisons of percentages of children without any serological markers confirm that in the group where there is moderate transmission of the virus as in the AA non transfused (transmission rate 30%) blood transfusion is definitely hazardous. However, the comparison between AA(NT)and SS(NT), shows that the transmission rate of HBV in families with sickle cell disease is much higher, even in absence of blood transfusion. This is further supported by the transmission rate observed among sickle cell trait.

The differences between two non transfused groups of SS and AA suggest that SS genes offer better sterile immunity as compared to normal HbAA carriers against developing chronic carrier state, though trait and transfused SS do not enjoy the benefit as SS (Table 6), suggesting that raised foctal Hb in SS non transfused may offer this protection.

The infectious group was significantly higher among the two transfused groups of children than their non transfused counterparts, with sickle cell trait in intermediate position. This further confirms the observations suggested by surface antibody results. When the HBV is encountered through transfusion, the complete sterile recovery is less likely, thereby resulting in higher pool of infectious subjects in the community. The infectious pool then spread the infection to other siblings and school mates residing in the same environment.

The results of this study explain why Lesi et al (4), did not find a significant difference in transmission rates between Nigeria sicklers and the general population. They compared only prevalence of the surface antigen. Had they looked at other Hepatitis B viral markers, some of the differences seen and explained above may have also been observed.

Liver Function Tests

12 (63%) of the 19 hepatitis B surface antigen positive children in the whole study had elevated SGPT. This association is more evident in transfused groups. This finding are contrary to the known fact that the majority of adult surface antigen positive carriers have normal LFTS (7). Since abnormal liver function tests reflect liver cell damage, all paediatric surface antigen carriers must be followed up.

Elevated SGPT levels without any Serological markers

It was observed in Table 6, that only the SS transfused and AA transfused children had elevated transaminase levels without any serological markers related to hepatitis B viral infection. In these cases other causes of chronic liver disease such as non A and non B hepatitis (1, 18), should be considered. Since there are serological markers specific for HBV, while those for non A non B are not available, its role in liver damage in these groups is based on exclusion.

Cossart et al (6), have recommended the exclusion of donors with HBsAb and HBcAb and elevated SGPT, in order to decrease both HBV and non A non B PTH in USA, and Australia. Such a recommendation is most unpractical here as over 50% of volunteer donors would have to be excluded. This would create a drastic shortage of blood in an already existence of shortages.

This study revealed higher HBV transmission rates among tribes where sickle cell gene predominates(regardless of transfusion) than non sickle cell families as shown in Table 8 . A larger proportion of these children are infected with HBV during pre-school age. On entering school, where all children mix, non infected children from other families acquire the infection from this group. This could explain the sudden rise in prevalence of HBV marker among Kikuyu children on school entry (Table 8).

TABLE 8

HBV Markers among non transfused children under 5 years compared to Kikuyu children and adult blood donors

	HBsAg	HBsAb	HBcAb	No HBV Markers	Total No Tested
5 year old children from present study who had never been transfused (Luo, Luhya)	14% (6)	38% (17)	9% (4)	52% (23)	44
1 - 5 year old Kikuyu children from Nairobi.	0%	6% (3)	6% (3)	94%(48)	51
5 - 15 year old Kikuyu children from Nairobi.	8% (3)	31% (12)	33%(13)	54%(21)	39
Adult blood donors from Nairobi.	(7)	50% (52)	55%(57)	38%(39)	104

48% of all the untransfused below 5 years of age children from the present study were found to have been already exposed to HBV, while only 6% of the Nairobi Kikuyu children in the same age group were exposed. The difference between the two groups was statistically significant ($X^2 = 45$, $p < .05$). Of the 5-15 year Kikuyu children 46% were found to have been exposed. The prevalence of HBV markers in this group was not significantly different from the untransfused under fives in this study. It confirms further the high rate of HBV transmission among sickle cell communities.

* Bowry. T, Personal communication.

This indicates that children from sickle cell families are exposed to the HBV at an earlier age than those from other communities in which sickle gene does not occur.

Comparisons between sickle cell trait and other non transfused groups show that sickle cell trait behave as if they had been transfused despite not having been transfused. (Table 6,7). The difference observed between sickle cell trait and AA may be a matter of how often one is exposed. The majority of AS children were siblings of sicklers, where as only a minority of normal haemoglobin children came from sickle cell families (see page 9). One wonders whether the observed differences between non transfused sicklers and sickle cell trait are genetic or environmental or both. It is reported that chronic carrier state results more often if virus infection occurs in early childhood (12), or in early postnatal life.

Kujwalire et al (16), in Dar es Salaam using CIEP found a significantly higher surface antibody prevalence among sickle cell trait adults than normal haemoglobin, (0.92% and 3.22% respectively). Results of this study differed from the Dar study, since the surface antibody prevalence among sickle cell trait did not differ significantly from that of normal haemoglobin. The differences in the observations may purely be related to the differences in sensitivity of the methods of detection used in the two studies.

In the Dar study the percentages were low, because the CIEP method picks up only high titres, treating low titres as negatives. The RIA method used is far more sensitive than CIEP and will pick up even very low titres. In Kenya the CIEP method is routinely used to screen donor blood for HBsAg. Use of the more sensitive third generation methods, such as RIA is highly recommended to reduce the risk of post transfusion Hepatitis B further.

CONCLUSIONS:

1. Blood transfusion in Paediatric population is not safe since asymptomatic hepatic damage which is detected by liver enzymes studies occurs in 37% of transfused as compared to 10% in the non non transfused group in tribes where HBV transmission rate is high anyway.

2. Post transfusion hepatitis is commonly due to HBV and non A non B, hepatitis viruses. Post Transfusion Hepatitis B is fortunately detected by examining for HBV markers. So far there are no serological markets for non A non B. There are other forms of viral and chemical hepatitis which may be indistinguishable from Post Transfusion Hepatitis, but appropriate controls in the study assist in their exclusion. HBsAg and core antibody are often associated with liver damage, but Post Transfusion Hepatitis by other viruses, possibly non A non B are even more commonly transmitted by transfusion.

3. The host responses suggest that course of HBV infection is adversely affected when virus is encountered through transfusion and possibly in high dose. Hence sterile immunity results more commonly in children with natural infections in the community than when it occurs via intravenous routes, especially via blood transfusion.

4. The HBV transmission in pre-school groups is higher in communities with sickle cell gene as compared to those which lack it. They are potentially hazardous to the non-immune children of other communities when they meet in schools where common sanitary facilities are shared.

RECOMMENDATIONS:

1. Blood donors should be better screened for HBV markers using more sensitive methods as Post-Transfusion Hepatitis B is generally preventable.
2. The HBV vaccination in tribes with sickle cell gene should be given a priority, it should be administered in early postnatal life.
3. These measures may reduce HBV transmission drastically in school children of Nairobi.

APPENDIX 1 HB MARKERS/SICKLE CELL PROJECT "H" NUMBER _____

IDENTITY DATE

NAME _____ AGE _____ SEX _____ TRIBE _____

CLINIC/UNIT _____ HOSPITAL NUMBER _____

DURATION OF RESIDENCE IN NAIROBI AREA _____

FAMILY: No. of SIBLINGS _____ F/H OF SICKLE CELL DISEASE/

TRAIT _____

HOW MANY AFFECTED _____

PERSONAL MEDICAL HISTORY

ANY CHRONIC ILLNESS:- DIABETES, LEUKAEMIA, LYMPHOMA, RENAL DISEASE,

CHRONIC LIVER DISEASE,

DERMATITIS OTHERS _____

ANY MEDICATIONS DRUG _____

DOSAGE _____

DURATION _____

ROUTE _____

IF WAS STOPPED, WHEN WAS IT STOPPED?

_____

ANY OPERATIONS/INSTRUMENTATION - DESCRIBE _____

_____

BLOOD TRANSFUSION NIL _____ YES _____

IF YES, HOW MANY _____

DATE (S) _____

WHERE _____

HISTORY OF JAUNDICE

PHYSICAL EXAMINATION

HISTORY OF JAUNDICE _____

PHYSICAL EXAMINATION

GENERAL REMARKS

TEMPERATURE

PALOR

JAUNDICE

LYMPHODES SITE (S) SIZE, MOBILITY, TENDERNESS

SKIN

RESP SYSTEM

CARDIOVASCULAR

P/A _____ LIVER

_____ SPLEEN

_____ OTHER

IF SICKLE IS PATIENT IN CRISIS _____ TYPE OF CRISIS _____

_____

LABORATORY Hb GENOTYPE _____ HAEMOGRAM _____ PTI _____

BILIRUBIN TOTAL _____ AIK. PHOSPH. _____ SGOT _____

SGPT _____

DIRECT _____

INDIRECT _____

HBsAS _____ HBsAb _____

HBcAb _____

OTHER INFORMATION _____

_____

_____

APPENDIX 11

CORAB is a competitive radioimmunoassay method, in which a given amount of commercial HBcAb tagged with radioactive Iodine (^{125}I), competes with the antibody in the test serum for binding sites on beads coated with HBcAg.

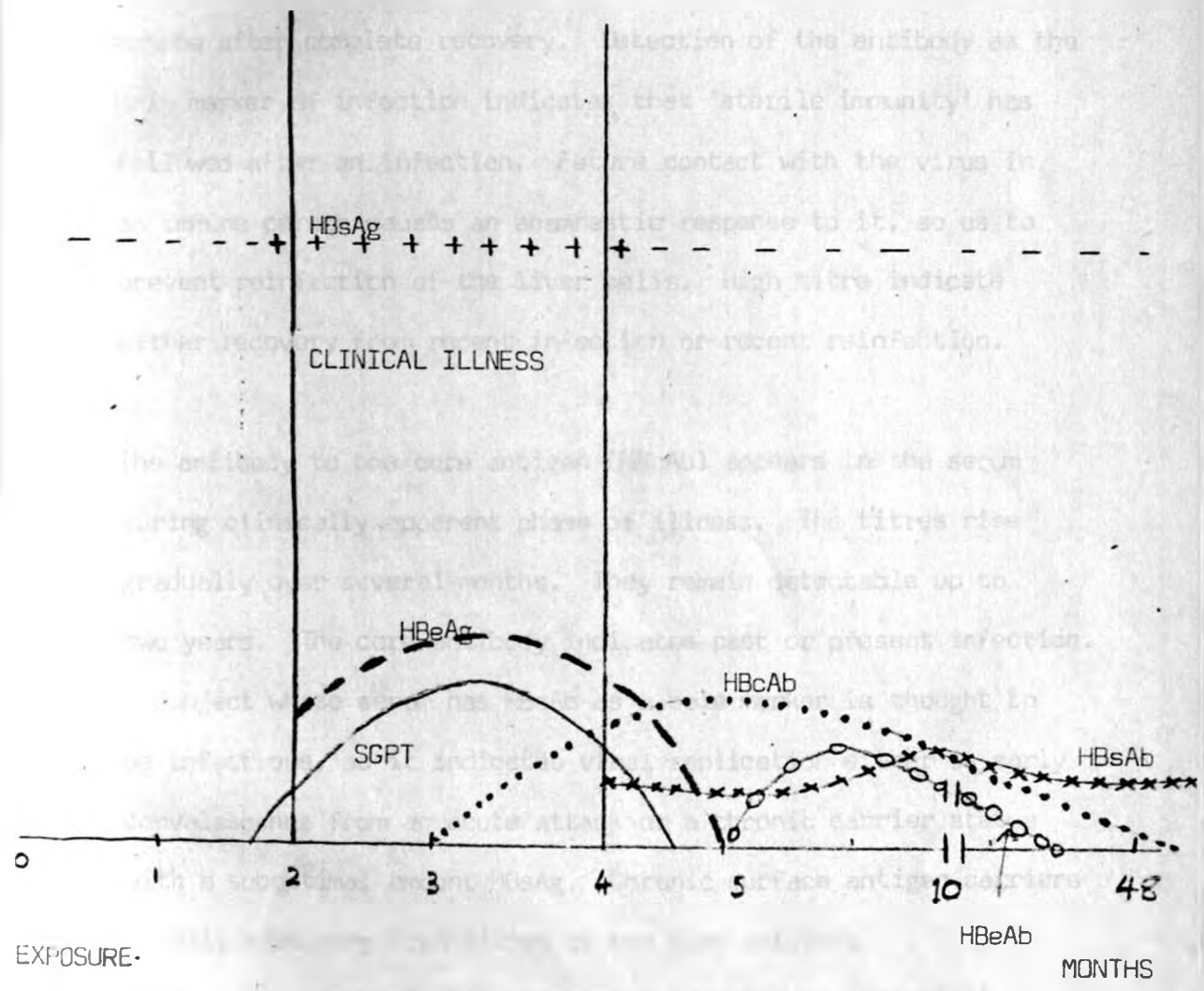
Beads coated with commercially prepared HBcAg are incubated with test serum. Radiolabelled HBcAb, when added to the system will bind any unoccupied sites on the bead. Thus the proportion of radioactive HBcAb on the bead is inversely proportional to the concentration of HBcAb in the test specimen.

Radioactive HBcAb is counted in a gamma scintillation counter, the lower the count, per minute, the greater the positivity after cutt off valve had been determined using known HBsAb positive and known HBsAb negative specimen respectively.

USAB is based on a "sandwich" principle. Plastic beads coated with human HBsAg from the kit is added to the test serum. HBsAntibody if present in the serum is fixed to the solid phase antigen. When other antigen labelled with radioactive Iodine is added, it binds to the unmenecomplex on the bead creating a radioactive antigenlantibody-antigen 1^{125} complex or "sandwich". Thus the higher the radioactive counts per minute the greater the positivity. Specimens were reported as positive or negative compared with standard controls. Positive results were repeated for confirmation. Anti HBc readings were only accepted as positive if the counts per minute were five times the negative control mean.

APPENDIX 111

igure 1: Typical Course of Clinical Hepatitis B



HBsAg: Hepatitis B surface antigen, was formerly called the Australian Associated Antigen. It is a particle from surface of the virus. It can be identified in serum one or two months after exposure and persists for variable periods. Up to 10-30% of the exposed progress to a chronic carrier state with persistent viraemia (2,7,8). The progression to this state is observed more frequently when exposed in early childhood.

HBsAb: The antibody to the surface antigen (HBsAb) appears one to three months after complete recovery. Detection of the antibody as the only marker of infection indicates that 'sterile immunity' has followed after an infection. Future contact with the virus in an immune person causes an anamnestic response to it, so as to prevent reinfection of the liver cells. High titre indicate either recovery from recent infection or recent reinfection.

HBcAb: The antibody to the core antigen (HBcAb) appears in the serum during clinically apparent phase of illness. The titres rise gradually over several months. They remain detectable up to two years. The core antibody indicates past or present infection. A subject whose serum has HBcAb as a sole marker is thought to be infectious, as it indicates viral replication either in early convalescence from an acute attack or a chronic carrier status with a suboptimal amount HBsAg. Chronic surface antigen carriers usually have very high titres of the core antibody.
Bowry (1), has used this phenomenon to estimate "potential infectivity" among blood donors.

The Hepatitis B E antigen (HBeAg): is another useful market of infectivity. The "e" positive carriers tend to develop active liver disease.

SGPT: rises just before onset of jaundice and returns to normal after about 3 weeks. Elevation indicates hepatocellular damage as in convalescent carriers or chronic active liver disease. Healthy carriers have, usually normal LFTS.

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