

**THROMBOCYTOPENIA IN HAART NAÏVE HIV INFECTED  
PATIENTS ATTENDING THE COMPREHENSIVE CARE  
CLINIC AT KENYATTA NATIONAL HOSPITAL.**

**DR ONG'ONDI. K. MATILDA**

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**A DISSERTATION SUBMITTED IN FULFILLMENT OF THE  
REQUIREMENT FOR THE DEGREE OF MASTER OF  
MEDICINE (INTERNAL MEDICINE)**

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**DECLARATION**

**I certify that this dissertation is my original work and has not been presented for a degree in any other university.**

Signed..........

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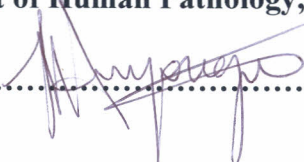
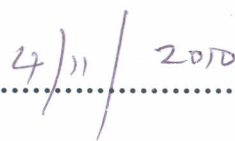
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## DEDICATION

**This book is dedicated to my loving parents Mr. Robert Mayaka and Mrs. Esther Ongóndi for their encouragement and support. You are the best.**

## ACKNOWLEDGEMENTS

I thank God for His faithfulness and never ending mercies that have ensured completion of this work.

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## ABBREVIATIONS

- AIDs - Acquired Immune Deficiency Syndrome
- AntiTB - Antituberculous treatment
- AntiHTN - Antihypertensives
- AZT - Azidothymidine (Zidovudine)
- CCC - Comprehensive Care Clinic
- CD4 - Cluster of Differentiation 4
- C3 - Complement factor 3
- C4 - Complement factor 4
- EDTA - Ethylene Diamine Tetra-acetate
- HAART -Highly Active Antiretroviral Therapy
- HIV - Human Immunodeficiency Virus
- IV - Intravenous
- ITP -Immune Thrombocytopenia
- IgG/ IgM – Immunoglobulin G or M
- KNH -Kenyatta National Hospital
- KEMRI- Kenya Medical Research Institute
- MGG stain - May Grunwald Giemsa stain.
- PBF -Peripheral blood film

PHAT -Primary HIV associated thrombocytopenia.

PMCTC- Prevention of mother to child transmission.

TCP - Thrombocytopenia

UNAIDs – Joint United Nations Programme on HIV/AIDS

VCT- Voluntary Counseling and Testing

WHO – World Health Organization

## **1. ABSTRACT**

### **Background:**

Haematological abnormalities are common in HIV infected patients. Thrombocytopenia has been associated with progression of disease. The presence of thrombocytopenia is significantly associated with decreased survival and is a predictor of mortality.

### **Objective:**

To study the prevalence of thrombocytopenia and clinical characteristics in HIV infected patients who are HAART naïve attending the Kenyatta National Hospital Comprehensive Care Centre.

### **Study Design**

A cross sectional Descriptive Study.

### **Study site:**

Kenyatta National Hospital Comprehensive Care Centre

### **Methods:**

HIV positive HAART naïve patients who fulfilled the study inclusion criteria were recruited. History and physical examination was done. Blood was drawn for total blood count, Peripheral blood film and CD4 count.

## **RESULTS**

340 HIV infected HAART naïve patients with a mean age of 37.3years and range of 18years to 72years were recruited. The male to female ratio was 1:1.6. The study population mostly comprised of; young patients (39.9% between 30-40yrs), females (61.6%) in WHO clinical stage I (57.6%) and with CD4 count between 200-500 cell/mm<sup>3</sup>. The mean platelet count was 230,000 cells/ul. The prevalence of thrombocytopenia in this population was 3.8%.

Most of the patients with thrombocytopenia had a bicytopenia with the rest having isolated thrombocytopenia or pancytopenia. Bleeding tendencies were more in the thrombocytopenia group (p= 0.011). Patients in WHO Clinical Stage IV were more likely to have thrombocytopenia (p <0.011) as well as those with CD4 count < 200cells/mm<sup>3</sup> (p <0.050).

### **Conclusion**

The prevalence of thrombocytopenia is low among HIV infected HAART naïve patients attending the Kenyatta National Hospital Comprehensive Care Clinic. This could be attributed to young age, predominant female gender and early disease in the study population.

## **2. LITERATURE REVIEW**

### **2.1 Introduction**

The HIV/AIDS pandemic remains a major source of concern worldwide with latest statistics in the 2009 AIDS Epidemic update by UNAIDS /WHO showing a steady rise in the number of people living with HIV to 33million in 2008<sup>1</sup>. 67.8% of these patients (22.4 million) are from the Sub Saharan Africa with an alarming AIDS related mortality of 1.4 million deaths. Kenya which has an estimated population of 36,913,721 as of 2007 is estimated to have 1.4- 1.8 million people living with HIV/AIDS<sup>2</sup>.

Haematologic manifestations of HIV is a well recognized complication and clinically important for many HIV infected patients. Indeed studies have shown that cytopenias associated with HIV infection are more prevalent and severe in those with advanced disease (WHO Clinical Stage III and IV)<sup>3</sup>. Efforts to establish the cause, severity and mechanism of cytopenias are important so as to ensure prudent choice of specific treatment or interventions as these may impact on patient outcome. Many studies have been done on anaemia which is the commonest hematological malignancy however there's increasing interest on thrombocytopenia and its effect on HIV/AIDS.

A relationship has been reported between platelet count, viral load and disease progression, raising questions on whether platelet count has any influence on natural history of HIV and disease progression.<sup>4</sup>

### **2.2 Platelet physiology:**

Thrombocytopenia is defined as platelet count of less than 150,000 cell/ul and is graded as mild (100-150,000cells/ul), moderate (>20-<100,000cells/ul) and severe (<20,000cells/ul).<sup>5</sup>

Thrombocytopenia has multi-factorial causative factors present during all stages of disease. The causes include: decreased platelet production, increased platelet destruction, massive transfusion or abnormal platelet distribution. Under normal conditions one third of all platelets are contained in the spleen. The life span of a platelet is 8-10days after which they are removed from circulation by macrophage-monocyte system and 15,000 - 45,000 platelets/ul are produced daily to maintain a steady state. Megakaryocytes which are pluripotent stem cells are influenced by

growth factors IL-3, IL-6, granulocyte macrophage colony stimulating factor (GM-CSF) and thrombopoietin (c-Mpl ligand) to produce platelets.<sup>6</sup>

## **2.3 EPIDEMIOLOGY**

### **2.3.1 Prevalence**

The prevalence of thrombocytopenia has been studied in various patient populations. Sloan et al between April 1986 and September 1989 studied 1004 HIV infected patients attending medical out-patient clinics in Washington, D.C America and found thrombocytopenia in 21.2% of those HIV patients who had AIDs compared to 9.2% in those who did not fit clinical criteria for AIDs.<sup>7</sup>

In Zimbabwe Adewuyi et al over a 12 month period studied the haematological features of HIV infected adults attending Parirenyatwa Hospital; a tertiary and referral hospital in Harare and found 47.5% of patients had cytopenias with 24.7% of these being thrombocytopenia. His study population included patients attending the HIV outpatient clinic, those receiving in patient care and asymptomatic persons who has donated blood at the blood transfusion service.<sup>8</sup>

Erharbor et al between June 2002 and July 2003 at the Port Harcourt Teaching Hospital sought to establish the Nigerian perspective of hematological parameters in HIV infected HAART naïve patients. In this study which comprised 100 patients with 88% being symptomatic the prevalence of thrombocytopenia was 10%.<sup>9</sup>

In the Middle East region Alaei K et al in 2000 studied thrombocytopenia in HIV infected patients in Islamic Republic of Iran and found a prevalence of 18.5-22.5%. Participants who were recruited from out-patient clinics were mostly male patients however none was on HAART.<sup>10</sup>

### **2.3.2 Patients characteristics and disease progression:**

The incidence of cytopenias correlates directly with the degree of immunosuppression however isolated thrombocytopenia may be the initial presentation of HIV infection. The incidence of platelet abnormalities appears to increase with progressive immunosuppression. A review of eleven studies determining the haematological aspects of HIV infection concluded that cytopenias tend to be more prevalent and severe in patients with advanced disease.<sup>3</sup> A similar

conclusion was made by Servias, Jean et al and their data indicated that haematological abnormalities correlate with HIV-1 plasma viral load in untreated patients.<sup>11</sup>

A study done at a rural hospital in Tanzania to determine the predictors of mortality in a cohort of 320 HIV infected patients starting HAART found anaemia, thrombocytopenia (<150,000 cell/ul) and severe malnutrition to be strong predictors of mortality.<sup>12</sup> The adjusted hazard ratio for thrombocytopenia was 2.30 with a 95% CI of 1.33- 3.99 and a p value of 0.003.

In a study done in Washington DC to describe the epidemiology of thrombocytopenia in HIV infection in 1004 patients at outpatient clinics; thrombocytopenia occurred more frequently in subjects with AIDs (21.2%) than HIV infected individuals who didn't fit the clinical criteria for AIDs (9.2%),  $p < 0.001$ . Patients with few CD4-positive cells and an advanced stage of disease were more likely to have low platelet counts. 30% with an absolute CD4 cell count lower than 200/mm<sup>3</sup> versus 8% with CD4 counts between 200 and 500 ( $p < 0.00001$ ) and 18.5% with Stage IV disease compared to 7.6% in Stage II ( $p < 0.001$ ) had platelet counts less than 150,000/mm<sup>3</sup>. 40% of patients with platelet counts <50,000 cells/ul reported bleeding.<sup>7</sup>

In Italy R. Caltaneo et al's study in 1988 concluded that thrombocytopenia associated with anaemia and or neutropenia was a manifestation of AIDs whereas the development of isolated thrombocytopenia didn't correlate with the evolution of the disease.<sup>13</sup>

A surveillance data longitudinal survey of medical records of 30,214 HIV infected patients from January 1990 to August 1996 in the US found 8.7% of patients with thrombocytopenia had clinical AIDs (1 or more AIDs opportunistic infections), 3.1% had immunological AIDs (CD4 <200 cells/mm<sup>3</sup>) and 1.7% had no clinical or immunological AIDs. After controlling for anaemia, clinical AIDs, CD4 count, neutropenia, antiretroviral therapy, PCP prophylaxis: thrombocytopenia was significantly associated with decreased survival (risk ratio 1.7; 95% CI, 1.6-1.8).<sup>14</sup>

Some of the characteristics that have been associated with the occurrence of thrombocytopenia include; intravenous drug use, age and race. A study done by Mientjes et al. in 1985 studied the prevalence of thrombocytopenia in HIV infected and non-infected drug users and homosexuals found the prevalence to be much higher in intravenous drug users however duration of drug injection, frequency of injecting or type of drug injected was not significant.<sup>15</sup> In May 2000-

April 2001 an Iranian study of 170 HIV infected patients all except seven females were injecting drug users.<sup>10</sup> The incidence of thrombocytopenia was associated with clinical AIDS (adjusted odds ratio [AOR] 2.2; 99% confidence interval [CI] 1.7-3.0), immunologic AIDS (AOR 1.5, CI 1.0-2.1), history of injecting drug use (AOR 1.4, CI 1.0-1.9), anemia (AOR 5.0, CI 3.8-6.7), lymphoma (AOR 3.7, CI 1.3-10.6) and black race (AOR 0.7, CI 0.5-0.9) in a multistate project looking at the thrombocytopenia in HIV patients. A higher occurrence of thrombocytopenia among whites than blacks and a higher occurrence of thrombocytopenia among older patients has been shown in the same study.<sup>14</sup>

A study done by Rieg et al in Los Angeles, USA examined the relationship between platelet number and the natural history of HIV-1 disease in the well-characterized Hemophilia Growth and Development Study cohort.<sup>16</sup> In the multivariate analysis platelets were found to be inversely related to plasma HIV-1 RNA with increasing platelets associated with lower plasma HIV-1 RNA levels ( $p < 0.001$ ). Despite this, increasing platelet count was independently associated with enhanced risk of progression to AIDS and death ( $p < 0.001$  for both). This paradox raises more questions on the true effect of platelets on the course HIV infection.

Analysis of clinical and haematological data from Multicentre Haemophilia Cohort Study, comparing thrombocytopenia in those who are HIV infected versus those that are not, concluded that thrombocytopenia was associated with increased risk of death (relative risk 1.7 95% CI 1.2-2.3) but little change in terms of progression to AIDS.<sup>17</sup>

The risk of bleeding is high in those with severe thrombocytopenia ( $<20,000$  platelet/ul) and that of fatal haemorrhage is likened to non-HIV related immune thrombocytopenia of 5%.<sup>7, 18</sup>

#### **2.4 Mechanisms of Thrombocytopenia in HIV**

Thrombocytopenia in HIV is divided into two groups; primary HIV associated thrombocytopenia (PHAT) and secondary thrombocytopenia. The aetiology of PHAT is complex with many studies that have shown both decreased platelet survival associated with platelet specific antibodies and reduced platelet production due to direct infection of the megakaryocytes. Secondary causes of thrombocytopenia are generally the result of underlying opportunistic infections, malignancy, medications and co-morbid conditions resulting in splenomegally with hypersplenism.

Thrombotic-Thrombocytopenic Purpura (TTP) and Haemolytic Uraemic Syndrome (HUS) are rare causes.<sup>19</sup>

In order for platelets to be removed from circulation there needs to be sufficient titers of offending antiplatelet antibody and ability of reticuloendothelial system to recognize and remove coated or damaged platelets. During HIV infection antibodies of different specificities are produced and these react to HIV antigens, lymphocyte antigens and platelets. HIV-1-ITP has been associated with markedly elevated platelet-associated IgG, IgM, and C3/C4, as well as presence of circulating serum immune complexes (CICs) and in some cases had predominant male incidence. The antibodies are directed against the platelet glycoprotein (Gp) IIIa peptide 49-66.<sup>20</sup>

Kinetic studies demonstrate shortened platelet life span in thrombocytopenic HIV-infected patients, suggesting that platelet production is not sufficiently expanded to compensate for accelerated platelet destruction in these patients.<sup>21, 22</sup> In South Africa Van Wyk et al. studied the kinetics of indium-111-labelled platelets in HIV infected patients with and without thrombocytopenia. The thrombocytopenic patients had a very short mean platelet life span (3.0+/-3.8 h) and a marked increase in platelet production (18.2+/-12.6x10<sup>9</sup>/l/h). The majority of these patients (5 of 7) had excessive sequestration of platelets in the spleen.<sup>23</sup>

Ultrastructural changes in platelet aggregates from HIV patients studied under scanning electron microscopy showed apoptotic changes with membrane blebbing.<sup>24</sup>

Zucker- Franklin et al in 1989 first observed ultrastructural megakaryocyte abnormalities and denuded megakaryocytes nuclei in all HIV infected individuals even in absence of low platelets.<sup>25</sup> Subsequently in the same year studies showed presence of HIV mRNA in megakaryocytes from AIDs patients suggesting possibility of direct infection of megakaryocytes leading to decreased platelet production.<sup>26</sup> Recently, Chelucci et al have provided in vitro data that indicate HIV infects megakaryocyte progenitors or precursors largely, but not exclusively, through CD4+ T-cell receptor sites.<sup>27</sup>

The plasma levels of thrombopoietin which stimulates both proliferation and maturation of megakaryocytes are elevated in HIV patients with thrombocytopenia as well as those without thrombocytopenia.<sup>28, 29</sup>



## **2.5 Bone marrow changes in thrombocytopenia**

Bone marrow abnormalities are found at all stages of HIV disease increasing in frequency as the disease progresses.<sup>30</sup> A study done in India by AK Tripathi et al looking at the bone marrow abnormalities in patients with HIV disease showed myelodysplasia in 32.4% of patients with higher incidence of dysplasia in those with advanced disease.<sup>30</sup> The higher incidence in the latter group being attributed to higher viral load, cytokine mediated effect of disease and the effect of opportunistic infections and drugs.

Detailed structural changes of megakaryocytes unique to thrombocytopenic HIV-1 infected patients include: large number of pyknotic, denuded megakaryocyte nuclei on light microscopy, extensive blebbing and vacuolization on electron microscopy with expression of viral RNA in megakaryocytes.<sup>25, 31</sup> In 1995 and 2001 Adediran et al. in Nigeria studied 72 HAART naïve patients and observed that the most characteristic bone marrow abnormality was abundance of naked nuclei of megakaryocytes in 60.1% of patients. Dysplastic changes were seen in 45.5% of bone marrow specimens and these included: - dysgranulopoiesis, pegler huet anomaly in some mature granulocytes, vacoulation of some erythroid cells and unilobular micromegakaryocytes and megaloblastic erythroid precursors.<sup>32</sup>

Brook et al in 1997 studied the utility of bone marrow examination in HIV patients and showed little value of this investigation in patients with isolated thrombocytopenia. However there is an indication for bone marrow examination if the patient presents with pancytopenia.<sup>33</sup>

## **2.6 Effect of HAART on thrombocytopenia**

Zidovudine (AZT) has been the mainstay of treatment for thrombocytopenia especially primary HIV associated thrombocytopenia and has been shown to increase platelet production.<sup>21</sup> In a prospective evaluation of HIV-infected patients with thrombocytopenia (mean platelet count, 53,000/ $\mu$ L; range 25 to 85,000/ $\mu$ L), the platelet count rose by more than 50,000/ $\mu$ L after eight weeks of AZT therapy (2 g/day for two weeks followed by 1 g/day for six weeks)<sup>34</sup> Higher daily doses are associated with more prominent and durable elevations in platelet count. This was demonstrated in an open label, randomized, multi-institutional study which compared the efficacy of two doses of AZT (0.5 and 1.0 g/day for six months) in HIV-infected patients with platelet counts below 50,000/ $\mu$ L<sup>35</sup>

HAART has been associated with improvement of HIV associated thrombocytopenia and there are several retrospective studies supporting this however no prospective controlled trials on the same.<sup>36,37</sup> A decline in platelet count was observed in a study in Thailand where patients underwent structured treatment interruption and 3 of the 23 enrolled patients developed recurrent thrombocytopenia during the periodic discontinuation of HAART.<sup>38</sup>

The use of additional therapies is based upon the severity of thrombocytopenia, evidence of bleeding (petechiae, epistaxis, hematuria) and other coincident conditions such as coagulation factor deficiencies. These therapies include; - intravenous immunoglobulin, anti-D immunoglobulin, danazol and dapsone, corticosteroids, interferon alfa, vincristine, splenectomy, splenic irradiation and growth factors.<sup>3</sup>

Thrombocytopenia is an important hematologic abnormality in HIV patients and it results from increased peripheral destruction of platelets or reduced production by the bone marrow megakaryocytes. Its occurrence is influenced by many factors such as age, stage of disease or use of intravenous drugs among others. It is a marker of disease progression and predictor of mortality.

### **3. STUDY JUSTIFICATION**

Thrombocytopenia in HIV patients has been found to have a prevalence ranging between 10-40% in several studies. Thrombocytopenia is associated with increased morbidity and mortality.

There's a knowledge gap of this condition and therefore this study will act as a baseline for other studies. The burden of disease and related patient variables will aid in providing useful data that can generate other entry points into studying this condition.

It will also help in raising clinicians' index of suspicion in identifying thrombocytopenia hence instituting appropriate management which will result in better patient outcome.

Results of this study will help in the review of policy guidelines on basic investigations of HIV infected patients for improved patient care.

### **4. STUDY OBJECTIVES**

#### **4.1 Broad objective**

To determine the prevalence of thrombocytopenia and its associated variables in HAART naïve HIV infected patients attending the Kenyatta National Hospital comprehensive care centre.

#### **4.2 SPECIFIC OBJECTIVES**

1. To determine the prevalence of thrombocytopenia in HAART naïve HIV patients attending the KNH CCC.
2. To describe the socio-demographic, clinical and laboratory characteristics of patients with thrombocytopenia.
3. To correlate thrombocytopenia with age, gender, WHO clinical staging, CD4 count and bleeding tendencies.

## **5. METHODOLOGY**

### **5.1 STUDY SITE**

Kenyatta National Hospital Comprehensive Care Centre- offers out-patient services to all HIV infected patients and those requiring post-exposure prophylaxis.

### **5.2 STUDY POPULATION**

HIV infected patients attending the KNH-CCC, 18 years and above.

### **5.3 STUDY DESIGN**

Cross sectional descriptive study

### **5.4 CASE DEFINITION**

HIV infection will be defined by a positive serology test: by long ELISA or rapid ELISA ( Bioline / Unigold).

### **5.5 DEFINITION OF THROMBOCYTOPENIA**

Thrombocytopenia will be defined as platelet count of less than 150,000 cells/ul as determined by haematology cell coulter counter and verified by peripheral blood film.

Severity of thrombocytopenia will be defined as follows:-

- Mild: 100,000-149,000cells/ul
- Moderate: 20,000-99,000 cells/ul
- Severe: <20,000 cells/ul

### **5.6 INCLUSION CRITERIA**

- HIV patients above 18 years attending CCC at KNH.
- HAART naïve.
- Informed written consent.

## **5.7 EXCLUSION CRITERIA**

- Patients known to have haematological malignancies or malignancies involving the bone marrow.
- Patients on cytotoxic medications.
- Pregnant women.
- Those who refuse to give consent.

## **5.8 SAMPLING METHOD**

Consecutive sampling of HAART naïve patients who meet the inclusion criteria.

## **5.9 SAMPLE SIZE DETERMINATION**

The sample size will be determined by the use of the following formula to achieve the determined sample to accurately estimate the prevalence and correlates of thrombocytopenia in the study population.

$$n = \frac{Z_{\alpha/2}^2 * P (1-P)}{D^2}$$

Where n = required sample size

P = estimated prevalence (24.7%), based on a similar study done in Zimbabwe.<sup>5</sup> This is the only study in Sub- Saharan Africa that has studied the prevalence of thrombocytopenia.

D = Precision with which to measure prevalence of the study, set at ±5%.

The  $Z_{\alpha/2}$  is 1.96 representing a 95% confidence interval.

Substituting the above in the formulae;

N = 286 patients

## **6. SCREENING AND RECRUITMENT**

The principal investigator with the help of the study assistant reviewed files of all HAART naive HIV infected patients who attended CCC daily. The files of patients who met the criteria were selected. The patients were given all the relevant information about the study and those that gave informed consent were recruited.

## **7. DATA COLLECTION**

### **7.1 CLINICAL METHODS**

The sociodemographic (age, gender, marital status, level of education and occupation) and clinical data; including history of intravenous drug use were obtained from the patient.

History targeted on presence of infections or conditions that would help clinically stage the patients disease as per WHO clinical staging (Appendix 4) as well as history of bleeding tendencies such as epistaxis, haematuria or petechiae were obtained. Physical examination was done to also ascertain the WHO clinical staging and evidence of bleeding tendencies. This information was filled in the study proforma (Appendix 3).

### **7.2 LABORATORY METHODS**

3mls of blood was collected from the antecubital fossa in each patient and immediately put in an EDTA bottle. It was taken to the CCC laboratory for evaluation and preparation of a peripheral blood smear that was read by a qualified Haematologist.(Appendix 5)

A total blood count was done within four hours using Haematology Cell counter (Beckam Coulter Ac.T5 diff) and peripheral blood film prepared, stained by Romanwsky stain/MGG and interpreted by haematologist using light microscopy.

**CD4 count** was done on the same sample using the Cyflow machine (Partec Model); flow cytometry within four hours of sample collection.

### **7.3 QUALITY ASSURANCE**

The recommended procedure for specimen collection, proper labelling and storage was followed strictly at all times to minimise sources of errors.

The laboratory runs internal and external quality controls whereby the internal controls are done daily and the latter monthly.

Monthly audits are done in collaboration with; KEMRI, National Reference Laboratories, Paediatric Department Lab and Kenyatta Main Lab.

External audits are done twice yearly by a German laboratory, Deutsche Vereinte Gesellschaft.

The lab is run by well trained qualified staff.

The peripheral blood films were read by qualified haematologist with vast experience.

## **8. DATA MANAGEMENT AND ANALYSIS**

### **8.1 DATA ENTRY AND MANAGEMENT**

Data was entered into the questionnaire by the principal investigator and study assistant. The forms were reviewed by the principal investigator to ensure they have been entered appropriately. Errors found were corrected and those that could not be corrected or incomplete were excluded.

The data was then entered and analysed using the Statistical Package for Social Scientists (SPSS USA Inc) Version 16.0.

## **8.2 DATA ANALYSIS**

Descriptive statistics such as frequencies, proportions, measures of central location and variation (mean, median, ranges and standard deviation) were used for most variables (age, gender, level of education, WHO clinical staging among others). The above data is presented in tables, pie charts or bar graphs. The prevalence of thrombocytopenia is expressed as a proportion in percentage. (number with TCP/ study population). The Student t-test was used for continuous variables such as age and Chi-square test/ Fischer' Exact test for categorical variables to compare socio-demographic data and other factors such as WHO clinical staging, CD4 count between patients who have TCP with those who do not.

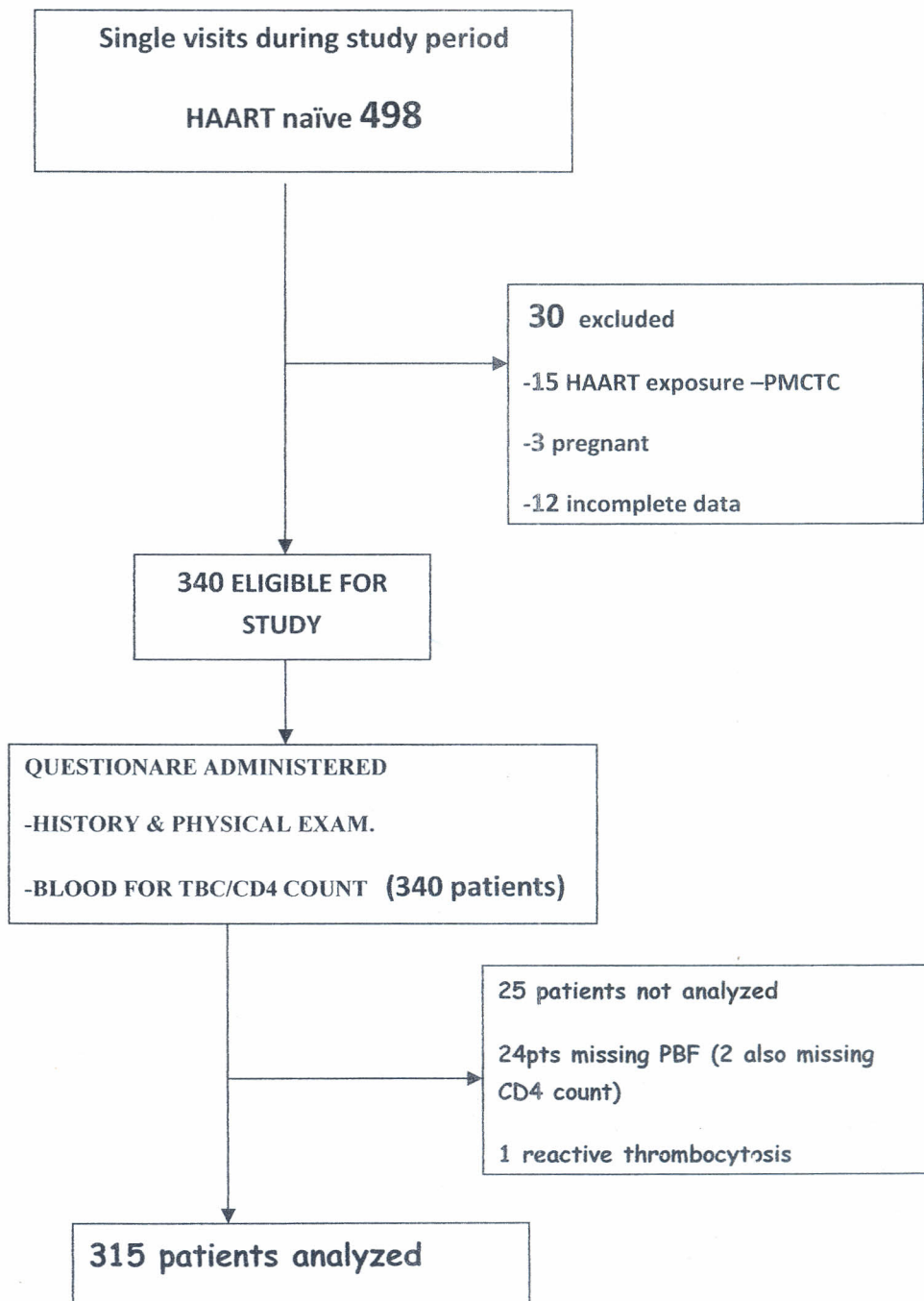
## **9. ETHICAL CONSIDERATIONS**

1. Permission to carry out the study was sought from the Kenyatta National Hospital/University of Nairobi Scientific and Ethical Review Committee.
2. Patients were enrolled into the study only after giving informed consent.
3. The usual care and evaluation of procedures was facilitated.
4. Results of the investigations were communicated to the primary health care providers at the Comprehensive Care Centre to facilitate HIV/AIDs care as required.
5. Confidentiality with each client was maintained.
6. The benefits for the patients who participated in the study was that they had thorough assessment ( history taking and physical examination) as well as blood tests on haematological parameters and CD4 count and for those found to have thrombocytopenia or other illnesses without adequate treatment advice was given to the primary care giver.



## 10. RESULTS

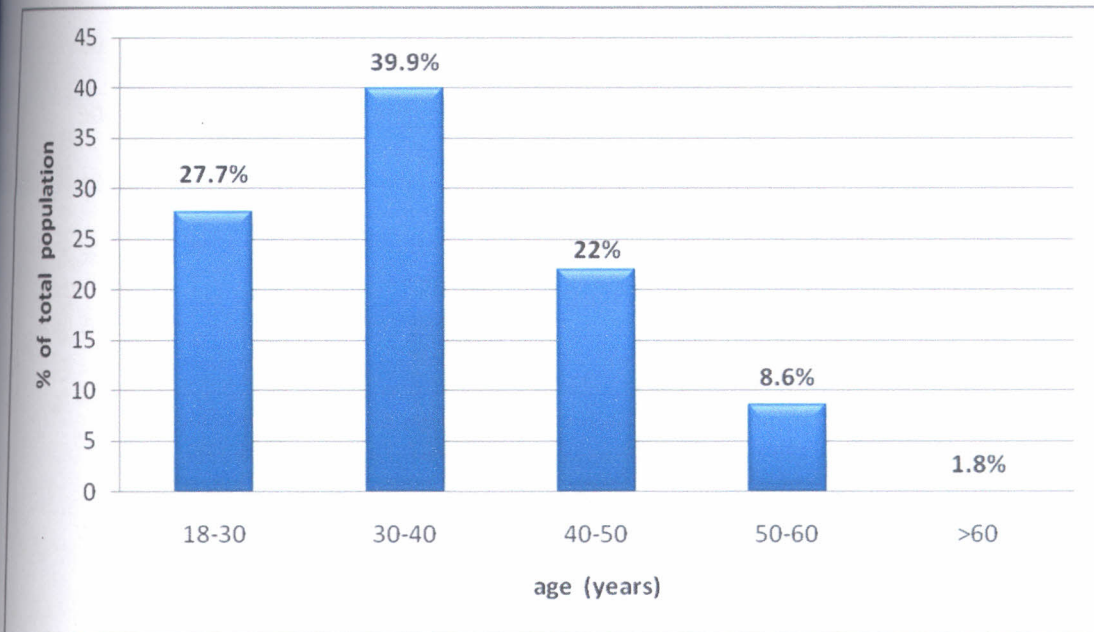
Between July 2009 and January 2010, 370 patients were evaluated for inclusion into the study. 18 were excluded as indicated in the flow chart below. 340 patients were analyzed.



( Figure 1: flow chart on screening and recruitment of study patients)

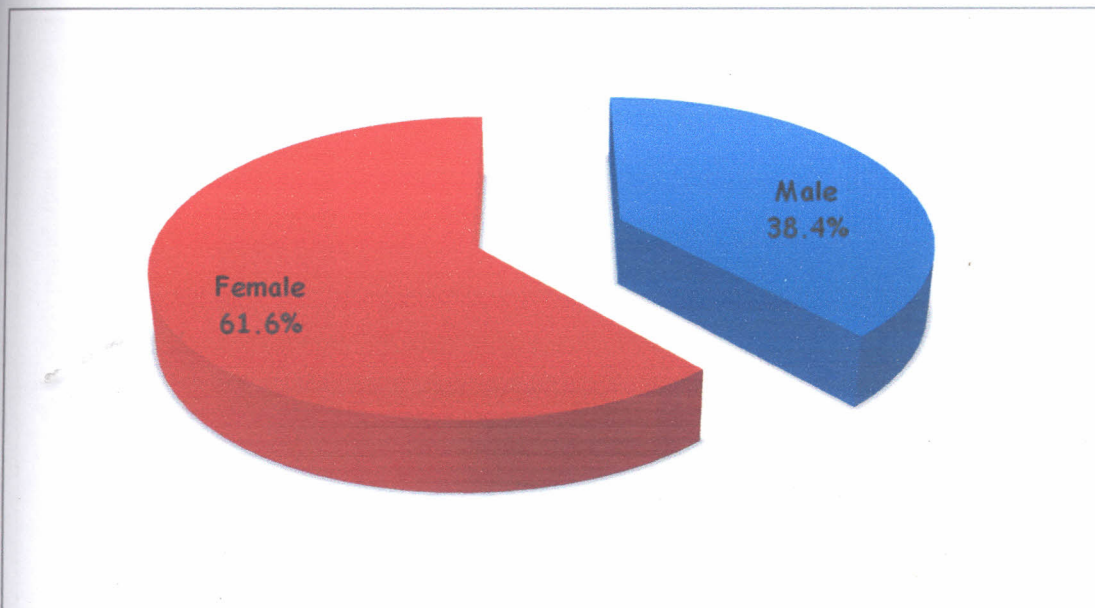
## 10.1 SOCIO-DEMOGRAPHIC CHARACTERISTICS

**Figure 2: Age distribution of study population**



The peak age group of the study population was 30-40yrs with a mean age of 37.3yrs (SD 9.8).

**Figure 3: Gender of study population**



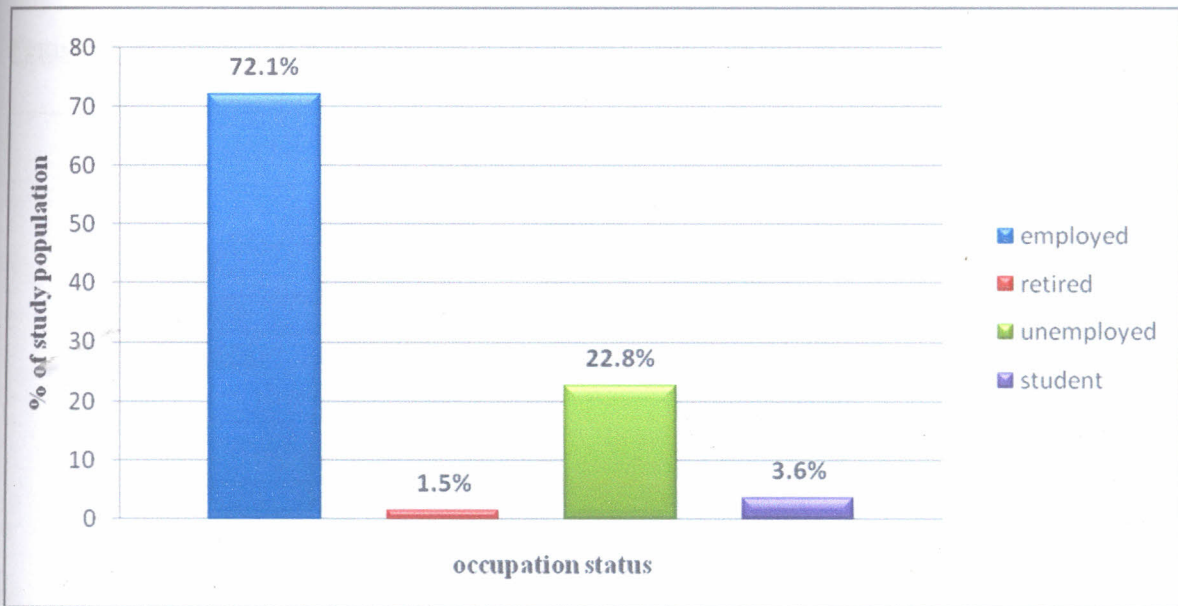
Majority of the patients were females (61.6%).

**Figure 4: Marital Status of the study population.**



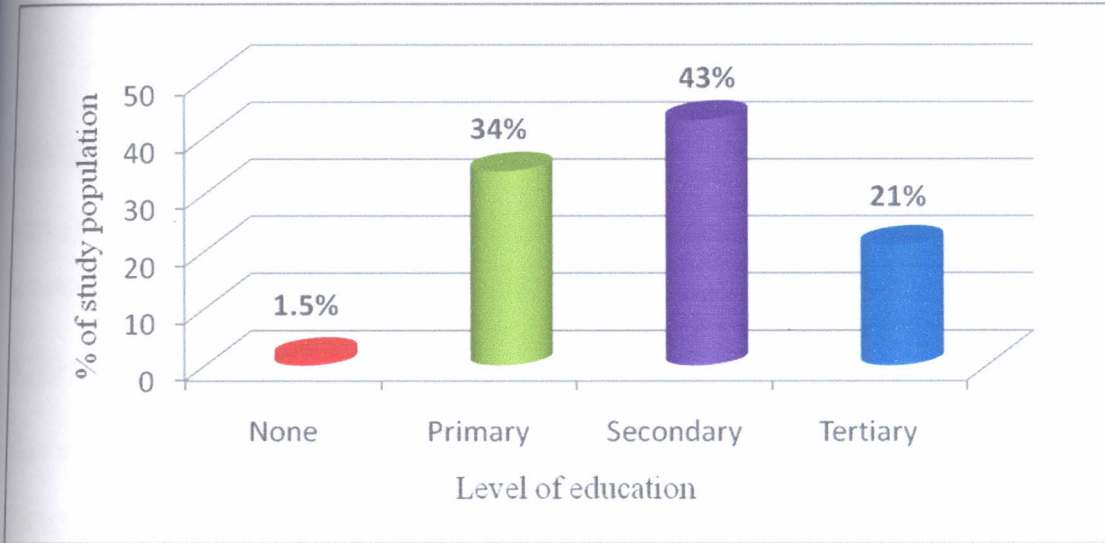
Most of the study population was married 58.6%.

**Figure 5: Occupation of study population**



Most of the patients were employed with 39.3% being formally employed and 32.7% being self employed.

**Figure 6: Level of Education of study population**



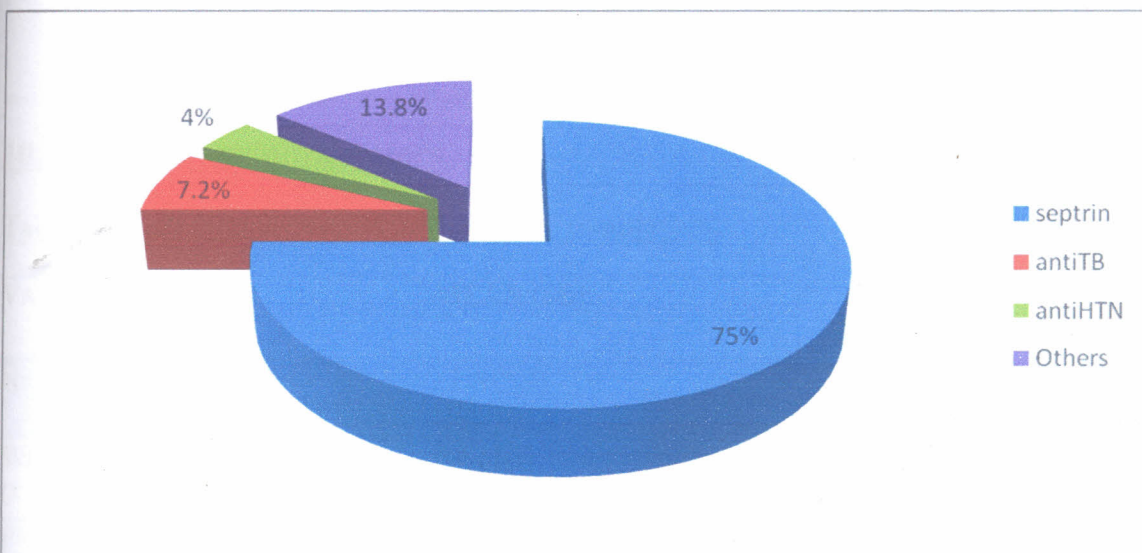
Majority of the patients were educated with most having secondary education (43%) whereas only 1.5% had no education.

**10.2 Other Baseline characteristics**

None of the patients in the study population reported being intravenous drug users.

62.9% were on prescription medications mostly:-

**Figure 7: Prescription medications used by the study population.**



- The other medications were: - dapsone (alternative to septrin due to adverse drug reactions), antibiotics, acid lowering treatment (Proton Pump Inhibitors, Histamine-2 receptor blockers), antacids, antibiotics, anticonvulsants, herbal medications.

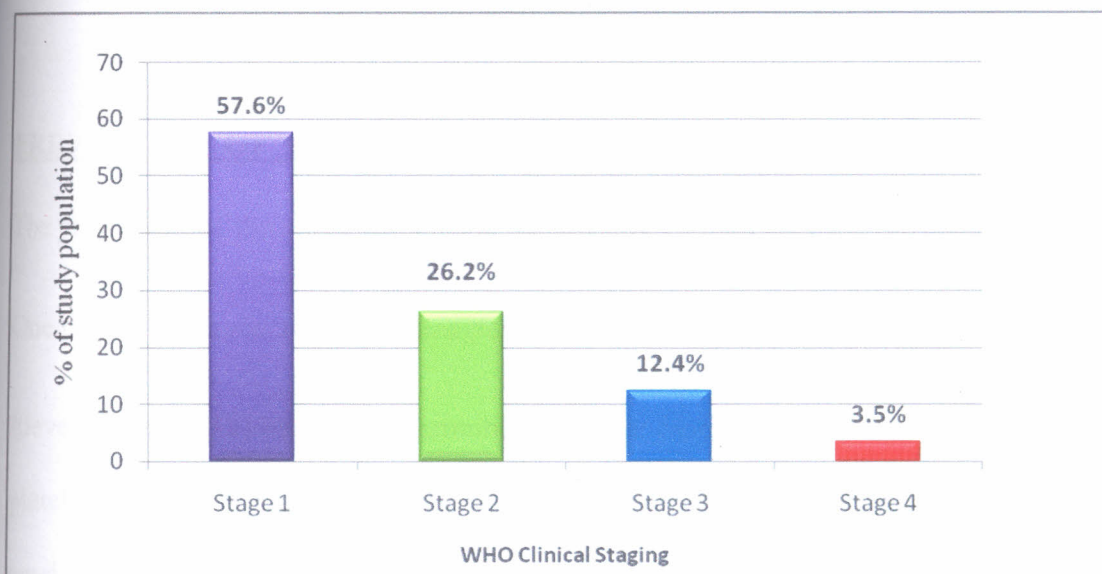
**Bleeding tendencies**

3.8% of the study population reported bleeding tendencies (mostly epistaxis, two cases of hematochezia, single cases of menorrhagia, hemoptysis & hematuria).

**WHO Clinical Staging**

Most of patients were in WHO Clinical Stage 1 (57.6%) as illustrated in the bar chart below.

**Figure 8: WHO clinical staging of study population**



**10.3 Laboratory parameters**

**Table 1: Total Blood Cell Count of study population.**

VARIABLE	MEAN (SD)
Haemoglobin (gm/dl)	12.8 (2.4)
White Blood Cell count (x 10 <sup>9</sup> /l)	5.6 (3.2)
Platelet count (x 10 <sup>9</sup> /l)	246.3 (104.4)

The mean CD4 count of the study population was 388.5cell/mm<sup>3</sup> (SD 228) and when stratified into three groups, majority of patients (54.4%) had CD4 count between 200-500 cells/mm<sup>3</sup>.

**Table 2: Distribution of CD4 count of study population.**

CD 4 count category	Frequency (%)
>500	85 (25%)
200-500	185 (54.4%)
<200	68 (20%)
Missing	2 (0.6%)

### **PREVALENCE OF THROMBOCYTOPENIA**

The prevalence of thrombocytopenia is 3.8%. Only 12 patients had confirmed thrombocytopenia.

One patient had a reactive thrombocytosis.

Eleven patients (11) had pseudothrombocytopenia: cases in whom coulter counter reading of platelet was low but on peripheral blood film the manual count was normal thus emphasizing the need of peripheral blood film. The table below better illustrates the cases found in this study.

**Table 3 : Cases of Pseudothrombocytopenia**

Platelet count (Coulter counter)	Number
<20,000 (19,200)	1
21-100,000 (93,000)	1
101-150,000 (range 136-149,000)	9

### Characteristics of those with thrombocytopenia

The mean age of these patients was 40yrs with male to female ratio of 1:1.

10 of the 12 patients were married and all were educated (five had primary and secondary, two tertiary). Majority were employed.

*There were no patients who reported use of intravenous drugs.*

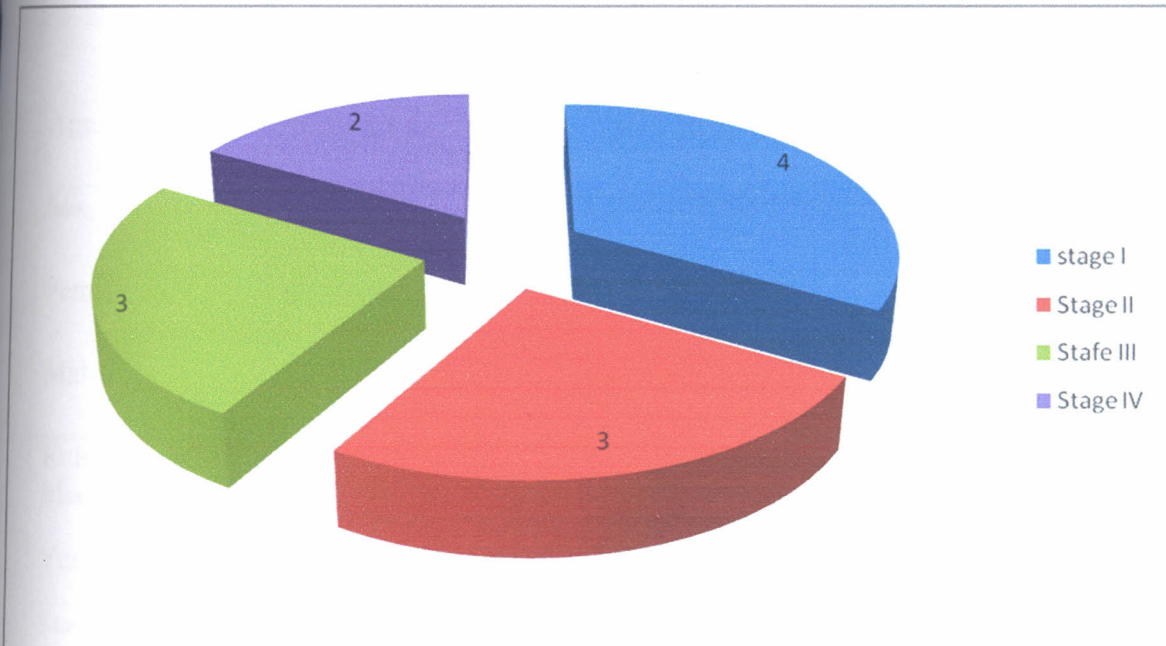
Two patients had evidence of bleeding. The first patient who reported haematuria, was a 28 year old male in WHO clinical stage III with CD4 count of 116cells/mm<sup>3</sup> and on septrin prophylaxis. His platelet count was 74,000cells/ul and he had a pancytopenia (haemoglobin 10.6gm/dl and white cell count 3.3). The second patient had epistaxis. He was in WHO clinical stage IV and not on any medications, his CD4 count was 189cell/mm<sup>3</sup> with platelet count of 60,000cell/ul. The total blood count and manual count on peripheral blood film of this patient was bicytopenia with anaemia of 6gm/dl.

Seven had history of drug exposure with most being septrin prophylaxis.

Four (4) patients were in WHO clinical stage I, three (3) in stages II and III each and two (2) in stage IV as illustrated in the figure 9.

**Figure 9: Distribution of thrombocytopenia in HIV WHO Clinical**

Stages



Thrombocytopenia was isolated in 2 cases. These two patients were both in WHO clinical stage I which mostly comprises those with Primary HIV Associated Thrombocytopenia (PHAT) which is an immune thrombocytopenia. In 8 patients bicytopenia was seen with platelets and red blood cells or leucocytes reduced. The patients who had bicytopenia were evenly distributed across all WHO Clinical Stages I- IV. Pancytopenia occurred in 2 cases in WHO clinical stage III.

There mean CD4 count was 310 with a range of 80- 369cells/mm<sup>3</sup>.



**Table 4: Correlation of thrombocytopenia with different variables**

Variable	Thrombocytopenia	No Thrombocytopenia	P value	OR (95% CI)
Age (mean yrs)	40.0 (8.1)	37.2 (9.7)	0.328	-
Sex				
Female	6 (50.0%)	192 (63.4%)	0.372	-
Male	6 (50.0%)	111 (36.6%)		
Evidence of bleeding				
Yes	2 (16.7%)	9 (3.0%)	0.011*	6.6 (1.3-34.4)
Staging				
Stage I	4 (33.3%)	180 (59.4%)	0.075	0.3 (0.1-1.2)
Stage II	3 (25.0%)	80 (26.4%)	0.919	0.9 (0.2-3.5)
Stage III	3 (25.0%)	34 (11.2%)	0.144	2.6 (0.7-10.3)
Stage IV	2 (16.7%)	9 (3.0%)	0.011*	6.6 (1.3-34.4)
Drug exposure				
Yes	7 (58.3%)	194 (63.8%)	0.763	0.8 (0.2-2.6)
Hb	11.6 (4.4)	12.9 (2.3)	0.074	-
WBC	6.7 (4.9)	5.6 (3.2)	0.265	-
CD <sub>4</sub> count	261.3 (177.0)	401.7 (226.7)	0.035*	-

\*p value of <0.05 was considered significant.

There was no difference in age and gender.

Patients with thrombocytopenia tended to have six times more evidence of bleeding compared to those with normal platelet count and this was statistically significant with  $p$  0.01. With a very wide confidence interval one cannot make a significant statistical inference therefore a study with larger numbers would be required.

The patients with thrombocytopenia were more likely to be in Stage IV, had a lower CD4 count which was statistically significant ( $p$  value 0.035).

**Table 5: Association of CD<sub>4</sub> count levels and thrombocytopenia**

Variable	Platelet count		P value
	Thrombocytopenia	Normal	
CD <sub>4</sub> count			
<200	5 (41.7%)	53 (17.5%)	0.050*
≥200	7 (58.3%)	249 (82.5%)	

**\*significant association  $p < 0.05$**

A lower CD4 count was significantly associated with thrombocytopenia when stratified as CD4 count of less than or more than 200.

## 11: DISCUSSION

Haematological abnormalities are common in HIV infected patients with higher prevalence as disease progresses.<sup>39</sup> HIV related thrombocytopenia is caused by immune destruction of platelet secondary to platelet antigenic mimicry or reduced bone marrow production. Previous studies report the prevalence of thrombocytopenia among HIV infected persons to be high ranging from 9% to 37% in various study populations. It is associated with progression of disease and is a predictor of mortality<sup>12, 14</sup>.

Like in most local studies our population was young with majority (39.9%) being between the age of 30-40years. This is similar to studies at the same clinic done by *Muita*<sup>40</sup> on the prevalence and correlates of anaemia in patients infected with HIV attending the KNH CCC. He found 48.8% being in the same age bracket (30-40yrs) and *Ngare*<sup>41</sup> who studied the prevalence of hypertension in HIV infected patients at the KNH CCC: mean age in that study was 39.3years (SD 0.4). The Kenya Demographic and Health Survey of 2007 reports that most of the HIV infected population in Kenya is young; 15-49years of age.<sup>42</sup>

Most of our study patients were female (61.6%), a finding similar to the studies by *Muita*<sup>40</sup> and *Ngare*<sup>41</sup>. This trend brings to question the issue of gender and health seeking behavior as has been reported by *Nyamongo et al*<sup>43</sup> in August 2003 looking at the Health Seeking Behaviour and Health Systems response in Kenya. He found that men delayed in seeking health services as this portrayed a sign of weakness. Similar conclusions were reached in March 2007 in a report in Capetown, South Africa by *Jolene Skordis et al*<sup>44</sup> that was looking at the role of Gender in Health seeking Behaviour and Expenditure. They observed that whereas men delayed in seeking treatment, female participants were more effective monitors of their own health. Studies on the

prevalence of HIV in Kenya during years 2008/2009 found a higher prevalence of 8% in women compared to 4.3% in men. This can account for the larger female population of HIV patients attending the clinic.<sup>47</sup>

Similar to other local studies majority of these patients had secondary education and most were employed.<sup>40, 41</sup>

The prevalence of thrombocytopenia in this study population was 3.8%. This is low compared to other studies. *Muita*<sup>40</sup> who was studying anaemia and its correlates reported similar low prevalence of 4.5%. This slightly higher figure could have been due to cases of pseudothrombocytopenia which were not excluded.

Factors that may have contributed to this low prevalence are majority of our patient population being of young age contrary to studies that have associated a higher incidence with increase in age. *Sloand et al*<sup>7</sup> in a three year study of 1004 HIV infected patients in two medical outpatient clinics found the incidence of thrombocytopenia to be higher in the older age group. Similar findings were reported by *Sullivan et al*<sup>14</sup> who did a surveillance of thrombocytopenia in 30,214 HIV infected patients in 100 different medical clinics in the USA.

Male gender has been strongly associated with the occurrence of thrombocytopenia in the studies by *Sloand et al*<sup>7</sup> and *Sullivan et al*<sup>14</sup> whereas in this study there was a predominant female population which may be another factor that led to the low prevalence found in the study.

Thirdly the absence of patients reported to be intravenous drug users could have affected our results as intravenous drug use has been positively correlated with increased incidence of thrombocytopenia. A possibility of patients withholding this information however cannot be ruled out. It has been noted by *Sloand et al*<sup>7</sup> in 1992 that thrombocytopenia was more common

in intravenous drug users. Similar findings were observed by *K.Alaei et al*<sup>10</sup> who studied the prevalence of thrombocytopenia in HIV infected patients in Iran. All but 7 out of 170 patients in the Iranian study reported to be intravenous drug users. *Mientjes et al*<sup>15</sup> in 1992 looked at two cohorts' homosexual and intravenous drug users with and without HIV and found both uninfected groups had thrombocytopenia but the prevalence markedly increased in those who were HIV infected. Another factor in consideration is that in Kenya the main mode of HIV transmission is heterosexual unlike the west where homosexual and intravenous drug use mode of transmission predominates.

Many of the patients were on septrin prophylaxis which is also known to cause blood dyscrasias including thrombocytopenia however this study did not set out to establish aetiology of the thrombocytopenia.<sup>46</sup> Nevertheless Sullivan et al found no association between thrombocytopenia and prescription of septrin.<sup>14</sup>

The other contributor to low prevalence was 57.6% of study population being in WHO clinical stage 1 whereas thrombocytopenia is more in advanced HIV disease<sup>3</sup>. This could be explained by the increasing awareness of the need for knowing ones' HIV status since most of the patients were referred from various VCT centers (mainly KNH and KEMRI). Most of the patients referred from the wards had already been initiated on HAART though the numbers at the CCC cannot account for all KNH in-patient referrals. This finding however confirms increased levels of HIV/AIDs awareness among the KNH catchment area population.

Having a predominant asymptomatic non-AIDs population could have been due to recruitment of patients primarily from outpatient clinic. *Adewuyi et al*<sup>8</sup> in Zimbabwe found a higher prevalence of 24.7% because he included in-patients who would have more co-morbidities compared to

outpatients hence this could contribute to higher numbers and there was a better representation by patients in all the WHO clinical staging more so stage III and IV . In Nigeria *Erharbor et al*<sup>9</sup> studied hematological parameters in HIV infected Nigerians and found prevalence of 10%. Most of the patients in his study had symptomatic HIV infection whereas in this study majority of patients were WHO Clinical Stage 1. In 1991 *Peltier et al*<sup>45</sup> in France studied the frequency and prognostic importance of thrombocytopenia in 435 symptom free HIV infected individuals (5yr prospective study) and found a 5.5% prevalence. This was a symptom free population similar to this study except that there were more intravenous drug users and the relative low figure supports the higher occurrence of thrombocytopenia in advanced disease.

Our study findings also support association of thrombocytopenia with advanced disease as it was more in those with WHO Clinical Stage IV (16.7% versus 3.0%) p value 0.011 and those with thrombocytopenia had lower CD4 count; mean 261.3 versus 401.7 with p value 0.035. The association being for those with CD4 <200cells/ul (p 0.005). This is supported by findings of *Luke Perkocha et al*<sup>3</sup> who in 1988 reviewed previous studies on hematopathology of AIDs concluded that cytopenias associated with HIV tend to be more prevalent and severe in groups with advanced disease. Study by *Sloand et al*<sup>7</sup> on Epidemiology of thrombocytopenia also found that patients with low CD4 count and advanced disease were more likely to have low platelets. Isolated thrombocytopenia was found in patients with WHO stage 1 and this is supported by Italian based study by *R. Calteneo et al*<sup>13</sup> who concluded that the presence of thrombocytopenia with other cytopenias (anaemia or leucopenia) was a manifestation of AIDs but isolated thrombocytopenia doesn't correlate with progression of disease.

Bleeding tendency was more in those with thrombocytopenia (16.7% versus 3% p 0.011).

Despite having few numbers thrombocytopenia was associated with more bleeding tendencies

(OR 6.6 95% CI 1.3-34) and this was statistically significant but the wide confidence interval indicates larger numbers would be required to ascertain with precision the association in our setup. The two patients with reported bleeding had relatively high platelets of 74,000/ml and 60,000/ml compared to levels below which spontaneous bleeding is expected. This can be explained by the fact that studies have shown that not only is platelet count important in predicting bleeding but the function also counts. Studies have also shown that platelet function is also impaired in HIV infected patients and that even those with normal platelet count may have platelet antibodies present in the serum. *Sloand et al*<sup>7</sup> reported 40% of those with platelet count <50,000cells/ul reported bleeding but 1 had fatal bleed; intracranial haemorrhage.

Although our study had few cases of thrombocytopenia the association with bleeding tendencies and advanced disease has implications on patient management. Despite platelet counts above 20,000 cell/ul patients had bleeding tendencies, its' occurrence with other cytopenias and further association with advanced disease may all be indicators for evaluation to consider early initiation of antiretrovirals.

## **12.0: Conclusions**

The prevalence of thrombocytopenia in the KNH CCC HAART naïve HIV infected patients is low.

Though limited by having few numbers, thrombocytopenia is associated with bleeding tendency at platelet counts  $>20,000$ cell/ul, advanced disease as evidenced by WHO stage IV and a lower CD4 count.

## **12.1 Recommendations**

1. Larger studies with all WHO clinical stages done to ascertain associations observed in this study or case control study.
2. Patients with thrombocytopenia especially in association with other cytopenias should be evaluated aggressively since this may indicate advanced disease.

## **12.2 Study limitations**

- Few of cases of thrombocytopenia therefore not able to make significant statistical inferences from the data.
- Excluding in patients underestimated burden of thrombocytopenia.
- Financial limitations hence could not do platelet antibodies and thrombopoietin levels to correlate with platelet count.
- Hospital based study hence not generalizable to the public.



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## **APPENDIX 1: CONSENT EXPLANATION**

My names are Dr. Matilda Ongóndi, a postgraduate student in Internal Medicine. I am conducting a research study on patients attending the Kenyatta National Hospital Comprehensive Care Clinic.

### **Purpose of the study**

To determine the prevalence of thrombocytopenia in HIV infected patients who are not taking antiretrovirals and describe the associated socio-demographic, clinical and laboratory characteristics.

### **Procedures**

If you agree to join this study you will be requested to;

1. Answer questions relating to your socio-demographics, present illnesses or significant past illnesses that will help categorize you based on WHO staging or suggest bleeding tendencies.
2. Undergo a physical examination inclusive of measurements of height and weight.
3. Have 3mls of venous blood drawn so as to get the following: total blood count, peripheral blood film and CD4 count.

Participation in this study is voluntary and you can choose to decline or withdraw from the study without any penalty.

### **Risks.**

Mild pain may be experienced during withdrawal of blood sample for laboratory tests.

### **Benefits.**

1. All the above examination and procedures shall be done free of charge.
2. A copy of the results shall be availed to your file and the doctor informed of these results.
3. For those found to have thrombocytopenia the primary care givers will be informed so as to institute the appropriate management.

## Confidentiality

Strict confidentiality will be maintained and all the data obtained will be securely stored and used for purposes of this study only.

If you have no objection to this study you will be required to sign an informed consent form.

If one has any questions concerning the study you can contact the following:

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**APPENDIX 2: CONSENT FORM**

I.....

after reading the consent explanation form and having been explained to by Dr Matilda Ongóndi (the principal investigator) do voluntarily agree to take part in this study on **THROMBOCYTOPENIA IN HAART NAÏVE HIV INFECTED PATIENTS ATTENDING THE COMPREHENSIVE CARE CLINIC AT THE KENYATTA NATIONAL HOSPITAL.**

I am also aware that I can withdraw from this study without losing any benefits or the quality of management of my medical problem being affected.

SIGNED.....

THUMB PRINT .....

WITNESS.....

DATE: \_\_\_\_\_



**APPENDIX 3: STUDY PROFORMA**

Study NO

DATE: ...../...../.....

IP NO:

Contact Details: P. O. Box \_\_\_\_\_

Mobile No: \_\_\_\_\_

Age: \_\_\_\_\_

Date of birth: ...../...../.....

**Demographics**

Gender: M\_\_\_ F\_\_\_

**Marital Status:**

Single\_\_\_ Married\_\_\_ Divorced\_\_\_ Widowed\_\_\_ Separated\_\_\_

**Occupation:** employed\_\_\_ unemployed\_\_\_ self employed\_\_\_ retired\_\_\_ student\_\_\_

**Level of education:** none\_\_\_ Primary\_\_\_ Secondary\_\_\_ Tertiary\_\_\_

**IV drug use (specify):** \_\_\_\_\_

**Exposure to other drugs and duration:**

.....

**History and Physical Examination**

Bleeding tendency: Nose bleeding \_\_\_\_\_

Petechiae \_\_\_\_\_

Purpura \_\_\_\_\_

Ecchymosis \_\_\_\_\_

GIT Bleeding \_\_\_\_\_

GUT bleeding: \_\_\_\_\_

Others: \_\_\_\_\_

**Physical Examination:**

WHO CLINICAL STAGE I \_\_\_\_\_

II \_\_\_\_\_

III \_\_\_\_\_

IV \_\_\_\_\_

Evidence of bleeding tendency: \_\_\_\_\_

(Petechiae/ ecchymosis)

**Laboratory measures**

Hb \_\_\_\_\_

WBC \_\_\_\_\_

Platelet count \_\_\_\_\_

CD4 count: \_\_\_\_\_ cells/ul

PBF \_\_\_\_\_

## **APPENDIX 4: WHO CLINICAL STAGING (infected adults and adolescents 2006)**

### **STAGE 1**

Asymptomatic

Persistent generalized

Lymphadenopathy

### **STAGE 2**

Moderate unexplained weight loss (<10% of presumed or measured body weight)

Recurrent respiratory tract infection (RTI's, Sinusitis, bronchitis, otitis media, pharyngitis)

Herpes zoster

Angular cheilitis

Recurrent oral ulceration

Papular pruritic eruptions

Seborrhoeic dermatitis

Fungal nail infections

### **STAGE 3**

Unexplained severe weight loss (>10% of presumed or measured Body weight)

Unexplained chronic diarrhea for longer than one month

Unexplained persistent fever (above 37.5 C intermittent or Constant for longer than one month)

Persistent oral candidiasis,

Oral hairy leukoplakia.

Pulmonary tuberculosis

Severe bacterial infections (e.g. pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteraemia)

Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis

Unexplained anemia (<8 g/dl), neutropenia (<0.5 x 10 /L) and/or chronic thrombocytopenia (<50 X 10 /L)

#### **STAGE 4**

HIV wasting syndrome

Pneumocystis pneumonia

Recurrent severe bacterial pneumonia

Chronic herpes simplex infection (Orolabial, genital or anorectal of More than one month's duration or visceral at any site)

Esophageal candidiasis (or candidiasis of trachea, bronchi or lungs)

Extra pulmonary tuberculosis

Kaposi's sarcoma

Cytomegalovirus infection (retinitis or infection of other organs)

Central nervous system toxoplasmosis

HIV encephalopathy

Extra pulmonary cryptococcosis including meningitis

Disseminated non-tuberculous mycobacteria infection

Progressive multifocal leukoencephalopathy

Chronic cryptosporidiosis

Chronic isosporiasis

Disseminated mycosis (extra pulmonary histoplasmosis, coccidiomycosis)

Recurrent septicemia (including non-typhoidal salmonella)

Lymphoma (cerebral or B cell non-Hodgkin)

Invasive cervical carcinoma

Atypical disseminated leishmaniasis

Symptomatic HIV associated nephropathy

Symptomatic HIV associated cardiomyopathy.

## Appendix 5: Preparation and reading of a peripheral smear

Place a small drop of blood at one end of the slide then using a spreader, spread the blood to the opposite end of the slide.

Air dry the films.

Fix by immersing in a jar of methanol for 10 minutes then transfer the slides to a jar with May-Grunwald stain and leave it in for another 10 minutes. Transfer the slides to a jar containing Giemsa and leave it for 10 minutes and a second jar with similar stain for same amount of time.

Transfer the slides to a jar with buffered water (PH 6.8) and leave for 10 minutes.

Finally transfer the slides to water and leave undisturbed for 2-5minutes then stand and leave to dry.

Examination of the peripheral blood film shall be done under light microscopy beginning with low power (X10) as progress to high power with oil emulsion.

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