THE UNIVERSITY OF ZAMBIA
SCHOOL OF MEDICINE

DEPARTMENT OF PAEDIATRICS AND CHILD HEALTH

THE SEROPREVALEANCE OF HUMAN IMMUNODEFIENCY VIRUS AND HEPATITIS B INFECTION IN PATIENTS WITH SICKLE CELL ANEMIA AT THE UNIVERSITY TEACHING HOSPITAL

A STUDY SUBMITTED TO THE DEPARTMENT OF PAEDIATRICS AND CHILDHEALTH, SCHOOL OF MEDICINE
UNIVERSITY OF ZAMBIA,
IN THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE MASTERS OF MEDICINE DEGREE IN PAEDIATRICS AND CHILDHEALTH

BY

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DECLARATION

I hereby declare that the work presented in this study for Masters of Medicine Degree in Pediatrics and Child Health has not been presented either wholly or partially for any other degree and is not being currently submitted for any other degree.

SIGNED: ...........................................

CANDIDATE

Dr. Andrew Lingililani Mbewe

SIGNED: ...........................................

SUPERVISOR

Professor Chifumbe Chintu.
DEDICATION:

This study is dedicated to the patients who suffer from Sickle Cell Anaemia and all those who look after them.
ACKNOWLEDGEMENTS

I wish to express my gratitude to the government of the Republic of Zambia for the Scholarship I got through the Directorate of Manpower Development and Ministry of Health. To all the taxpayers, I say thank you for supporting and enabling me to study for the Masters of Medicine in Pediatrics and Child Health at the University of Zambia.

I sincerely thank professor Chintu for his valuable Supervision and Candid Suggestions regarding the content of the study. I would like to thank Dr. Francis Onyango for his valuable advice as well.

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ABSTRACT

The aim of this study was to determine the seroprevalence of Human Immunodeficiency virus (HIV-1) and Hepatitis B virus (HBV) in patients with Sickle Cell Anaemia (SCA). The other, was to determine the role of blood transfusions and herbal skin scarification in the transmission of HIV-1 and HBV in the SCA Patients.

This was a cross-sectional study of patients with SCA, who attended the Sickle Cell Clinic at the University Teaching Hospital (UTH), Lusaka, Zambia. An informed consent was obtained from the patients or the guardians of the patients. A presented questionnaire was administered to these patients and their responses and laboratory results were recorded on the questionnaire. One hundred and fifty four SCA patients who were randomly assigned to the Investigator were included in the study between March, 1990 and March, 1991. Data obtained was analysed by computer, the Chi Square and the Fischeres Exact Test, were used for significance testing. A p value of less than 0.05 was taken to be significant.

Overall, 22 (14.3%) were positive for HIV-1 antibodies using both ELISA (Recombigen and Wellcozyme). Nine (5.8%) were positive for HBs Ags and 38 (24.7%) had HBs Abs using Haemagglutination tests.
There was only a significant correlation between blood transfusion and HIV-1 positivity in the SCA patients as compared to those patients with SCA who did not receive blood transfusion ($P=0.02$).

There was no significant correlation between other cross tabulations. HIV-1 with herbal skin scarifications

($P = 0.50$) HBs Ags with blood transfusions

($P = 0.72$), HBs Ags with scarifications

($P = 0.84$), HBs Abs with blood transfusions

($P = 0.97$), and HBs Abs with scarification ($p = 0.92$).

In conclusion, 95% of Sickle Cell Anaemia patients who are found positive for HIV-1 antibodies had received blood and were older than 14 years of age. More patients with SCA has been exposed to HBV than HIV-1. It is advisable to carry out longitudinal studies in such high risk patients in order to determine the magnitude of HIV-1 and HBV infection and to evaluate the current control progra- mmes and polices.
CHAPTER ONE

INTRODUCTION AND HYPOTHESIS

1. INTRODUCTION:

Human Immunodeficiency Virus (HIV-1) and Hepatitis B Virus (HBV) are both transmitted through blood transfusions, contaminated Syringes and needles, sexual intercourse and from mother to child (during Intrauterine life, during delivery or postnatally) 1,2,3,4.

Sickle Cell Anaemia (SCA) patients are at risk of contracting HIV-1 and HBV because they receive repeated transfusions and Herbal Skin Scarifications as part of their management.

There has been no study done to determine the seroprévalences of HIV-1 and HBV in patients with SCA in Zambia. Therefore, since HIV-1 has become one of the most dreaded infections in Zambia and the world over (5), and that patients with SCA are at risk of contracting the virus through the treatment given to them, it was felt necessary to determine the seroprevalence in such a high risk group of patients. Similarly HBV is another virus which is transmitted in a similar manner with HIV-1 and it was therefore also felt necessary to include it in this study.
2. **HYPOTHESIS**

The hypotheses for the study were:

1. Since patients with Sickle Cell Anemia receive repeated blood transfusions than the normal population it was postulated that they may have a higher prevalence of HIV-1 and HBV.

2. Due to the chronicity of Sickle Cell Anaemia, several treatment regimes are tried which include among others, Herbal Skin Scarifications which may be a route of transmission of HIV and HBV. In this regard, these patients are postulated to have a higher seroprevalence of HIV-1 and HBV.

3. Given that screening for HIV-1 and HBV of blood intended for transfusions only began in 1985 and 1989 respectively, there is a possibility that many SCA patients who were born before 1985 and 1989 could have been infected by the viruses. This fact may contribute to higher Seroprevalences of HIV-1 and HBV in SCA patients.
CHAPTER TWO

OBJECTIVES AND SIGNIFICANCE OF THE STUDY

1. OBJECTIVES

(a) **Main Objective**

To determine the Seroprevalence of Human Immunodeficiency Virus and Hepatitis B. Virus in patients with Sickle Cell Anaemia.

(b) **Specific Objectives**

(i) To identify the general epidemiological data in patients with Sickle Cell Anemia
   - Age and Sex
   - Residence
   - Number of previous admissions in the past five years.

(ii) To determine HIV-1 antibodies, HBV Surface Antigens and antibodies in the Sickle Cell Anaemia patients.

(iii) To determine the Association of blood transfusions and traditional herbal skin scarifications and the transmission of HIV-1 and HBV in the patients with Sickle Cell Anaemia.

(iv) To determine whether the same risk factors were equally important in acquisition of HIV and HBV.
2. THE SIGNIFICANCE OF THE STUDY

Currently and world wide, HIV-1 Infection is one of the major public health hazards. In Blantyre, Malawi, Lusaka, Zambia and Kigali, Rwanda, recent studies of pregnant women show rates of HIV - Seropositivity between 22 percent and 30 per cent. (6).

In Uganda, the Ministry of Health reports that there were estimated 800,000 Ugandans with HIV - Seropositivity in 1990 while in Zambia the number of HIV-1 Seropositive case by March, 1991 was 4,000 known AIDS cases and at least 12,000 AIDS related - Complex patients was reported to the Ministry of Health of Zambia (7). Under reporting can not be ruled out since HIV-1 testing is not compulsory and voluntary testing is not practiced widely. With such a large pool of HIV-Seropositive persons in the population, it only becomes necessary to estimate the Seroprevalence of HIV-1 and related Infections in populations that are at a higher risk of contracting the infections.

Patients with SCA are at risk of contracting HIV-1 and related Infections because they receive blood transfusions repeatedly as part of the Management of their condition. It may be possible that they may receive Infected blood and also engage in Sexual Activity.
This study will be significant for it will show the magnitude of Infections and evaluate in part, the control programmes that are currently in place among a high risk group of patients.
1. SICKLE CELL ANAEMIA AND INFECTIONS

Sickle Cell disease is a condition which results from the inheritance of two abnormal genes controlling the formation of hemoglobin at least one of which is responsible for the formation of haemoglobin S(8).

Sickle Cell Anaemia is therefore a condition resulting from the Inheritance of two Sickle genes responsible for the haemoglobin SS (HBSS). The diagnosis of SCA is based on clinical features and a confirmatory hemoglobin electrophoresis test. Prenatal diagnosis is now possible (9).

One of the features of patients with Sickle Cell Anaemia is that they have an impaired resistance to Infection. Some of the cause of Impaired resistance to Infection include the following (10,11):

(a) The presence of a defect of the alternative pathway of the complement activation and opsonization system.

(b) A reduction in the neutrophil and macrophage activity and functional hyposplenism.
(c) Occurrence of hyperhemolysis of the red blood Cells that makes iron available to organisms that require iron as a nutrient for their growth and multiplication.

(d) A diminished Cell Mediated Immunity.

These impaired Immune mechanisms in patients with SCA makes them vulnerable to infection.

In Zambia, Studies determining common infections in patients with SCA are few. Osborne and Chintu in Lusaa, Zambia in 1981, found that the common infections in 770 Sickle Cell Anaemia patients were acute respiratory Infections (9.2%), Osteomyelitis (8.7%), Malaria (6.8%) tuberculosis and Meningitis with lower percentages (12). HBV and HIV Seroprevalences were not estimated in this report, however, HIV-1 was a new infections and diagnostic tests not yet available in 1981. Barret, Kabins, Adeyounnu reported in 1982 after Nigerian studies that common infections occuring in patients with SCA were pneumonia, meningitis, diplococcus pneumonia, salmonella and mycoplasma. The organisms causing meningitis were diplococcus Pneumonia and Hemophilus Influenzae. Osteomyelitis was commonly caused by salmonella in 45% of Cases, the rest were by staphylococcus, klebsiella and pseudomonas (13,14,15). Viral Infections in patients with SCA were not reported in these studies.
Evans in 1990 in England reported that the risk of pneumococcal infection has been estimated to be at least 600 times than that in the normal population. He advocated that patients with SCA should receive prophylactic penicillin from the age of four months to adult life (16). Viral infections in Sickle Cell Anaemia patients was not reported.

2. HUMAN IMMUNODEFICIENCY VIRUS

In Zambia, Studies determining the Seroprevalence of HIV-1 have been done in several groups of patients other than the SCA patients. The Ministry of Health of Zambia has reported (1992) that the average seroprevalence of HIV-1 in Zambia is 20% (17). Luo (18) found a seroprevalence of HIV-1 to be 7.5% amongst health blood donors in 1989 in Zambia. Hira Etal reported Seroprevalences of 43.7% of patients attending the STD Clinic were HIV-1 Seropositive in Lusaka (19) in 1989.

Allison Elliot studied 346 patients with tuberculosis in Zambia and found a Seroprevalence of HIV-1 to be 60%. (20).

Other studies have been done by other workers in breast feeding mothers (21), neonates (22) and Surgical patients (23). And in all these the Seroprevalence of HIV-1 has been estimated to be between 20 and 35 per cent.
Studies determining the Seroprevalence of HIV-1 in Sickle Cell Anaemia patients has not been done before except in few patients during hospitalization.

It is also the same with Hepatitis B Virus whose markers have only been determined in pediatric and Adult patients (24) Neonates (25) and general pediatric patients (26). In all these groups the Seroprevalence of HBs Ags has been less than 5%.

3. **BLOOD TRANSFUSIONS**

The benefits of blood transfusions in patients with SCA with severe anaemia is well documented. C. Lambote reported that in severe anaemia blood transfusions are unavoidable and are beneficial (27).

Behrman and Vaughan supports the reports by C. Lambote on the use of blood transfusions in SCA patients especially in cases of severe anaemia, aplastic and hypoplastic crises and sequestration crises (28, 29, 30).

Lambote, Behrmann and Vaughan also warned of the adverse effects of repeated blood transfusions in SCA patients as well in non SCA patients. Some of the side effects of blood transfusions are viral infections such as HIV-1 and HBV.
Transmission of HIV-1 through infected blood is a well documented fact. Transmission of HIV-1 through blood transfusions has been studied extensively in patients with Hemophilia (31, 32, 33, 34).

Hemophilia is similar to SCA, as patients suffering from both diseases have to receive repeated blood products as part of management of their condition and its complications. Therefore, studies like this will show how blood transfusions are responsible for the transmission of HIV- in Zambia.

Transmission of Hepatitis B Virus through blood transfusions is also well documented (35, 36, 37). This has been done in patients other than SCA patients in Zambia.

Sheila Sherlock has also given detailed information concerning the natural history of hepatitis B Virus. She has reported that the carrier rate of HBV varies worldwide. In countries like Britain, USA and Scandinavian Countries, the estimated Carrier rate of HBs Ag has been estimated to be 0.1% to 0.2%; more than 3% in areas lie Greece, Southern Italy, and for Africa and the Far East of Asia 10-15% (38).
4. **SKIN SCARIFICATIONS**

The administration of herbal powders through skin scarification has been practiced for a long time in Zambia. It is presumed that about 50% of children attending the clinics or hospitals at any given time may have herbal skin scarifications.

These Scarifications are made by sharp objects like razors, sharp steel objects and other sharp metals. These sharp objects are usually not sterilized after use. With the knowledge that HIV-1 and even HBV may be transmitted by contaminated needles and syringes among drug users, it may be postulated that the sharp objects used for scarifications may also be a source of infections if they are shared without proper sterilization.

The role of Skin Scarifications as a route of HIV-1 infections and HBV has not been studied in Zambia, let alone in developed countries where such practices are rare except for tattooing.
CHAPTER FOUR

METHODOLOGY

1. RESEARCH SETTING

This study was done at the Sickle Cell Clinic which is at the University Teaching Hospital in Lusaka, Zambia. This clinic was established in 1973 and currently has nearly 1000 patients with sickle cell disease in attendance. SCA patients are seen every Monday in the Morning. A Maximum of four doctors see the patients who are randomly assigned to them by clerks. Before they are seen by the doctor, the SCA patients are first weighed and the hemoglobin estimated. The doctors screen these patients for any other illness and if found very ill, the patients are admitted to the haematology unit of the department of Pediatrics at the University Teaching Hospital. Those who are well are sent home on malaria chemoprophylaxis and folic acid.

2. RESEARCH DESIGN

This was a cross sectional study.
3. **SAMPLE SELECTION**

The sample for the study was randomly selected from among patients who had sickle cell anaemia (confirmed by haemoglobin electrophoresis). The sample size was calculated using a computer based formula on the assumption that 700 to 1000 patients with Sickle Cell Anaemia attend the clinic and that estimated Seroprevalences for HIV-1 and HBV were between 10 to 15% and less than 15% respectively in SCA patients at the time of the study.

4. **DATA COLLECTION AND RESEARCH INSTRUMENT**

All patients who were randomly assigned to the Investigator and not on any other basis were included in the study between March 1990 and March 1991. Demographic and specific data concerning history of having blood transfusions and scarification marks was obtained by interviewing the patients or the guardians in case of children. Information concerning blood transfusions was verified by checking the patients hospital files. Each patient was also examined for skin scarification and other physical findings. Information obtained was recorded on questionnaires which later on acted as data bases (Appendix 1).
5. PILOT STUDY

A pilot study was done at the beginning of April, 1990 to test the suitability of questionnaire. Several adjustments were made without losing the intended objective for which the questionnaire was intended for.

6. LABORATORY INVESTIGATION

All the patients included in the study were those with sickle Cell Anaemia diagnosed by the Cellulose Acetate membrane Haemoglobin Electrophoresis Tests (Appendix 5).

Four millilitres of venous blood was collected from each patient. Two millilitres of this were put in two separate plain bottles. The blood was kept in refrigerators and allowed to clot. Later each sample was centrifuged and serum removed from the bottles.

In one sample serum, antibodies to HIV-1 were estimated using the ELISA (Recombinant Cambridge Corporation USA, Appendix 6). A positive result was counter-checked with the ELISA (Wellcozyme). In the second sample, HBV surface antigens and antibodies were estimated using the Hemagglutination tests (Appendix 7).
7. **RESEARCH ETHICS**

Pretest counselling was done before blood samples for HIV-1 antibodies testing was collected. The counselling was done by the investigator. A verbal consent was also obtained from the patients or guardians before including them in the study.

This study was approved by the Research and Ethics Committee of the School of Medicine and University Teaching Hospital (UTH).

8. **DATA ANALYSIS**

The data was analysed by computer employing the Data Base II plus the SPSS computer software packages. The Chi-Square and Fischers Exact tests were employed for significant testing.

A P value of less than 0.05 was considered significant.
CHAPTER FIVE
RESULTS

1. GENERAL CHARACTERISTICS
One hundred and fifty four patients with Sickle Cell Anaemia were screened for HIV-1 antibodies and HBV surface antigens and antibodies. Their mean age was 13.3 years with the oldest being a female of 33 years of age. (see table 1).

There were 76 (49.4%) male and 78 (50.6%) females. Sixty four percent of all sickle cell anaemia patients came from the high density areas of Lusaka. (See table 2).

Seventy two (46.8%) of the patients had been admitted to the University Teaching Hospital for more than five times. The majority of these admissions were due to vasocclusive crisis 115 (75.7%) followed by Osteomyelitis 7(4.5%). (See tables 3 and 4).

Of the 154 patients 103(66.9%) had received blood transfusions and 82 (53.2%) had herbal skin scarification. (see table 5 and 6). Their mean weight was 25.49kg and their mean hemoglobin was 6.90, g/dl.
### TABLE 1

**AGE RANGE (n = 154)**

<table>
<thead>
<tr>
<th>AGE RANGE IN YEARS</th>
<th>NUMBER OF PATIENTS</th>
<th>PERCENTAGE OF THE TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ≤ 5</td>
<td>5</td>
<td>3.3</td>
</tr>
<tr>
<td>5 ≤ 10</td>
<td>27</td>
<td>17.5</td>
</tr>
<tr>
<td>10 ≤ 15</td>
<td>49</td>
<td>31.8</td>
</tr>
<tr>
<td>15 ≤ 20</td>
<td>36</td>
<td>23.5</td>
</tr>
<tr>
<td>20 ≤ 25</td>
<td>26</td>
<td>16.8</td>
</tr>
<tr>
<td>25 ≤ 30</td>
<td>6</td>
<td>3.8</td>
</tr>
<tr>
<td>30 or more</td>
<td>5</td>
<td>3.3</td>
</tr>
<tr>
<td>TOTAL</td>
<td>154</td>
<td>100.0</td>
</tr>
</tbody>
</table>

### TABLE 2

**RESIDENCE (n = 154)**

<table>
<thead>
<tr>
<th>AREA OF RESIDENCE</th>
<th>NUMBER OF PATIENTS</th>
<th>PERCENTAGES OF THE TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Density</td>
<td>93</td>
<td>60.4</td>
</tr>
<tr>
<td>Low Density</td>
<td>31</td>
<td>20.1</td>
</tr>
<tr>
<td>Town Centre</td>
<td>15</td>
<td>9.7</td>
</tr>
<tr>
<td>Peri Urban</td>
<td>6</td>
<td>3.9</td>
</tr>
<tr>
<td>Lusaka Rural</td>
<td>9</td>
<td>5.8</td>
</tr>
<tr>
<td>TOTAL</td>
<td>154</td>
<td>100.0</td>
</tr>
</tbody>
</table>
TABLE 3

CAUSES OF PREVIOUS ADMISSIONS

<table>
<thead>
<tr>
<th>ILLNESS</th>
<th>FREQUENCY</th>
<th>PERCENTAGES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasoocclusive Crises</td>
<td>115</td>
<td>74.7</td>
</tr>
<tr>
<td>Osteomyelitis</td>
<td>7</td>
<td>4.5</td>
</tr>
<tr>
<td>Acute Respiratory Infection</td>
<td>5</td>
<td>3.2</td>
</tr>
<tr>
<td>Malaria</td>
<td>5</td>
<td>3.2</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>22</td>
<td>14.4</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>154</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

TABLE 4

FREQUENCY OF PAST ADMISSIONS

<table>
<thead>
<tr>
<th>NUMBER OF PAST ADMISSIONS</th>
<th>FREQUENCY</th>
<th>PERCENTAGES</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>3.2</td>
</tr>
<tr>
<td>1</td>
<td>52</td>
<td>33.8</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>10.4</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>4.5</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>More than 5 times</td>
<td>73</td>
<td>47.4</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>154</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>
2. **SEROREVLENCES**

(a) **HIV-1 Antibodies**

Out of the 154 Sick Cell Anaemia Patients 22 (14.3%) were positive for HIV-1 antibodies. Of the 22 patients 20 (90.9%) were older than 15 years, 16 (72.7%) were female, 14 (63.6%) came from the high density areas of Lusaka, 20 (90.9%) had received blood transfusions and 10 (45%) had received herbal skin scarifications.

Two SCA patients were positive for HIV-1 antibodies out of 51 who had not received blood transfusions. While 20 patients were positive for HIV-1 antibodies out of the total of 103 patients who had received blood transfusions. There was significant difference between these two groups with a P value of 0.02 (Yates Corrected), Odds ratio of 5.90 and Relative Risk of 4.08.

Twelve SCA patients were positive for HIV-1 antibodies out of 72 patients who had no scarifications. Ten patients were positive for HIV-1 antibodies out of 82 who had scarifications. There was no significant difference between the two groups of patients with P value of 0.57 (Yates Corrected), Odds ratio of 0.69 and Relative Risk of 0.83.
Hepatitis B Viral Surface Antigens and Antibodies

(i) Hepatitis B Surface Antigens (HBs Ags)

Out of the 154 SCA patients 9 (5.8%) had HBs Ags. Seven were below the age of 15 years of age, 5 were male, 7 had received blood transfusions and only 4 had herbal scarifications.

Cross tabulations between those who had HBs Ags and blood transfusions showed that there was no significant difference between those who had HBsAgs but had no blood transfusion and those who had HBsAgs and had blood transfusions with a P value of 0.72, Odds ratio 1.79 and a Relative Risk between the two groups of 1.52.

Five SCA patients had HBs Ags out of 72 who had no scarifications. Four patients had HBs Ags out of 82 patients who had scarifications. There was no significant difference between the two groups with a P V value of 0.84 (Yates Corrected), Odds ratio of 0.69 and Relative Risk of 0.93.

Since the numbers were small, the fischers test was done and the 1 tailed P value was 0.41 and 2 tailed was 0.73.
(ii) **Hepatitis B Viral Surface Antibodies (HBs Abs)**

Thirty eight (24.7%) SCA patients out of 154 were found to have HBsAbs. Of the 38 patients 23 (60.5%) were older than 15 years, 22 (57.9%) were female, 27 had received blood transfusions and 19 had herbal skin scarifications.

Twelve patients were positive for HBs Abs out of 51 who had not received blood transfusions. While 26 patients had HBs Abs out of 103 who had received blood transfusions. There was no statistical significant difference between the two groups with a Yates Corrected P value of 0.97, Odds ratio of 1.10, and Relative Risk of 1.06.

Eighteen patients were positive for HBs Abs out of 72 patients who had no herbal skin scarifications. Twenty patients had HBsAbs out of 82 patients who had scarifications. There was no statistical significant difference between the two groups; with a P value of 0.92 (Yates Corrected) and, Odds ratio of 0.97 and a Relative Risk of 0.98.
(iii) Comparisons of the characteristics of patients with antibodies only and HIV/HBV Antibodies.

A group of SCA patients who were HIV Seropositive only were compared with those who had both HIV-1 Antibodies and HBV Antibodies.

Comparing the patients characteristics seem to indicate that the same risk factors do play in the transmission of HIV and HBV in Sickle Cell Anaemia (See table 7).

The question however, is that why do the two Viruses have different prevalences. It is possible that they may be another mode of transmission or the late start (1989) of screening for HBV markers in Blood for transfusion could have contributed to the difference.

Table 7: Comparison of characteristics of patients with HIV-1 Seropositivity and those with HIV-1 antibodies and HBV markers. n = 8

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HIV Seropositive</th>
<th>HIV-Seropositive + HBV positive marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>All above 15 years</td>
<td>7/8 were above 15 years</td>
</tr>
<tr>
<td>Sex</td>
<td>6/8 were female</td>
<td>6/8 were female</td>
</tr>
<tr>
<td>Previous admission</td>
<td>6/8 had been admitted for Vaso-occlusive Crisis.</td>
<td>6/8 had been admitted for VOC.</td>
</tr>
<tr>
<td>Blood Transfusion</td>
<td>7/8 had blood Transfusion</td>
<td>All had blood transfusion</td>
</tr>
<tr>
<td>Herbal Skin Scarification</td>
<td>4/8 has Skin Scarification</td>
<td>4/8 had scarification</td>
</tr>
</tbody>
</table>
TABLE 6
FREQUENCY OF BLOOD TRANSFUSIONS AND HERBAL SKIN SCARIFICATIONS

<table>
<thead>
<tr>
<th>BLOOD TRANSFUSIONS</th>
<th>FREQUENCY</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Received</td>
<td>103</td>
<td>66.9%</td>
</tr>
<tr>
<td>Not Received</td>
<td>51</td>
<td>33.1%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>154</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SCARIFICATIONS</th>
<th>FREQUENCY</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>82</td>
<td>53.2%</td>
</tr>
<tr>
<td>Not Present</td>
<td>72</td>
<td>46.8%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>154</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

TABLE 7
CROSSPREVALENCE OF HIV-1, HBsAgs and HBsAbs, n = 154

<table>
<thead>
<tr>
<th>INFECTION</th>
<th>SEROPOSITIVITY</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1</td>
<td>22</td>
<td>14.3%</td>
</tr>
<tr>
<td>HBsAg</td>
<td>9</td>
<td>5.8%</td>
</tr>
<tr>
<td>HBsAb</td>
<td>38</td>
<td>24.7%</td>
</tr>
</tbody>
</table>
### TABLE 8

**SEROPREVALENCES OF HIV-1 IN VARIOUS STUDY GROUPS IN ZAMBIA**

<table>
<thead>
<tr>
<th>STUDY GROUP</th>
<th>SEROPREVALENCE IN PERCENTAGES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sickle Cell Anaemia* Patients (n = 154) (present study)</td>
<td>14.3%</td>
</tr>
<tr>
<td>Tuberculosis Patients (n = 346) (20)</td>
<td>60.0%</td>
</tr>
<tr>
<td>General Pediatric Patients (n = 250) (45)</td>
<td>23.9%</td>
</tr>
<tr>
<td>Neonates (n = 250) (22)</td>
<td>32.2%</td>
</tr>
<tr>
<td>Surgical Patients (n = 114) (23)</td>
<td>26.3%</td>
</tr>
<tr>
<td>Blood Donors (31 hospitals) (18)</td>
<td>7.5%</td>
</tr>
<tr>
<td>STD Clinic attenders (19)</td>
<td>46.5%</td>
</tr>
<tr>
<td>General Population, Zambia MOH (1992)</td>
<td>20.0%</td>
</tr>
</tbody>
</table>

### TABLE 9

**THE SEROPREVALENCE OF HBsAgs and HBsAbs IN ZAMBIA AND KENYA**

<table>
<thead>
<tr>
<th>COUNTRY</th>
<th>SEROPREVALENCE IN HBsAg</th>
<th>PERCENTAGES HBsAb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present Study (SCA Patients) Zambia</td>
<td>5.8%</td>
<td>24.7%</td>
</tr>
<tr>
<td>Zambia Hospital Workers (2)</td>
<td>3.0%</td>
<td></td>
</tr>
<tr>
<td>Pediatric Population (25)</td>
<td>2.5%</td>
<td>3.0%</td>
</tr>
<tr>
<td>Kenya (NAIROBI) AIDS Patients (47)</td>
<td>12.2%</td>
<td>24.4%</td>
</tr>
<tr>
<td>Kenya Rural Dwellers (46)</td>
<td>3.0%</td>
<td>23.6%</td>
</tr>
<tr>
<td>Kenya Medical Students (48)</td>
<td>18.0%</td>
<td>33.0%</td>
</tr>
</tbody>
</table>
CHAPTER SIX

DISCUSSION, CONCLUSIONS, RECOMMENDATIONS

DISCUSSION

Age and Sex

The Mean Age of the Sickle Cell Anaemia patients studies was 13.3 years; the oldest was 33 years of age. The apparent longevity of life of SCA patients is in contrast with earlier findings by several researchers and writers. Lombote in 1970 (27) had reported that a majority of SCA patients die before the age of two years.

Macleod (09) had also reported that in Africa, with Inadequate medical facilities and attentions few children with SCA survive to adult life.

Krupp and Chalton (42) wrote that SCA patients often die early because of severe complications and repeated acute respiratory infections.

This apparent longevity of life in the SCA patients studies could contribute to improved medical facility and attention, given to these patients since 1973 when the Sickle Cell Clinic was opened. This fact has been supported by Macleod (09) who reported that with improved medical facilities and improved social economic circumstances many patients survive longer.
Roseline (43) reported that although previously SCA patients used to die in infancy and childhood, they currently live longer because of improved diagnostic procedures and medical care.

In 1970 Barclay (44) recorded the age range of 414 Zambian SCA patients to be 0 to 18 years. In this study the age range has increased and is 0 to 33 years.

In 1981 Osborne and Chintu (12) studied in Zambia 770 SCA patients and found a mean age of 12 years compared to one found in the present study which was 13.3 years. There is a definite increase in the survival of patients with SCA.

In the present study the female to male ratio was almost 1:1 and 54% of the patients were older than 15 years of age.

RESIDENCE
The majority of the SCA patients came from the high density areas of Lusaka; indicating that our study sample was a true representation of the population in Lusaka since the majority of people in Lusaka are living in the high density areas.
CLINICAL PROFILE
The Clinical Presentation of SCA patients studied did not differ much with those described by other workers (12) in Zambia and elsewhere in the world (13,16). SCA Patients usually are admitted because of acute respiratory infection and vasocclusive crises. And those were the main causes of admissions in our study population.

BLOOD TRANSFUSION AND SCARIFICATIONS
It is very apparent that patients with SCA receive many blood transfusions. 103/154 (66.9%) of the patients studied received blood transfusion during their stay in hospital. This confirms the fact that these patients are at risk of acquiring infections that may be transmitted through infected blood. This actually supports the reports by Ansary et al who in 1989 classified the patients with Sickle Cell Anaemia as a group who are at risk of HIV-1 infection because they receive repeated blood products (1).

On the other hand, 82 (53%) of the patients received herbal skin scarifications. This as well, confirms our postulations indicated earlier on.

Very little work has been done in determining the role of scarification in the transmission of infections. The findings in this study contribute to the availability of knowledge on the role of scarification in the transmission of infection.
SEROPREVALENCE OF HIV-1 AND HBV HUMAN IMMUNODEFICIENCY VIRUS

The seroprevalence of HIV-1 in patients with Sickle Cell Anaemia was 14.3%.

This seroprevalence is relatively low when compared to seroprevalences determined in other groups of patients at the same institution in Zambia.

Shilalukey et al in 1989, in Lusaka studied 223 neonates in the neonatal intensive care Unit and found a seroprevalence of HIV-1 antibodies to be 32.2% (22).

Allison Elliot et al, in 1990 in Lusaka studied 346 patients with tuberculosis and found the seroprevalence of HIV-1 antibodies to be 60%. This year, 1992 she reported that the figure has risen to 70% in recent studies. (21)

Mugala et al in 1990, in Lusaka, Zambia studied 114 patients with surgical conditions and found a seroprevalence of 26.3% for HIV-1 antibodies (23).

Luo C. et al, in 1990, in Lusaka studied 250 children in the department of pediatrics and child health and found a seroprevalence of HIV-1 antibodies to be 23.9% (45)
As may be seen all the seroprevalences in the other study groups are higher than the one in our study group. The only exception was seroprevalence found in blood donors which was 7.5% (18).

The apparent low seroprevalence in SCA patients would be due the Control programme initiated in 1985 where by only screened blood is given to patients to correct severe Anaemia. There is however, a possibility that some of the SCA patients could have received unscreened blood before 1985. The results seem to support this postulation as only 2 patients who were positive for HIV-1 antibodies out of the 22 were younger than 5 years. 20/22 (95%) of seropositive patients were older than 15 years of age. This large group could have acquired the HIV-1 from blood transfusions before 1985 or through sexual intercourse.

The association of scarification and transmission of HIV-1 was found to be statistically insignificant. The same results were found in Luo's study (45). Scarifications play no significant role in the transmission of HIV-1 and HBV.

HEPATITIS B VIRUS
The Seroprevalence of HBsAg was 5.8% while that of HBsAb was 24.7%. With both Seroprevalences; there was no significant difference between those who had HBs Ags and HBsAb but had not received blood or Scarification and those who had HBsAgs and HBsAb and had received blood or Scarifications.
Comparatively, the present study results showed higher Seroprevalences of HBs Ags and HBsAbs in SCA patients than in groups of patients done by earlier workers.

Bhagwat and Palmer in 1983 found a Seroprevalence of HBsAg to be (3.0%) among hospital staff working in University Teaching Hospital, Lusaka (2).

Nomura et al in 1989 studied 500 children with general pediatric conditions at UTH, Lusaka and found the seroprevalences of 2.5% for HBsAgs and 3.0% for HBsAbs (25).

In another cross sectional study of children and adults admitted to UTH done by the present investigator, the seroprevalences for HBs Ag was 9.2% while that of HBs Ab was 11.3% (24).

It is apparent that Seroprevalences for HBs Ag and HBs Ab were higher in patients with Sickle Cell Anaemia than those of persons and patients investigated in the same institution.

The reasons for this may be due to repeated blood transfusions given to SCA patients which may be infected, realizing that screening for hepatitis B virus markers only started in 1989. On the other hand, when the results of the present study are compared to studies elsewhere in Africa. The seroprevalences of HBs Ag is only higher than those who live in Rural areas.
Okoth et al in Kenya, in 1990 studied rural dwellers and found the Seroprevalences of HBs Ag to be 3.0% while that of HBs Ab to be 23.6% (46).

Ogutu et al in 1990, in Kenya found a Seroprevalence of HBs Ag to be 12.2% and that of HBs Ab to be 24.4% in patients with AIDS (47).

Notably, the Seroprevalences of HBs Abs in the two groups is almost the same as in the one found in the present study.
CONCLUSION
This study shows that patients with SCA have repeated blood transfusions and more than half had scarifications.

Comparatively, the Seroprevalence of HIV-1 was lower than the Seroprevalences determined in other study groups in the same institution and lower than that in the general population of Zambia. However, the Seroprevalences for HBV were comparatively higher. The majority of Sickle Cell Anaemia patients who had HIV-1 antibodies were older than 15 years of age; suggesting another possible route of transmission other than blood transfusion.

It was apparent that more patients with SCA had been exposed to HBV more than to HIV-1 and that the same risk factors do play a role in the transmission of both viruses.

RECOMMENDATION
Blood meant for blood transfusion should continue to be screened for HIV-1 and HBV in order to further reduce the transmission of the viruses through blood transfusions to persons at risk. The present policy of giving blood only when the haemoglobin levels are below 5g/dl should also continue. Blood safety programmes should be given the highest priority in planning health systems. Longitudinal studies should be encouraged in risk groups in order to evaluate the magnitude of epidemics and also evaluate current preventative and control programmes.
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Appendices

1. Questionnaire.


3. The sickling Test.

4. Solubility test for Haemoglobin.

5. Routine cellulose acetate electrophoresis for separation of haemoglobin.


APPENDIX

QUESTIONNAIRE: Seroprevalences of HIV - 1 and HBV in patients with Sickle Cell Anaemia.

A.  1. Serial Number:.............. Date:...........................
    2. Hospital Number:..................
    3. Interviewer:..................
    4. Name of Patient:..................
    5. Age: 1.............(years) 2. ..............(months)
    7. Residential Address:..........................
        ..........................................
        ..........................................

B. HISTORY

8. Past Medical Admissions in the last five years.
        ..........................................
        ..........................................
        ..........................................

    1. Yes 2. No.

10 Injections
    1. Yes 2. No.

11. Intravenous Fluids
    1. Yes 2. No.

12. Scarification Marks
    1. Yes 2. No.

13. Sexual Intercourse
    1. Yes 2. No.
C. Physical Examination
14. 1. Pallor Yes/No
    2. Jaundice Yes/No
    3. Cyanosis Yes/No
    4. Lymphadenopathy Yes/No
15. Weight

D. Systemic Examination
16. Head and Neck: ..........................................
    ........................................................
17. CNS: ......................................................
18. CVS: ......................................................
19. RS: .......................................................  
20. GUT: .....................................................
21. Endocrine: .............................................
22. GIT: ......................................................
23. MSS: .....................................................
24. Others: ..................................................

E. Investigations
25. Sickling 1. Yes 2. No
26. Electrophoresis 1. Yes 2. No
27. Haemoglobin: ......................................g/dl
28. ELISA 1. Positive 2. Negative
29. HBs Ag 1. Positive 2. Negative
30. HBs Ab 1. Positive 2. Negative

F. Follow up
31. Date of specimen Collection: .........................
32. Date of receiving Results: ...........................
33. Date of review of patients: ..........................

Andrew L. Mbowe
1990/1991
APPENDIX 2:

HAEMOGLOBIN ESTIMATION: MODIFIED CYANMETHEMOGLOBIN METHOD

1. REAGENTS:
   1. 0.9% Sodium Chloride (NaCl).
   2. Lysate: Lysing and Haemoglobin Reagent (Coultronics, France)

2. METHOD:
   Add 10ml of 0.9% NaCl to 0.02 mls of blood sample
   Add 3-4 drops of Lysate
   Read haemoglobin estimation in a Colorimeter (252) at 540nm.

3. CALCULATION:
   Haemoglobin values of blood Specimens are read from the Calibration graph.
APPENDIX 3:
THE SICKLING TEST:
PRINCIPLE:
The Sickling test is based on the fact that when red cells containing Sickle Haemoglobin are exposed to reduced oxygen tension, they assume the characteristic sickle shape.

PROCEDURE:
A drop of peripheral venous blood on a microscope slide is mixed with two drops of freshly prepared 2% Sodium Metabisulphite. The preparation is covered with a cover slip which is sealed with petroleum jelly to prevent drying, and examined under the microscope after 15 minutes, one hour and 24 hours.

INTERPRETATION:
Cells containing 15 per cent or more Hb-S will sickle; this test does not distinguish between homozygotes and heterozygotes. False positive results may be obtained when poikilococytes are confused with Sickle-Cell forms or when using Sodium Metabisulphite Solution which is greater than 2%.
False negative results are usually due to stale Metabisulphite Solution. They also occur frequently in the neonatal period because of inadequate Hb-S in the cells that are required to deform cells.

Sickle Cell Anaemia (Hb-SS): All the haemoglobin is precipitated on the surface, and the solution shows a clear straw color. However, these results may be difficult to interpret since Sickle Cell Haemoglobin Disease and Sickle Cell betathalassaemia results may resemble that of Hb-AS. So there is again a need for confirmatory tests.
APPENDIX: 4

SOLUBILITY TEST FOR HAEMOGLOBIN:

PRINCIPLES:
The test depends on the low solubility of Hb-S in the reduced state. In the method, Hb-S forms a turbid suspension which can flocculate when centrifuged or when left standing over night.

REAGENTS:
1. STOCK BUFFER:
   Anhydrous Potassium Dihydrogen Phosphate - 33.78g
   Anhydrous Dipotassium Hydrogen Phosphate - 59.33g
   White Saponin - 2.50g
   Distilled Water to 250 ml
   The Sock Buffer should be stored at 4°C.

2. WORKING SOLUTION:
   This must be prepared fresh immediately before performing the test.
   Sodium Metabisulphite - 100 mg
   Stock Buffer - 10 ml
   This is sufficient for 10 tests.

METHOD:
Blood may be collected by finger prick or heel prick or by venipuncture and added to an EDTA anticoagulated container if other tests are to be performed. One drop (about 0.02 ml) of blood is added to 1 ml of the Working Solution in a 8 x 75 mm tube and mixed thoroughly.
APPENDIX 5

ROUTINE CELLULOSE ACETATE ELECTROPHORESIS FOR SEPARATION OF
HAEMOGLOBIN (II)

EQUIPMENT:
1. electrophoresis Chamber (e.g. Shandon) and power supply
2. D.C. Power pack (e.g. Shandon Vokam)
3. Shandon multi-applicator plate
4. Shandon Multi-applicator
5. Filter paper - whatman No. 1 (46x57cm)
6. Cellulose acetate strips (e.g. Shandon Celagram II)
7. Two kidney dishes (large size)
8. Pasteur pipettes (long type)

REAGENTS

Analar grade reagents are used throughout.

1. Buffer: Tris-EDTA-Borate (TEB) buffer PH 8.9
   Tris (hydroxymethyl) Amino methane 14.5g
   Ethylene diaminetetracetic acid (EDTA) 1.5g
   Boric Acid 0.9g
   Distilled H$_3$O to 1 litre.

2. Stock Solutions
   (a) Potassium Cyanide (KCN) 12.5g
      Distilled Water to 250 ml
   (b) EDTA
      Distilled Water to 50 ml

3. Working Solution
   KCN Stock Solution 2.0 ml
   EDTA Stock Solution 0.3 ml
   Distilled H$_3$O to 120 ml

4. Ponceau - S Stain
   Ponceau - S 0.2g
   Trishloroacetic acid (TCA) 5.0g
   Distilled Water to 100ml
5. **Rinsing Solution (5% glacial acetic Acid)**

Concentrated glacial acetic acid 5ml
Distilled Water to 100ml

**PROCEDURE**

Preparation of electrophoresis chamber and cellulose acetate strips

1. Fix two pieces of filter paper of appropriate size across each shoulder and into each outer compartment of electrophoresis chamber.

2. Pour 100 ml of TEB buffer into each Compartment and 50 ml into each inner compartment of electrophoresis chamber.

3. Pour 50 ml of TEB buffer into a large kidney dish

4. Immerse and leave a cellulose acetate strip (about 17cm long) in buffer in the kidney dish for at least 10 minutes but preferably for 15 to 20 minutes.

**PREPARATION OF HAEMOLYSATE**

1. Set up a row of 16 small (2.5 ml) glass or plastic tubes

2. Pipette 0.5 ml working solution into each tube

3. Using a long pasteur pipette, add one drop of whole blood from each sequestrene (EDTA) anticoagulated blood sample remembering to rinse the pipette thoroughly with distilled water between Samples. In severely anaemic patients, centrifuge 1ml of the sequestrene blood specimen, and remove plasma with a pasteur pipette until the PCV is approximately 0.33 (33 per cent); re-suspend these packed Cells by shaking, and use of drop of this suspension instead of the original whole blood.

4. Mix the contents of each tube by moderately vigorous tapping of the base of each tube so as to lyse the red cells.

**APPLICATION OF HAEMOLYSATES TO SHANDON MULTI-APPLICATOR PLATE**

1. The applicator plate should be placed flat on a smooth surface of the laboratory bench.
2. Using a long Pasteur pipette, apply an extremely small amount (less than half a drop) of each haemolysate sample to each of the 16 segments on the plate. It is helpful if a known Control HB-AA sample is applied to segment 1 and a known Control Hb AS sample to segment 16.

3. With blunt forceps, remove the wet Cellulose strip from the kidney dish in which it had been immersed in buffer and remove excess buffer by blotting the strip between two pieces of filter paper.
4. Lay the Cellulose acetate strip absolutely flat on a smooth piece of Card board.
5. Apply the teeth of the Shandon multi-applicator to the haemolysates on the respective segments of the applicator plate.
6. Place the teeth of the multi-applicator on the buffer impregnated Cellulose acetate strip along a line 1.5 cm from one of the lateral margins of the strip. This manoeuvre should be gentle but firm.

**Electrophoresis Run**

1. Remove excess buffer from the parts of the filter paper lying on the shoulders of the electrophoresis chamber using any absorbent material e.g. tissue paper.
2. Fix the Cellulose acetate strip containing the haemolysate samples on both shoulders of the chamber so that samples 1 to 16 are aligned in parallel with the shoulders. Hold the strip in position with supports provided so that it covers the inner chamber and rests on both shoulders.
3. Cover the chamber, make the appropriate electrical connections, switch on the DC power pack and carryout electrophoresis from cathode to anode at 250 volts (5 to 10 mA) for 15 to 30 minutes. At the end of this period, good separation of the haemoglobin should be obtained.

**STAINING**

1. After the electrophoresis 'run' stain the Cellulose acetate strip in a small volume of Ponceau S. Stain in a large kidney dish for 5 to 10 minutes. Rinse the stained strip in 5 percent glacial acetic until excess stain is removed (this usually involves one or two rinses)
2. Remove excess fluid by blotting the strip between two pieces of filter paper.
3. Dry the strip face downwards
4. Mount the dry stained cellulose acetate strip on a stiff card of appropriate size so as to obtain a permanent record.
APPENDIX 6

TEST USED FOR DETECTING HIV-1 ANTIBODY RECOMBICEN R - HIV EIA (39)

**Bank ground Information**
The Cambridge Bioscience Corporation RECOMBICEN-R-HIV-EIA is a qualitative enzyme immunoassay for the detection of antibody to Human Immunodeficiency virus (HIV) in human serum or plasma. In using the RECOMBICEN-R-HIV-EIA, when a specimen reacts in an initial test (initially reactive), the test should be repeated in duplicate on the same specimen. Reactivity in either or both of these duplicate tests (repeatably reactive) is highly predictive of the presence of the antibody in people at increased risk of Infection (e.g. homosexual men, hemophiliacs, or intravenous drug users).

**Principles of the Procedure**
The Cambridge Bioscience Corporation RECOMBICEN-R-HIV-EIA is manufactured from genetically engineered gp 120 and gp 41 (env) gene products of HIV, expressed in *Escherichia Coli*. All detectable contaminating bacterial proteins have been removed, and the recombinant antigen is coated onto polystyren microwells to be used in an Indirect enzyme Immuno assay (EIA).

1. Diluted patients sample or controls are incubated in coated microwells.
2. Antibodies to the virus **env** antigens, if present, will bind to the antigen-coated wells.
3. After removal of unbound antibody by aspiration and washing, enzyme-labelled anti-human antibody is added to each well and allowed to incubate.
4. Unbound conjugate is removed by aspiration and washing and a substrate solution is added to all wells.
5. A blue color will develop when anti-HIV antibody is present; addition of a stop solution changes this color to yellow.
6. The intensity of the color, read spectrophotometrically is proportional to the amount of antibody bound to the microwell.
APPENDIX 7

PASSIVE HEMAGGLUTINATION TESTS

(a) Detection of Hepatitis B Surface Antibody (HBs Ab)

Requirements
1. HBsAg coated erythrocyte (5ml)
2. Buffer Solution (50 ml)
3. HBs Ab positive Control (1ml)
4. HBs Ag for confirmatory Assay (25 ml)

Preparation of HBs Ag Coated erythrocyte (1:10 dilution)
1. Remove the supernatant of HBs Ag coated erythrocyte
2. Add 5.0 ml of the buffer solution and mix them using a pipette
3. Transfer this Solution to a Centrifuge test tube
   Centrifuge at 2,000 r.p.m for 5 minutes
   Remove the supernatant after Centrifuge
4. Add the buffer solution and mix again
   Raise to 50 ml of the HBs Ag coated erythrocyte
NB. The HBs Ag coated erythrocyte should be kept for more than 6 hours after preparing. (It is stable for one month).

Method of the Screening test
1. 4 vertical wells of a microtray are used for each sample.
2. Add 25 UL of the buffer solution to each well using a dropper
3. Take 25 ul of serum specimen by dipping the tip of a dilutor into serum specimen. Serially, dilute from the first well till the fourth well mixing well. HBs Ab positive control is done similar to sample.
4. Add 25 ul of the HBs Ag coated erythrocyte to every well using a dropper. Rotate for 5 to 10 seconds immediately.
5. Leave for one hour in room temperature. Read haemagglutination.

Confirm:
(a) if agglutination is present only in the first well, confirmatory test need not be done.

(b) If agglutination is present in

(b) If agglutination is present in:
(i) First and Second wells or
(ii) First, Second and Third wells or
(iii) all wells,
then proceed to the confirmatory test.

Confirmatory and Quantitative ests.

1. Add 25 ml of the buffer solution to each left side wells using a dropper.

2. Add 25 UL of the HBs Ab for confirmatory assay to each right side wells using another pipette.

3. Take 25 ul of serum specimen by dipping tips of two dilutors into serum specimen. Serially dilute from the first well till the forth well mixing well. HBs Ag postive Control is done similar to the sample.

4. Add 25 ul of the HBs Ab coated erythrocyte to every well using a dropper. Rotate for 5-10 seconds immediately.

5. Leave for 1 hour in noon temperature
Read haemagglutination

Confirm:

(a) Final agglutinate titre to HBs Ag positve Control up to the fourth well.

(b) No agglutination in HBs Ab for confirmatory assay.
READING OF RESULTS

1. Non agglutination (-)
   . Blood Cells precipitate into the bottom of the wells
   . Most of blood Cells precipitate into bottom of the well

2. Agglutination (+)
   . few blood cells precipitate
   . Haemagglutination can be seen around like a net
   . A ring of hemagglutination gets bigger
   . Haemagglutination around a ring can be seen like a net clearly
   . Haemagglutintion is pread like a net all over.

(b) Detection of Hepatitis B Surface Antigen (HBs Ag) (Using Reversed passive Hemagglutination).

Requirements:
1. ABs Ab coated erythrocute (5ml)
2. Buffer Solution (50ml)
3. HBs Ag Positive Control (1ml)
4. HBs Ab for confirmatory Assay (25ml)

Preparation of HBs Ab coated Erythrocytes (1:10 dilution)
1. Remove the supernantant of HBs Ab coated erythrocyte
2. Add 5.0 ml of the buffer solution and mix them using a pipette.
3. Transfer this solution to a centrifuge test tube.
   After centrifuging at 2000 r.p.m. for 5 minutes, remove the supernatant.
4. Add the buffer Solution and mix again.
   Raise to 50 ml of the HBs Ab coated erythrocyte.

NB: HBs Ab coated erythrocyte should be kept for more than 3 hours after preparing. (It is stable for one month at 2-8°C).

Method of the Screening test.
1. Vertical 4 wells of the microtray are used for sample.
2. Add 25 ul of the buffer solution to each well using a dropper
3. Take 25μl of serum specimen by dipping tip of dilutor into serum specimen. Serially dilute form the first well till the forth well mixing well. HBs Ag positive Control is done similar to the Sample.

4. Add 25 μl of the HBs Ag coated erythrocyt to every well using a dropper. Rotate for 5 to 10 seconds immediately.

5. Leave for one hour in room temperature. Read haemagglutination.

   Confirm:
   (a) if agglutination is present only in the first well, confirmatory test not necessary.
   (b) If agglutination is present in:
       (i) First and Second wells or
       (ii) First, Second and Third wells or
       (iii) all well, then proceed to the confirmatory test.

**Confirmatory and Quanitative ests.**

1. Two collums of the microtray are used for each Sample.

2. Add 25 ml of the buffer solution to each left side wells using a dropper.

Add 25 μl of the HBs Ab for confirmatory assay to each right side well using another pipette.

3. Take 25 μl of serum specimen by dipping tips of two dilutors into serum specimen. Serially dilute from the first wells till the sixth wells mixing well.

   HBs Ag positive control is done similar to Sample.

4. Add 25 μl of the HBs Ab coated erythrocyte to every well using a dropper. Rotate for 5-10 seconds immediately.

5. Leave for 1 hour in noon temperature

   Read haemagglutination

   Confirm:
   (a) Final agglutinate titre to HBs Ag positive Control up to the fourth well.
   (b) No agglutination in HBs Ab for confirmatory assay.