CORRELATION BETWEEN THROMBOCYTOPENIA AND ANAEMIA IN *Plasmodium falciparum* MALARIA AMONG PATIENTS IN KISUMU COUNTY-WESTERN KENYA

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Correlation between Thrombocytopenia and Anaemia in *Plasmodium falciparum* malaria among patients in Kisumu County-Western Kenya

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A Thesis Submitted in partial fulfilment for the Degree of Master of Medical Laboratory Sciences (Clinical Haematology and Blood Transfusion option) in the Jomo Kenyatta University of Agriculture and Technology

2016
DECLARATION

This thesis is my original work and has not been presented in any institution leading to the award of a degree or any other award.

Sign……………………………….. Date………………………………………………

Kosiyo Paul Mboya

We confirm that this thesis was written by the above named student and has been submitted with our approval as supervisors.

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Finally, I am thankful to my entire family members for their emotional support and encouragement that made this a success.

God bless you abundantly
DEDICATION

This work is sincerely dedicated to my dear mother the late Jenipher Atieno for her inspiration into academic career.
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OPERATIONAL TERMS

Anaemia: Is a haematological disorder characterised by the reduction of the haemoglobin level to below the reference range depending on age, sex and altitude.

Thrombocytopenia: is a haematological disorder characterised by the reduction in the number of platelets in the peripheral blood to below the reference range, depending on age.

Malaria: Is disease/infection caused by a protozoan called Plasmodium of different species.

Haemoglobin: this is a conjugated protein with high affinity for oxygen and low affinity for carbon dioxide within red blood cells.

Lysis This is the mechanical breakdown of a cell resulting in the release of its contents.

Prevalence: is the proportion of individuals at risk in a specified population who have the disease of interest at a given time.

Incidence: Is number of new cases of a disease in a specified period of time in a population at risk.

Differential: is a systemic diagnostic method or the process of weighing the diagnosis probability of one disease versus that of other disease possibly accounting for patient’s illness.

Cytopenia: Is a general reduction of all the blood cells in the peripheral blood.
**Endemicity:** (or disease intensity) is a measure of disease prevalence in a particular region.

**Protozoa:** Are unicellular, eukaryotic organisms.
## ACRONYMS AND ABBREVIATIONS

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBC</td>
<td>Complete blood count</td>
</tr>
<tr>
<td>DOMC</td>
<td>Division of Malaria Control</td>
</tr>
<tr>
<td>DIC</td>
<td>Disseminated intravascular haemolysis</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene Diamine Tetra acetic Acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme linked immunoabsorbent assay</td>
</tr>
<tr>
<td>g/dL</td>
<td>Grams per decilitre</td>
</tr>
<tr>
<td>G-6PD</td>
<td>Glucose 6 Phosphate Dehydrogenase</td>
</tr>
<tr>
<td>Hb</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency syndrome</td>
</tr>
<tr>
<td>JOOTRH</td>
<td>Jaramogi Oginga Odinga Teaching and Referral Hospital</td>
</tr>
<tr>
<td>KMIS</td>
<td>Kenya Malaria Indicator Survey</td>
</tr>
<tr>
<td>KNBS</td>
<td>Kenya National Bureau of Statistics</td>
</tr>
<tr>
<td>Mls</td>
<td>Millilitres</td>
</tr>
<tr>
<td>MPS</td>
<td>Malaria parasites</td>
</tr>
<tr>
<td>MPV</td>
<td>Mean platelet volume</td>
</tr>
<tr>
<td>MTG</td>
<td>Malaria treatment guidelines</td>
</tr>
<tr>
<td>OPD</td>
<td>Outpatient Department</td>
</tr>
<tr>
<td>PBF</td>
<td>Peripheral blood film</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>PCT</td>
<td>Plateletcrit</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PDW</td>
<td>Platelet distribution width</td>
</tr>
<tr>
<td>Pf</td>
<td><em>Plasmodium falciparum</em></td>
</tr>
<tr>
<td>PI</td>
<td>Principal Investigator</td>
</tr>
<tr>
<td>Plt</td>
<td>Platelet</td>
</tr>
<tr>
<td>RBC</td>
<td>Red blood cells</td>
</tr>
<tr>
<td>RDW</td>
<td>Red cell distribution width</td>
</tr>
<tr>
<td>SMA</td>
<td>Severe malaria anaemia</td>
</tr>
<tr>
<td>WHO</td>
<td>World health Organization</td>
</tr>
</tbody>
</table>
ABSTRACT

Malaria is associated with haematological complications which may be avoided by early diagnosis and treatment. Microscopic diagnosis showing presence of malarial parasites is needed for confirmation which requires technical expertise. The study was therefore carried out to determine the diagnostic value of haemoglobin and platelet parameters in malaria patients. The study was conducted in Kisumu County-Kenya a holoendemic malaria region. *Plasmodium falciparum* infection status was determined using both thick and thin smears in infected; n=157 and non-infected; n=71 patients presenting with acute febrile illness at JOOTRH. Severe to moderate anaemia was present in 78 % (n=122) of malaria infected patients against 47 % (n=33) of the non-infected group (p<0.001) while thrombocytopenia was present in 87 % (n=137) of the infected patients against 10 % (n=7) of the non-infected group (p<0.001). All participants’ demographics and haematological parameters i.e. haemoglobin level, platelet count, mean platelet volume and platelet distribution width were analysed. Spearman correlation test revealed a positive correlation between anaemia and thrombocytopenia during malaria infection (ρ= 0.26, p<0.001). Non-parametric analysis showed that both haemoglobin and platelet counts were significantly lower in the malaria infected group compared to non-infected group [Hb; 7.7g/dl vs. 10.2g/dl and platelet counts; 88x10⁹/l vs. 297x10⁹/l respectively, (p<0.001)]. Conversely, mean platelet volume and platelet distribution width were higher in infected group relative to non-infected group [MPV; 17.77fL vs. 17.08fL and PDW; 9.81% vs. 7.98% respectively, (p<0.001). Taken together, these results show that platelet count and haemoglobin levels are important predictors of *P. falciparum* malaria when used in combination with other clinical manifestations and can be used in diagnosis of malaria.
CHAPTER ONE

INTRODUCTION

1.1 Background information

In the year 2013, 97 countries had on-going malaria transmission. An estimated 3.4 billion people are at risk of malaria, of which 1.2 billion are at high risk. In high-risk areas, more than one malaria case occurs per 1000 population (WHO, 2013). In 2012, malaria killed an estimated 482 000 children under five years of age in sub-Saharan Africa. This translates to one child almost every minute (WHO, 2013). There were an estimated 207 million cases of malaria in 2012 (uncertainty range: 135 – 287 million) and an estimated 627 000 deaths (uncertainty range: 473 000 – 789 000) of which 90% of all malaria deaths occur in sub-Saharan (WHO, 2013).

Malaria infection is associated with various haematological abnormalities including anaemia and thrombocytopenia ((WHO, 2013).however, the possible pathogenesis of the haematological abnormalities may be parasite, products-cell-derived cytokines, macrophage activation, macrophage-derived factors such as tumour necrosis factor-α, and macrophage functional abnormalities dysfunction (Wickramasinghe & Abdalla, 2000). Platelets play a critical role in the pathogenesis of malaria by causing sequestration of infected red blood cells (Wickramasinghe & Abdalla, 2000). Platelets attach malarial-infected red cells and kill the parasite within; indicating a protective function of platelets in the early stages of erythrocyte infection distinct from their role in cerebral malaria (McMorran et al., 2009).

Thrombocytopenia is a haematological disorder referring to the reduction in the number of platelets in the peripheral blood to below the reference range. The normal platelet count in adult is between 150 x 10⁹/ L and 400x10⁹/L. Severe thrombocytopenia results in excessive bleeding when a minor injury occurs to a blood vessel. The mechanism behind thrombocytopenia during malaria infection, however, remains unclear. Both immunological as well as non-immunological destruction of platelets have been implicated (Jadhav, Patkar, & Kadam, 2004). The aim of this study was to determine the frequency and severity of abnormal Hb and platelet parameters and to identify their significance in malaria patients in Kisumu County. The study also determined the
relevance of these haematological parameters as an early diagnostic tool in malaria and how the two variables correlate.

1.2 Problem statement of the study

The Ministry of Health considers malaria a national priority and remains firmly committed to malaria control, diagnosis and treatment in Kenya. This is in line with the Ministry’s vision of a nation free of preventable diseases and ill health and the national development agenda as outlined in Kenya Vision 2030, and the aims of Millennium Development Goals (MDG’s).

Malaria has been a major cause of morbidity and mortality in Sub Saharan Africa and in Kenya especially among children below 5 years (KNBS, 2011) but a cheaper effective laboratory diagnosis is still challenging which might lead to unnecessary treatment of patients with antimalarial drugs hence encouraging and worsening drug resistance.

1.3 Justification of the study

Anaemia and thrombocytopenia may hint at malaria infection and platelet count may be of major clinical value to be requested by the clinicians. Microscopic diagnosis is the perfect gold standard for malaria parasite detection and speciation. This technique requires technical expertise and is time-consuming in repeated smear examinations (WHO, 2000). However, it is a valuable technique when performed correctly in the right hands but can be unreliable and perceived as wasteful when poorly executed.

Haematological changes in malaria, such as anaemia, thrombocytopenia and leucocytosis or leucopaenia are well recognized. The extent of these alterations varies with level of malaria endemicity, background haemoglobinopathy, nutritional status, demographic factors, and malaria immunity. Presumptive anti-malarial treatment is widely practiced and studies show that it is wrought with significant misuse of anti-malarial drugs.

The information gained from this study may be helpful to healthcare providers, especially the clinicians in implementing an empirical differential diagnosis of malaria. This may be performed through the analysis of Hb levels and platelet counts and its parameters such as MPV and PDW besides the clinical manifestations. This will supplement both clinical and conventional laboratory
diagnosis without a replacement. Molecular techniques such PCR remains highly expensive for a resource limiting set up in Kenya and Sub-saharan Africa where malaria is holoendemic.

1.4 Research questions

1. What is the correlation between anaemia and thrombocytopenia in patients with *Plasmodium falciparum* malaria?
2. What is the utility of anaemia and thrombocytopenia as an early indicator in the laboratory diagnosis of malaria in patients infected with *Plasmodium falciparum*?
3. What is the applicability of platelet indices such as MPV and PDW in differential diagnosis of patients suffering from *Plasmodium falciparum* malaria?

1.5 Hypotheses

This was a hospital-based cross-sectional descriptive study so no hypothesis was adopted because descriptive studies are also known as hypothesis generating studies (Grimes and Schulz, 2002).

1.6 Objectives

1.6.1 General Objectives

To correlate anaemia and thrombocytopenia in *Plasmodium falciparum* malaria patients attending JOOTRH.

1.6.2 Specific Objectives

1. To determine the correlation between anaemia and thrombocytopenia in patients with *P. falciparum* malaria.
2. To assess the presence and severity of anaemia and thrombocytopenia in patients with *P. falciparum* malaria.
3. To determine the role of platelet indices in the differential diagnosis of patients with *P. falciparum* malaria.
CHAPTER TWO

LITERATURE REVIEW

2.1 Definition of malaria, Anaemia and thrombocytopenia.

Malaria is an infectious disease/infection caused by a protozoan called *Plasmodium* of different species i.e. *P. vivax*, *P. ovale*, *P. falciparum* and *P. malariae*. Anaemia is a haematological disorder characterised by the reduction of the haemoglobin level to below the reference range depending on age, sex and altitude ((Hoffbrand, Moss, & Pettit. 2006)and *et al*., 2006). Thrombocytopenia is a haematological disorder resulting in the reduction of the number of platelets in the peripheral blood to below the reference range ((Hoffbrand, Moss, & Pettit. 2006). The normal platelet count is 150 – 400 x 10^9/L (Hoffbrand, Moss, & Pettit. 2006)and *et al*., 2006).

2.2 Aetiology and Pathogenesis of malaria.

Malaria is caused by a protozoan called *Plasmodium* of different species which is transmitted by female anopheles mosquito as a vector. In Western Kenya, the main cause of severe malaria is *P. falciparum* (KNBS, 2011). *Plasmodium vivax*, *P. ovale*, and *P. malariae* cause low levels of parasitaemia, mild anaemia, and, in rare instances, splenic rupture and nephrotic syndrome. *Plasmodium falciparum* causes high levels of parasitaemia, severe anaemia, cerebral symptoms, renal failure, pulmonary oedema, and death (Kumar, Abbas, & Aster, 2012). The life cycles of the *Plasmodium* species are similar, although *P. falciparum* differs in ways that contribute to its greater virulence (Kumar, Abbas, & Aster, 2012).

The infectious stage of malaria, the *sporozoite*, is found in the salivary glands of female mosquitoes. When the mosquito takes a blood meal, sporozoites are released into the human's blood and within minutes attach to and invade liver cells by binding to the hepatocyte receptor for the serum proteins thrombospondin and properdin (Mikolajczak and Kappe, 2006). Within liver cells, malaria parasites multiply rapidly, releasing as many as 30,000 *merozoites* (asexual, haploid forms) when each infected hepatocyte ruptures. *P. vivax* and *P. ovale* form latent *hypnozoites* in hepatocytes, which cause relapses of malaria long after initial infection (Haldar, Murphy, Milner ., & Taylor, 2007).
2.2.1 Epidemiology of malaria.

Nearly 28 million Kenyans live in areas of malaria risk, a majority of them children under the age 15 years (WHO, 1999; KNBS, 2011). The lake endemic zone comprising of lowland counties of Nyanza and Western provinces has the highest prevalence of malaria overall of 38% while the prevalence in other zones is less than 5 % (KNBS, 2011). High intensity of malaria transmission in this region is experienced during the seasonal rainfalls in April to August and November to December (Beier et al., 1994). *P. falciparum* is the most prevalent species at 96% of which 16% comprises mixed infections with *P. ovale, P. malariae* or both (KNBS, 2011).

2.2.2 The life cycle of malaria parasites

When an infected female anopheles mosquito bites, the parasites are injected into the human body through the saliva of the mosquito. On an average 5 to 10 uni-nucleated sporozoites are injected. Within 30 minutes these sporozoites migrate to the liver and invade hepatocytes (liver cells) and develop into schizonts (Kumar, Abbas, & Aster, 2012). The schizonts multiply in the liver cells until there is no space left. Within one week of entering the liver cell, mature liver stage schizonts rupture spilling merozoites into the blood stream. Their numbers could range between 10,000 and 40,000 (Kumar, Abbas, & Aster, 2012). These merozoites in the blood stream invade circulating erythrocytes and develop into a trophozoite, secreting proteins that form knobs on the erythrocyte membrane. With the help of these knobs it attaches itself to the capillary wall affecting the microcirculation. On maturity the erythrocyte ruptures and 8 to 32 merozoites spill out from each one which in turn invades other erythrocytes which have been unaffected till now (Mikolajczak and Kappe, 2006).

The entire process of the merozoite invading the erythrocyte to maturity to ultimate rupture of erythrocyte takes approximately 48 hours (Mikolajczak and Kappe, 2006). This is one asexual life cycle of the schizont. The symptoms of malaria such as chills, fever, sweating along with nausea, vomiting and headache occur during the blood stage. If left untreated it may lead to severe anaemia, convulsion, coma and ultimately death. Some erythrocytic-stage parasites develop into sexual stage parasites called gametocytes. The bite of another female anopheles mosquito takes in these gametocytes along with its blood meal and the process of transmission to another human is repeated.
2.3 Clinical complications of malaria

Malaria is known to cause haemolytic anaemia as major haematological disorder. Other complications include thrombocytopenia and leucopenia. Children with severe malarial anaemia (SMA) have a mortality rate of about 8.6% compared with 3.6% in children with severe anaemia due to other causes (Obonyo et al., 2007). Anaemia is due to lytic destruction of red blood cells by the *P. falciparum* which is an intracellular parasite. Decreased production results from marrow hypoplasia seen in acute infections, reduced erythropoiesis and dyserythropoiesis, a morphological appearance, which in functional terms results in ineffective erythropoiesis, specific/nonspecific immune responses whereby red cell survival is shortened (Erhabor et al., 2014). Severe forms of anaemia may develop faster in the course of a malaria illness in the presence of high parasite densities and co-pathogens such as bacteria and HIV-1 (Newton et al., 1997; Otieno et al., 2006; Were et al., 2011). High parasitemia mainly in immunocompromised individuals can result into massive lysis and clearance of erythrocytes leading to profound anaemia (Phillips et al., 1986).
The cause of thrombocytopenia in malaria remains unclear. However, various causes of thrombocytopenia ranging from immunological to non-immunological have been postulated including bone marrow failure. An earlier study by (Fajardo, 1974) demonstrated that *P. vivax* within platelets by electron microscopy and suggested a direct lytic effect of the parasite on the platelets. Immune-mediated lyses, sequestration in the spleen and a dyserythropoietic process in the marrow with diminished platelet production have all been postulated for the cause of thrombocytopenia (Jadhav et al., 2004). Platelets play an important role in the formation of the mechanical plugs during the normal haemostatic response to vascular injury. This involves platelet-vessel wall (adhesion) and platelet-platelet (aggregation) interactions. Low platelet count will therefore result in haemostatic disorder ((Hoffbrand, Moss, & Pettit. 2006) rand et al., 2006; (Hoffbrand, Moss, & Pettit. 2006) rand et al., 2010). A finding of thrombocytopenia should increase the suspicion of malaria and lead to performance of more specific tests, including multiple Peripheral smears and ELISA for parasite-specific antigen (Patel et al., 2004). Platelet indices i.e. MPV, PDW and PCT could play an important role in the differential diagnosis of malaria.
CHAPTER THREE
MATERIALS AND METHOD

3.1 Introduction

The investigations were performed on both capillary and venous blood sample drawn into EDTA tubes (BD and Company, WJ 07417 USA) for preparation of the thick and thin smears for malaria parasites and automated determination of Complete Blood Counts (CBCs). Blood counts were performed using ACT5 Diff Haematology Analyzer (Beckman Coulter Inc, Miami, Florida, USA) as per local Standard Operating Procedures (SOPs) within one hour.

Daily Quality Assurance checks were performed and recorded; commercial controls were used in accordance with manufacturer’s recommendations for the blood counts. The Analyzer provided data on WBCs, RBCs, haemoglobin level, platelet counts, mean platelet volume (MPV), platelet distribution width, (PDW) red cell distribution width (RDW) and five part differentials was determined. The Analyzer also detected and flagged as platelet aggregation and cosld agglutinins based on the particle size using a 256-channel pulse–height analyzer of platelet histogram region. Since sickle cell disease affects the Hb levels, Sickling test was done to demonstrate haemoglobin S for children below 15 years and those found positive were excluded in the study.

Two blood slides both thick and thin were prepared and stained with Giemsa, alongside control slides i.e. both negative and positive controls. The negative control slide did not have any asexual form of *Plasmodium falciparum* while the positive control slide had the asexual forms of *Plasmodium falciparum* which was either trophozoites or schizonts. One thick and one thin slide from each study participant were examined independently by the Medical Laboratory Scientist and another set of a thick and thin slide was counter checked by another Laboratory Scientist for the purpose of quality assurance. A semi-quantitative assessment of parasitaemia was performed according to the WHO standard of reporting (appendix 3).

Patients with thrombocytopenia were graded into three categories i.e.

i. Mild thrombocytopenia <150X10⁹/L but >50X10⁹/L
ii. Moderate thrombocytopenia<50x10⁹/L but > 20X10⁹/L
iii. Severe thrombocytopenia < 20x10^9/L.
Reference values for platelet indices.

<table>
<thead>
<tr>
<th>Index</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPV</td>
<td>6-10 fL</td>
</tr>
<tr>
<td>PDW</td>
<td>15.5-17.1%</td>
</tr>
</tbody>
</table>

3.2 Evaluation of anaemia.

The degree of anaemia was evaluated through blood haemoglobin quantification and analysis. The following parameters were used:

i. Male adult individuals were considered anaemic when the haemoglobin level was less than or equal to 13 g/dl of blood.

ii. Adult women, adolescents, and children (more than 2 years of age) of both sexes were considered anaemic when the haemoglobin level was less than or equal to 12 g/dl of blood.

iii. Children of between 3 months and 2 years were considered anaemic when the haemoglobin level was less than or equal to 14g/dl.

Classification of various Degrees of Anaemia

Anaemia was classified as:

i. Mild Anaemia when the haemoglobin level was between 10 and 12 or 13 g/dl of blood depending on the sex and on the age.

ii. Moderate Anaemia when the haemoglobin level was equal to or above 7 and below 10 g/dl.

And

iii. Severe Anaemia when the haemoglobin level was lower than 7 g/dl.

3.3 Study Site

This research work was carried out at Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH) which is located within the headquarter of Kisumu county in Kenya. The laboratory analyses of clinical specimens were done at the three major laboratories of this hospital namely: Obama (Paediatric laboratory), main laboratory and casualty laboratory. High intensity of malaria
transmission in this holoendemic area is evident during the seasonal rainfalls in April to August and November to December (Beier et al., 1994).

3.4 Study design and population

This was a cross-sectional hospital-based descriptive study. Both in-patients and out patients with history of acute febrile illness (temperature above 37°C) attending this health facility from the month of August 2013 to April 2014. Those who turned positive upon the demonstration of the asexual forms of *P. falciparum* through microscopic examination of their thick and thin blood films formed the study population. Patients with history of acute febrile illness and confirmed negative upon microscopic examination of thick and thin smears attending this health facility from the month of August 2013 to April 2014 but met the inclusion criteria were included as study as control population.

3.4.1 The inclusion Criteria

Naive patients of both sexes who were 3 months and above, who presented with acute febrile illness among other clinical symptoms of malaria were included in the study. Besides, patients found to have a positive slide (showing asexual *P. falciparum* parasitaemia) or negative slide for malaria upon microscopic examination of either thick or thin smear of peripheral blood. Patients who were clinically stable and guardians of children willing and able to sign the consent form were included in the study.

3.4.2 The Exclusion Criteria

Patients with acute febrile illness and positive or negative for malaria parasite on peripheral blood examination but with cases of cerebral malaria (rare in holoendemic area) or prior hospitalization were not included in the study. Patients with known history of haematological disorders, immunosuppression, co-infection with bacterial, viral infections or HIV positive individuals as well as those unwilling to participate in the study and unable to sign the consent form were excluded.
3.5 Determination of sample size.

The study was carried out in a total of 228 naive patients (aged 3 months and above) who presented with acute febrile condition at Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH). The sample size (n=228) was based on actual number of patients above 3 months who presented themselves between 6 months during the study period.

The sample size (n=228) was calculated using Cochran’s formula with finite population correction for proportions as shown below (Cochran, 1963).

\[ n = \frac{t^2 \times p \times (1-p)}{M^2} \]

Where:

- \( n \) = required sample size
- \( t \) = confidence interval at 95 \% (standard value of 1.96)
- \( p \) = estimated prevalence of the disease in the project area at 38\% (KNBS, 2011).
- \( M \) = margin of error at 5\%

\[ n = \frac{(1.96)^2 \times 0.38 \times (1-0.38)}{0.05^2} \]

\[ n = 3.8416 \times 0.38 \times 0.62 \times 0.05 \times 0.05 \]

\[ n = 362. \]

Given a population size (N) of 615 during the study, the sample size (n) was therefore adjusted using the equation below; (Cochran, 1963).
\[ n = \frac{n_0}{1 + \frac{(n_0 - 1)}{N}} \]

n=362

\[ 1 + \frac{362-1}{615} \]

n=228 participants

### 3.6 Sampling Method

Every patient who met the inclusion criteria was included in the study as they present themselves until the calculated sample size was attained. The patients and parents/guardians of the children were taken through the informed consent form (Appendix 4). The adult patients and parents/guardians of the children were taken through the structured questionnaire (Appendix 5) before sample collection.
Figure 3.1: Flow chart showing the experimental design of the study.
3.7 Laboratory Procedures

Complete blood count was done for all the patients using ACT5 Diff Haematology Analyzer (Beckman Coulter Inc, Miami, Florida, USA) as per manufacturer’s instructions. Thick and thin smears were made for identification and speciation of malaria parasite species respectively. The slides were stained with 10% Giemsa (Sigma-Aldrich, Germany), alongside quality control slides and examined using × 100 objective. Thin smears were examined in the ideal area of thickness for trophozoites, gametocytes and schizonts and graded according to the WHO guidelines (WHO, 2009).

Blood collection for automated count:

Capillary blood in children: Pre-cleaned slides were labelled (preferably at the frosted-end) with the patient’s name and lab number, date and time of collection. The site was cleaned well with 70% alcohol and allowed to air dry. A deep prick was made at the side of the pulp of the 3rd or 4th finger and the first drop of blood wiped away with clean gauze and squeezed to obtain a free flow of capillary blood into 1.5 ml EDTA vacutainer tube (BD and Company, WJ 07417 USA) and a clean dry swab was applied to prevent excessive bleeding as the content of vacutainer tube properly mixed to prevent clotting.

Venous blood in adults: Venous blood was obtained from the median cubital vein as per established SOPs. The blood was transferred to a 5mls EDTA vacutainer tube and mixed thoroughly to prevent clotting.

Automated Complete Blood count: Automated blood count was preceded by mixing of anticoagulated whole blood in a mixer. The patients’ details e.g. name, lab number, age and sex were fed manually through the touch screen monitor of coulter counter followed by putting the vacutainer tube into the tube holder. This was followed by pressing the run button as the automated counter does the self-priming, sizing, enumeration blood cell and calculation of blood cell indices. The results were automatically displayed on the coulter counter screen and printed on the machines printer.
**Procedure for thick and thin smear:** Both thick and thin smears were made on the same slide. The frosted end of the slide was labelled with the patient number and a drop of blood put next to the frosted end and another smaller drop at the middle of the slide. The first drop was spread using a corner of the spreader in one circular direction to make even thick film, of a 1 cm diameter in size. For the thin film the spreader slide was placed in contact with second drop at an angle and gently pushed backwards to obtain even spread of blood and pushed gently towards the other end of the slide. The slide was allowed to air dry.

**WHO Grading of Malaria Parasites:** The WHO grades malaria parasitemia upon examination of slide through battlement method as follows: 1-10 parasites per 100 high power fields (+), 11-100 parasites per 100 high power fields (++), 1-10 parasites per high power field (+++) and >10 parasites per high power field (++__). The three important haematological parameters analysed included Hb level, platelet count and platelet indices i.e. mean platelet volume (MPV) and platelet distribution width (PDW).

### 3.8 Data Management

The reports were first captured in a proforma questionnaire. Data was then fed into computer spreadsheets, cleaned, verified for consistency. SPSS® version-17 (IBM SPSS, Inc., Chicago, IL) statistical software package was used in the analysis of data. The data were presented as medians and interquartile ranges. Comparisons of the difference in the means were calculated by student’s t-test and the difference in proportions by chi-square test ($\chi^2$). Differences were considered statically significant at $p \leq 0.05$.

### 3.9 Data Analysis and Presentation

Statistical analyses were performed using SPSS® (Version 17.0) (IBM SPSS, Inc., Chicago, IL) and Graph pad prism software (Graph Pad software, Inc., San Diego, CA.). Student’s t-test with two tailed p values was used to compare normally distributed data. Quantitative comparisons involving unpaired data not conforming to normal distribution were made using Mann–Whitney U test and Kruskal-Wallis test. Association between two continuous variables were computed by Spearman’s rank correlation. The diagnostic values of Hb level and platelet count were determined by computing sensitivity, specificity and predictive values of the two variables using sensitivity
and specificity calculator (Table 4.4). The precision of these parameters was evaluated using 95% confidence interval. Tables and graphs were used for presenting data.

3.9.1 Quality Assurance Measures.

Daily Quality Assurance checks were performed and recorded; commercial controls were used in accordance with manufacturer’s recommendations for the blood counts. Two blood slides both thick and thin were prepared and stained with Giemsa, alongside control slides, both negative and positive controls. One thick and one thin slide from each study participant were examined independently by the PI and another set of a thick and thin slide was counter checked by one of the supervisors.

3.9.2 Limitations of the Study

i. Co-infections that affect bone marrow can equally cause thrombocytopenia, cytopenia and other haematological abnormalities.

ii. Malaria patients who have been put on treatment might not give a true picture of haematological profile in malaria.

iii. It was very expensive to determine the presence of haemoglobinopathies (α-thalassaemia trait, G6PD status and Hb type) which could be potential cofounders in elucidating haematological complications in malaria.

iv. Polymerase chain reaction (PCR), osmotic fragility test and screening for bacterial and viral infections like Dengue fever which could cause haematological alterations were not done.

3.9.3 Confounders in the study

Platelet aggregation

Platelet aggregation refers to the clumping together of platelets in the blood and comprises homogenous clusters of 3-12 individual platelets. This might have resulted in false thrombocytopenia. When present in the blood sample platelets are detected by the Analyzer on the
basis of their size using a 256-channel pulse–height analyzer. Platelet aggregate flag were generated by the Analyzer when platelet aggregation was detected. This prompted thorough examination of peripheral blood film to check for platelet morphology and distribution and to rule out false thrombocytopenia due to aggregation.

**Cold Agglutinin**

Cold agglutinins refer to the circulating antibodies directed against own red blood cells and which bind to RBC at low temperatures. These were detected by the haematology analyzer and the cold agglutinin flag generated.

**Haemoglobinopathies**

Haemoglobinopathy refers to abnormalities in globin chain synthesis of haemoglobin. It includes thalassemias and are characterised by low haemoglobin levels. This potential confounder was controlled by performing peripheral blood film examination as a golden store of haematological information. Any participant of known or suspected haematological disorder was excluded in the study as indicated in the exclusion criteria.

**3.10 Ethical Considerations**

Approval was obtained from Jaramogi Oginga Odinga Teaching and Referral Hospital Scientific and Ethics Review Committee (accreditation NO.01713) having reviewed my protocol and found it ethically satisfactory (appendix 1). An informed, written and voluntary consent was sought from the patients and parents/guardians of children before obtaining blood samples for malaria, anaemia and complete blood count testing. No extra costs were charged to the participants because of this study. Strict confidentiality was maintained and all personal identifiers were removed from data during analysis. The risks and benefits of the participants in the study were clearly explained to the participants in a language they could understand during the process of informed consent. The risk of participation for both children less than five years and adults was minimal, since it was limited mostly to temporary discomfort associated with finger prick blood collection.
The benefits of participation in the study included identification and immediate treatment of malaria and referral for further treatment where necessary. No incentives were offered to participants in this study.

Participants were allowed to withdraw from the study at any time during the study and did not suffer any consequences.
CHAPTER FOUR

RESULTS

4.1 Laboratory, demographic, and clinical characteristics of the study participants.

Cross sectional analysis was conducted on both out patients and inpatients (n=228, age 3 months to 54 years) presenting with acute febrile conditions (temperature above 37.5°C). Clinically the study participants were classified into two categories based on infection status as the infected and non-infected group. The distribution of gender was significantly different between the clinical categories (p=0.007). Moreover, the proportions of Hb level, platelet count, MPV and PDW were also significantly different between clinical groups (p<0.001) (Table 4.1). Haematological parameters of the malaria parasitaemic group were compared with that of control using Mann-Whitney U test. The median values for Haemoglobin, platelet count were significantly lower for the parasitaemic group compared with the controls(p<0.001 in both cases). Conversely, the mean platelet volume (MPV) and platelet distribution width (PDW) were significantly higher in the parasitaemic group with p =0.001 in both cases (Figure 4.5; (A) and (B) respectively).

Table 4.1. Laboratory, demographic and clinical characteristics of the study participants.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Non-infected group (n=228)</th>
<th>Infected Group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of participants</td>
<td>71</td>
<td>157</td>
<td></td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>33 (46.9)</td>
<td>109 (69.4)</td>
<td>0.007a</td>
</tr>
<tr>
<td>Female</td>
<td>38 (53.1)</td>
<td>48(30.6)</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>3.5 (4.8)</td>
<td>3.5 (6.0)</td>
<td>0.76b</td>
</tr>
<tr>
<td>Hemoglobin level, g/dL</td>
<td>10.2 (2.7)</td>
<td>7.75 (3.9)</td>
<td>&lt;0.001b</td>
</tr>
<tr>
<td>Platelet count×10^9/L</td>
<td>297 (137)</td>
<td>88.0 (67.8)</td>
<td>&lt;0.001b</td>
</tr>
<tr>
<td>MPV,fl</td>
<td>7.7 (1.3)</td>
<td>8.8 (0.7)</td>
<td>&lt;0.001b</td>
</tr>
<tr>
<td>PDW,%</td>
<td>16.7(1.5)</td>
<td>12.9(1.1)</td>
<td>&lt;0.001b</td>
</tr>
</tbody>
</table>
Data are the median (interquartile range; IQR) unless otherwise stated. Patients with acute febrile illness (n=228) were categorized on the basis *P. falciparum* infection status. Non-infected group (n=71) and Infected group (n=157).  

a Statistical significance was determined by the $\chi^2$ test.  

b Statistical significance was determined by the Mann-Whitney U test. Values in bold are statistically significant at $p \leq 0.05$.

### 4.2 Association between Anaemia and thrombocytopenia.

Since anaemia and thrombocytopenia have profound haematological complications in malaria, the association between these two variables was assessed (Figure 4.1). There was a positive correlation between platelet count and haemoglobin level in malaria as confirmed by Spearman’s rank correlation ($\rho=0.26$, $p<0.001$).

![Graph of platelet count against degrees of anaemia.](image)

**Figure 4.1: Graph of platelet count against degrees of anaemia.**

Data are represented in box-plots. The boxes represent interquartile range; the line through boxes is the median while the whiskers show the 10th and the 90th percentiles. Across group comparisons were determined using Kruskal-Wallis test. **SA:** Severe anaemia, **ModA:** Moderate anaemia, **MA:** Mild anaemia
4.3 Association between anaemia and malaria.

Anaemia was defined as haemoglobin level <13 g/dl for adult males, <12g/dl for women and adolescents and children (more than 2 years of age). It was further classified as mild, moderate and severe (Table 4.2). Severe malarial anaemia was eventually defined as Hb<7g/dl in the presence of parasitaemia. The median Hb value of the malaria infected group was significantly lower than the negative group (7.7g/dl vs. 10.2g/dl, p=0.001, Figure 2). One hundred and twenty two (78%) of the malaria infected patients had severe to moderate anaemia compared to thirty three (46%) of the non-infected group (Table 4.2).

Table 4.2: Distribution of Haemoglobin level among malaria infected and non-infected patients in the study group.

<table>
<thead>
<tr>
<th>Category of anaemia</th>
<th>Hb level in g/dl</th>
<th>Malaria positive (n=157)</th>
<th>Malaria negative (n=71)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe</td>
<td>&lt;7g/dl</td>
<td>64(41%)</td>
<td>10(14%)</td>
</tr>
<tr>
<td>Moderate</td>
<td>7-10 g/dl</td>
<td>58(38%)</td>
<td>23(33%)</td>
</tr>
<tr>
<td>Mild</td>
<td>10-13g/dl</td>
<td>29(18%)</td>
<td>37(51%)</td>
</tr>
<tr>
<td>Non anaemic</td>
<td>&gt;13g/dl</td>
<td>6(3%)</td>
<td>1(1%)</td>
</tr>
</tbody>
</table>

The percentages of the Hb levels in malaria negative and malaria positive were determined using cross-tabulations.
Figure 4.2: Graph of haemoglobin concentration against malaria infection status.

Data are represented in box-plots. The boxes represent interquartile range; the line through boxes is the median while the whiskers show the 10\textsuperscript{th} and the 90\textsuperscript{th} percentiles. Median Hb level in NEG=10.2 g/dl while median Hb level in POS=7.7 g/dl. Between groups comparisons were determined using Mann-Whitney U test. NEG-Negative, POS-positive.

4.4. Association between thrombocytopenia and malaria.

Thrombocytopenia was defined as platelet count <150x10\textsuperscript{9}/l and further defined as severe if the platelet count is <20x10\textsuperscript{9}/l. The median platelet count for the malaria infected group was significantly lower than non-malaria infected (88x10\textsuperscript{9}/l vs. 297x10\textsuperscript{9}/l); (p<0.001) (Figure3). Thrombocytopenia was reported in 87\% (n=136) of the malaria infected patients with 17 \% being severe to moderate as compared to 10 \% of non-infected group which had no cases of severe or moderate thrombocytopenia (Table 4.3).
Table 4.3: Distribution of platelet among the malaria infected and the non-infected patients in the study group

<table>
<thead>
<tr>
<th>Degree of thrombocytopenia</th>
<th>Platelet count</th>
<th>Malaria positive N=157</th>
<th>Malaria negative N=71</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe</td>
<td>&lt;20×10^9/l</td>
<td>5(3.2%)</td>
<td>0%</td>
</tr>
<tr>
<td>Mild</td>
<td>20-50×10^9/l</td>
<td>22(14%)</td>
<td>0%</td>
</tr>
<tr>
<td>Moderate</td>
<td>50-150×10^9/l</td>
<td>110(70.1%)</td>
<td>7(10%)</td>
</tr>
<tr>
<td>Non thrombocytopenia</td>
<td>&gt;150×10^9/l</td>
<td>20(12.7%)</td>
<td>64(90%)</td>
</tr>
</tbody>
</table>

The percentages of the platelet counts in malaria negative and malaria positive were determined using cross-tabulations.

Figure 4.3: Graph of platelet count against the non-infected and the infected patients with *Plasmodium falciparum* in the study groups.
Data are represented in box-plots. The boxes represent interquartile range; the line through boxes is the median while the whiskers show the 10th and the 90th percentiles. Median platelet count in NEG=297×10⁹/l while the median platelet count in POS=88×10⁹/l. Between groups comparisons were determined using Mann-Whitney U test. NEG-Negative, POS-positive

4.5 Correlation between platelet counts and MPV.

Since malaria infection is likely to alter platelet counts and MPV, analysis was also done to determine the relationship between platelet counts and MPV in both malaria infected and non-infected individuals. The spearman’s rank correlation analysis revealed a strong inverse correlation between platelet count and MPV (r=-0.36, p<0.001). There was a marked increase in MPV as platelet count decreased in both malaria infected and the non-infected group (Figure 4.4).

Figure 4.4: Graph of relationship between MPV and platelet count in both non-infected and infected patients with Plasmodium falciparum patients.

The line through the plots measures and shows a negative correlation between MPV and platelet count.
4.6. Association between MPV, PDW and malaria.

Since mean platelet volume (MPV) and platelet distribution width (PDW) could play an important role in the differential diagnosis of malaria, these two variables were also analyzed. Both mean platelet volume (MPV) and platelet distribution width (PDW) were increased in the infected group compared to the non-infected group [median MPV; 17.77fL vs. 17.08fL and median PDW; 9.81% vs. 7.98% respectively] as opposed to platelet count among the *P. falciparum* infected patients (Figure 4.5). The Mann Whitney U test confirmed a significant association of both variables with a p<0.001.

![Graph of platelet MPV against infection status](image1)

**Figure 4.5; A and B: Graphs of MPV against infection status and graph PDW against infection status respectively.**

Data are represented as box-plots. In both cases, the boxes represent interquartile ranges; the line through each box is the median while the whiskers show the 10th and the 90th percentiles. Differences in mean platelet volume (MPV) and platelet distribution width (PDW) were considered significant at *p*<0.05 using Mann-Whitney U test. NEG- negative, POS- positive.
4.7 Sensitivity, Specificity and Predictive values of Haematological parameters

The diagnostic values of Hb level and platelet count were determined by computing sensitivity, specificity and predictive values of the two variables using sensitivity and specificity calculator (Table 4.4). Hb level and platelet count had good sensitivity and specificity (77.71% and 53.52% vs. 87.26% and 90.14%) respectively to diagnose *P. falciparum* malaria.

Table 4.4: Table of sensitivity, specificity and predictive values of Haematological parameters.

<table>
<thead>
<tr>
<th>Diagnostic values</th>
<th>Hb level</th>
<th>Platelet count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>77.71%</td>
<td>87.26%</td>
</tr>
<tr>
<td>Specificity</td>
<td>53.52%</td>
<td>90.14%</td>
</tr>
<tr>
<td>PPV</td>
<td>78.71%</td>
<td>95.14%</td>
</tr>
<tr>
<td>NPV</td>
<td>52.05%</td>
<td>76.19%</td>
</tr>
</tbody>
</table>

**PPV**: positive predictive value, **NPV**: negative predictive value
CHAPTER FIVE

DISCUSSION

5.1 Introduction

This study vividly confirms that haematological abnormalities are more pronounced in P. falciparum infection. According to this study, the major haematological changes include, lower Hb levels and platelet count and other platelet abnormalities among other parameters generated from complete blood count resulting in defective thromboplastic and disseminated intravascular haemolysis (Richards et al., 1998; Wickramasinghe and Abdalla, 2000). One of the most common complications in malaria especially in younger children and pregnant women is anaemia in high transmission areas (Menendez et al., 2000). This could be due to haemolytic mechanisms and accelerated removal of both parasitized and non-parasitised red blood cells and ineffective erythropoiesis (Wickramasinghe and Abdalla, 2000; Weatherall et al., 2002). Abnormally high tumor necrosis factor (TNF), in malaria has been associated with impairment of bone marrow activity (Wickramasinghe and Abdalla, 2000) and imbalance in RBC surface markers such as CR1 (Waitumbi et al., 2000). Severe anaemia (Hb <7g/dl) was only seen in 41% of the infected group and in 14% of the non-infected group. This could be due to increased haemolysis of the parasitized red cells and reduced erythropoiesis (Menendez et al., 2000). However, severe anaemia in the infected group and the non-infected group could in part be contributed to by other anaemia promoting conditions in P. falciparum infected individuals such as HIV-1 and bacteraemia (Otieno et al., 2006; Were et al., 2011). Mild anaemia reported in 51% in the non-malaria infected group and 18% of the malaria infected patients may partially reflect poor nutritional status, background haemoglobinopathy and previous and/or repeated malaria infections in this area (Maina et al., 2010). Sickle cell trait incidence of 28% (Aluoch, 1997) is common in Western Kenya. This compounded by high malaria transmission poses significant health problems in this region due to hereditary and acquired haemolytic anaemia although these patients are protected from severe disease.

Both qualitative and quantitative platelet abnormalities occur in malaria. In this study, platelet counts were markedly reduced in malaria infected patients compared to non-malaria infected patients. Thrombocytopenia occurred in 87% of the infected patients. Thrombocytopenia occur
through peripheral destruction (Ladhani et al., 2002) platelet consumption by disseminated intravascular coagulation process (Essien., 1989) as well as through excessive platelet removal by plenic pooling (Beale et al., 1972; Skudowitz et al., 1973). This study equally confirms lack of bleeding in thrombocytopenic malaria patients as previously reported (Gerardin et al., 2002). Reports of adequate or increased number of megakaryocytes in the bone marrow, makes decreased thrombopoiesis an unlikely cause of thrombocytopenia in malaria (Beale et al., 1972). Immune-mediated destruction of circulating platelets has been postulated as a cause of thrombocytopenia seen in malaria. Platelets have also been shown to mediate clumping of *P. falciparum* infected erythrocytes (Pain et al., 2001). This could lead to pseudo-thrombocytopenia. Malaria infected patients have elevated levels of specific IgG in their blood which binds to platelet-bound malaria antigens (Moulin et al., 2003) possibly leading to accelerated destruction.

Clumping of platelets was the most important platelet functional abnormality seen in this study. A large number of small platelets are seen mixed or clumped with a few giant platelets possibly due to the cytokine interference of megakaryopoiesis (Kelkar et al., 2004) since cytokine milieu is a common feature of severe malaria anaemia. Virtually all the peripheral blood smears from samples with platelet aggregate flag revealed small platelet aggregates mixed with giant platelets (platelets that nearly approach or exceed the size of a red cell), which could have triggered the platelet aggregation. The platelets clumps comprising three to 10 platelets are falsely counted as single platelet by the analyzers thus causing pseudo-thrombocytopenia. These observations suggest that, in as much as patients with malaria are likely to develop thrombocytopenia, a reduced platelet count in some patients may be attributed in part to pseudo-thrombocytopenia further explaining the lack of bleeding tendency. Although, presence of giant platelets as well as increased MPV argues against pseudo-thrombocytopenia being the most likely cause of thrombocytopenia in malaria-infected individuals. Besides, a number of samples did not have microscopically detectable platelet aggregates despite having significant thrombocytopenia. Hb level positively correlated with platelet count. This was consistent with results of a similar study in Nigeria (Adedapo et al., 2007) which showed that baseline platelet counts were related to day haematocrit, but no correlation between platelet counts and parasite densities.

The MPV was significantly higher and inversely correlated to the platelet counts in the parasitaemic group consistent with the finding of a similar study involving the investigation of
haematological parameters in children living in Western Kenya (Maina et al., 2010). MPV increased as the platelet count decreased in both the infected and non-infected patients. This may reflect an early release of platelets from the bone marrow in a compensatory response to reduced platelet levels in the peripheral blood (Maina et al., 2010). The raised MPV may be explained by the presence of the giant platelets observed in some of the peripheral blood films examined. A previous report indicates 60% sensitivity and 88% specificity for malaria diagnosis in acute febrile patients (Lathia and Joshi, 2004) to which our finding is consistent with. From this study, we found out that a combination of thrombocytopenia with high sensitivity and specificity and anaemia with moderate sensitivity and specificity (87.26% and 90.14% vs. 77.71% and 53.52%) respectively, can be a better predictor towards malaria when clinical manifestations are taken into consideration as a prerequisite.
CHAPTER SIX

CONCLUSION

6.1 Introduction

Significant changes on haematological parameters occur in *P. falciparum* infections which are of crucial diagnostic value. Most commonly affected are platelet count and haemoglobin level concentration among other platelet parameters. Thrombocytopenia is associated with anaemia in *P. falciparum* malaria and may be a marker of disease severity. Pseudo-thrombocytopenia may be partially related to low platelet count in some patients but immune mediated thrombocytopenia may not be ruled out. Combination of all these haematological findings together with clinical presentations from malaria endemic areas may be useful diagnostic tool in situations where conventional microscopy and rapid diagnostic tests may be sub-optimal as may be the case with low parasite density. This can prompt timely initiation of anti-malarial therapy.

6.1 RECOMMENDATIONS

Further studies are required to characterize the platelet aggregates and explain their association with falciparum malaria. A more robust research should be undertaken to elucidate both cellular and molecular mechanism of thrombocytopenia in *P. falciparum* malaria.
REFERENCES


APPENDICES

Appendix 1: Ethical clearance form

MINISTRY OF HEALTH
JARAMOGI OGINGA Odinga Teaching & Referral Hospital
P.O. Box 849
KISUMU

Telegrams: “MEDICAL”, Kisumu
Telephone: 057-2020801/2020803/2020321
Fax: 057-2024337
E-mail: medsupntph@yahoo.com

ERC IB/VOL.1/62
Ref: .......................................................... Date ....................................................

3rd October, 2013

Paul Mboya Kosio,
Jomo Kenyatta University of Agriculture & Technology,
NAIROBI

RE: FORMAL APPROVAL TO CONDUCT RESEARCH TITLED: “CORRELATION BETWEEN THROMBOCYTOPENIA AND ANAEMIA IN MALARIA AMONG PATIENTS ATTENDING JARAMOGI OGINGA ODINGA TEACHING AND REFERRAL HOSPITAL IN KISUMU COUNTY”

The JOOTRH ERC (ACCREDITATION NO. 01713) has reviewed your protocol and found it ethically satisfactory. You are, therefore, permitted to commence your study immediately. Note that this approval is granted for a period of one year (2nd October, 2013 to 3rd October, 2014). If it is necessary to proceed with this research beyond the approved period, you will be required to apply for further extension.

Also note that you will be required to notify the committee of any protocol amendment(s), serious or unexpected outcomes related to the conduct of the study or termination for any reason.

Finally, note that you will also be required to share the findings of the study in both hard and soft copies upon completion.

The JOOTRH ERC takes this opportunity to thank you for choosing this institution and wishes you the best in your endeavours.

Yours sincerely,

DR. MARY A.ONYANGO,
For: FRED O. AKWATTA,
SECRETARY – ERC,
JOOTRH – KISUMU.
Appendix 2: Malaria Endemicity Map.

Malaria endemicity map in Kenya. Source: (Noor et al., 2009)
Appendix 3: Procedures for Analysis

Blood collection for automated count

Capillary blood in Pre-cleaned slides were labelled (preferably at the frosted-end) with the patient's name (or other identifier) and date and time of collection. The site was cleaned well with 70% alcohol and allowed to air dry. A deep prick was then be made at the side of the side of the pulp of the 3rd or 4th finger and the first drop of blood wiped away with clean gauze and squeezed to obtain a free flow of capillary blood into 1.5 ml EDTA vacutainer tube and a clean dry swab to be applied to prevent excessive bleeding as the content of vacationer tube is properly mixed to prevent clotting.

Venous blood in adults.

Median cubital vein was cleaned with 70% alcohol swab and allowed to air dry. A 21 guage needle attached to a 5ml syringe was inserted into the median cubital vein inclined at an angle of 25° with the bevel facing up and plunger pulled backwards to obtain 5 mls of blood and pressure applied using a dry swab to prevent excessive bleeding. The blood was then transferred to a 5mls EDTA vacutainer tube and mixed thoroughly to prevent clotting.

Automated Blood count

Automated blood count was preceded by mixing of anticoagulated whole blood in a mixer. The patient details e.g. name, lab number, age and sex were fed manually through the touch screen monitor of coulter counter followed by putting the vacutainer tube into the tube holder. This was followed by pressing the run button as the automated counter does the self-priming, sizing, enumeration blood cell and calculation of blood cell indices. The results were automatically displayed on the coulter counter screen and printed on the machines printer.
**Procedure for thick and thin smear**

Both thick and thin smears were made on the same slide. The frosted end of the slide was labelled with the patient number and a drop of blood put next to the frosted end and another smaller drop at the middle of the slide. The first drop was spread using a corner of the spreader in one circular direction to make even thick film, of a 1 cm diameter in size. For the thin film the spreader slide was placed in contact with second drop at an angle and gently pushed backwards to obtain even spread of blood and then pushed gently towards the other end of the slide. The slide was then allowed to air dry.

**Giemsa staining procedure**

The smears were put on a flat staining rack to air dry. Giemsa stain of 10% dilution was used to flood the air dried slides alongside the quality control slides for 10-15 minutes. This step was followed by washing the slides using phosphate buffer of pH 7.2 then allowed to air dry and examined using oil immersion objective.

**WHO Grading of MPs**

The WHO grades malaria parasitaemia upon examination of slide through battlement method as follows:

1-10 parasites per 100 high power fields.....................+

i. 11-100 parasites per 100 high power fields...............++

ii. 1-10 parasites per high power field........................+++  

iii. >10 parasites per high power field............................++++
Appendix 4: Informed consent form.

This research study is being conducted by Kosiyo Paul Mboya of Jomo Kenyatta University of Agriculture and Technology to determine the Correlation between Thrombocytopenia and Anaemia in malaria among patients attending Jaramogi Oginga Odinga teaching and Referral Hospital in Kisumu County.

Procedures

You will be requested to give permission for withdrawal of approximately 1.5 ml to 5ml of blood from you or your child. This sample shall be analysed to determine your haematological parameters or that of your child in malaria. Research findings shall be made available through reports which shall be free.

You will also be asked to complete a short questionnaire or the questionnaire will be read for you and your response shall be recorded. Questions will include mainly details about your demographic background.

Risks/Discomforts

There are minimal risks for participation in this study. However, you may feel physical discomfort during sample withdrawal.

Benefits

The benefits of participation in the study will include identification and immediate treatment of malaria and referral for further treatment, where necessary. No incentives will be offered to participants for study.

Confidentiality

All information provided will remain confidential and will only be reported as group data with no identifying information. All data, including questionnaires will be kept in a secure location and
only those directly involved with the research will have access to them. After the research is completed, excess samples will be discarded and the questionnaires will be destroyed.

**Participation**

Participation in this research study is voluntary and you have the right to refuse to participate or withdraw without suffering any dire consequences.

**Questions about the Research**

If you have questions regarding this study, you may contact Kosiyo Paul Mboya at paulkosiyo@gmail.com or 0720 459 582.

a. Certificate of consent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I have been explained the purpose of this study and I do understand the risk and benefits of this study.

I hereby consent voluntarily on my behalf/on behalf of the subject to participate as a participant in this study.

**You are making a decision whether or not to participate. Your signature indicates that you have decided to participate, having understood the information provided above.**

Signature…………………… Date…………………………

Time…………………… Relationship to Subject if a child (If applicable)…………

I have accurately read or witnessed the reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that consent was given freely.

Signature of Witness……………… Signature of Investigator……………………..
Signature of PI................................................... Date..............................................
Appendix 5: Proforma Questionnaire.

Study..................................................................................................................No..................
Date.................................Name..............................................................Age..............Sex..........

Marital status (where applicable).................................................................

County of Birth........................................ County of Residence

COULTERGRAM

Hb.................. RBC.............. PCV............

MCV....... MCH............ MCHC........

WBC........... Plt................ MPV............

PDW.......... PBF

RBC............................................................... WBC Differential.................................

Malaria pigment in monocytes YES/NO

Plt............................................................... Malaria parasites: Trophozoites: YES/NO Schizonts: YES/NO Gametocytes: YES/NO

Grading of parasitaemia...........................

Thick smear

Trophozoites.................................

Grading of parasitaemia.................................
Appendix 6: Principle investigator performing a cytomorphological study on a peripheral blood film using a light compound microscope at JOOTRH.
Appendix 7: A template of a complete blood count test result generated from a coulter counter

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result</th>
<th>Unit</th>
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</thead>
<tbody>
<tr>
<td>WBC</td>
<td>4.7</td>
<td>10^3/μL</td>
</tr>
<tr>
<td>LY</td>
<td>34.1</td>
<td>%</td>
</tr>
<tr>
<td>MO</td>
<td>7.2</td>
<td>%</td>
</tr>
<tr>
<td>GR</td>
<td>50.7</td>
<td>%</td>
</tr>
<tr>
<td>LYN</td>
<td>1.6</td>
<td>10^3/μL</td>
</tr>
<tr>
<td>MON</td>
<td>0.5</td>
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<td>GRN</td>
<td>2.9</td>
<td>10^3/μL</td>
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<tr>
<td>RBC</td>
<td>3.66 L</td>
<td></td>
</tr>
<tr>
<td>Hgb</td>
<td>11.8 L</td>
<td>g/dL</td>
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<tr>
<td>Hct</td>
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<td>%</td>
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<tr>
<td>MCV</td>
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<tr>
<td>MCH</td>
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<tr>
<td>MCHC</td>
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</tr>
<tr>
<td>RDW</td>
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<td>%</td>
</tr>
<tr>
<td>MPV</td>
<td>7.9</td>
<td>fl</td>
</tr>
<tr>
<td>PCT</td>
<td>106 L</td>
<td>%</td>
</tr>
</tbody>
</table>

WBC Histogram

RBC Histogram

PLT Histogram

11/14

The results show normal values for all parameters within the specified limits.