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Addis Ababa University

**ADDIS ABABA UNIVERSITY COLLEGE OF HEALTH SCIENCE SCHOOL OF ALLIED HEALTH SCIENCES DEPARTMENT OF MEDICAL LABORATORY SCIENCES**

# Assessment of platelet count in malaria suspected patients and associated factors in Arba Minch Health center, Arba Minch, Ethiopia


**By**

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## Abstract

**Background**: Malaria infection is a major public health problem and cause of morbidity and mortality in tropical and subtropical regions of the world. It has been consistently reported as one of the three leading causes of morbidity and mortality in Ethiopia. Changes in hematological parameters are likely to be influenced by any disease condition including endemic diseases, such as malaria. Anemia and thrombocytopenia are the most frequent malaria associated hematological complications and they play a major role in malaria pathogenesis. The presence of thrombocytopenia in malaria positive patients has become a highly sensitive clinical marker for malaria diagnosis. significant correlation between malaria and the presence of thrombocytopenia is mandatory before taking it as a hematological parameter of the disease. However in Ethiopia the relation between thrombocytopenia and malaria pathogenesis is not well studied.

**Objective**: To assess platelet count among malaria suspected patients and associated factors in Arba Minch Health Center, Arba Minch, Ethiopia.

.**Methodology:** Aninstitutional based cross sectional study was conducted in Arba Minch Health center from February to April, 2015. Data was collected from 424 malaria suspected patients that came during the 3 months of data collection period. Using estimation of single population proportion formula and sequential sampling technique we selected 424 study participants. In addition data on sex, age, occupation and associated factors was collected using structured checklist.Data was entered and analyzed by SPSS Version 20.Frequency and cross tabulation was conductedto describe relevant variables in relation to the outcome variables; bivariate and multivariate logistic regression analysis was conducted to identify significant predictors based on p-value less than 0.05 with 95% confidence level.

**Result**: Among 424 malaria suspected patients 117 (27.6%) [95% CI 23.3 - 31.9] were positive for malaria and the rest 307(72.4%) [95%CI 68.1- 76.7] were negative. Prevalence of thrombocytopenia was 36.1% and among this 19.8% were malaria positive. The mean platelet count in *Plasmodium vivax*was 163,000/µl (SD 115,000) with a range of 18,000-403,000/µl as against *Plasmodium falciparum* malaria 128,000(SD72, 324/µl) with a range of 33,000-311,000/µl). A significant reduction of platelet count was seen in this study (AOR 15.7, P<0.001) in malaria positive patients than that of malaria negative patients. Among the associated factor being positive for bacterial infection [AOR= 2.14 (95%CI 1.22-3.76)] (P=0.008), malaria positivity [AOR=15.8 (95%CI 8.7-28.66)] (p <0.001) have a statistically significant correlation to thrombocytopenia.

**Conclusion & Recommendation**: Thrombocytopenia is a common hematological finding in malaria. Reduced number of platelet count was noticed in 71.8% of cases of malaria. So this study also connotes the importance of thrombocytopenia in malaria infection.

## Acknowledgment

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## Abbreviations

| | |
|---|---|
| AOR | Adjusted Odds Ratio |
| BM | Bone Marrow |
| CBC | Complete Blood Count |
| CI | Confidence Interval |
| CMV | Cytomegalo Virus |
| COR | Crude Odds Ratio |
| DIC | Disseminated Intravascular Coagulation |
| DRERC | Departmental Research and Ethics Review Committee |
| EBV | Epstein Bar Virus |
| EQA | External Quality Assessment |
| ETB | Ethiopian Birr |
| Hb | Hemoglobin |
| Ig | Immunoglobulin |
| MBF | Malaria Blood Film |
| MCH | Mean Cell Hemoglobin |
| MCHC | Mean Cell Hemoglobin Concentration |
| MCV | Mean Cell Volume |
| MLR | Monocyte Lymphocyte Ratio |
| MPFT | Malarial Parasite Fluorescent Technique |
| NLR | Neutrophil Lymphocyte Ratio |
| OPD | Outpatient Department |
| OR | Odds Ratio |
| P. Falciparum | Plasmodium Falciparum |
| P. Ovale | Plasmodium Ovale |
| P.Vivax | Plasmodium Vivax |
| RBC | Red Blood Cell |
| SNNPR | Southern Nation Nationalities and Peoples Region |

| | |
|---|---|
| SPSS | Statistical Package for Social Science |
| WBC | White Blood Cell |

## Operational definition and definition of terms

**Anemia:** A physiologic or/and clinical condition where an individual exhibits inability to maintain normal tissue oxygenation because of decreased hemoglobin content (normal Hg RI, 11.5-15.5g/dl), RBC (RI 3.8-5.2 trillion/liter) number or packed red blood cells ( 33-45%)/ (42-48% for adult Ethiopians (32).

**Endemic disease:** Disease that occurs continuously or recurrently in a particular geographic region or continued prevalence of a disease in a specific population or area.

**EQA**: A system whereby a reference laboratory sends stained blood films to a laboratory for examination. The laboratory receiving the slides is not informed of the correct result of the slides until the laboratory has reported their findings back to the reference laboratory.

**Hematopoietic Response:** production of blood cells by blood cell forming organs like BM, spleen and liver in response to any loss of blood.

**Leukopenia:** Any situation in which the total number of leukocytes in the circulating blood is less than normal. Normally 4-11 billion of WBC is present in a liter of circulating blood of an individual. In adult population of Ethiopians however decreased amount is registered that is 6-6.2 billion WBC per liter of circulating blood (32).

**Malaria Suspected patients:** A patient with a clinical sign and symptoms of malaria infection, like Headache, pain of bone joints, Fever with chills, backache and rigors.

**Quality Control**: Measures the quality of a test or a reagent. For malaria microscopy, the most common form of quality control (QC) is the cross-checking of routine blood slides to monitor the accuracy of examination.

**Splenomegaly:** Enlargement of the spleen. Many diseases affect the spleen and malaria parasite infection is one of them making the spleen to enlarge and hold too many platelets than normal (normally 1/3 of the circulating platelet is present in the spleen).

**Thrombocytopenia:** A condition in which there is an abnormally low number of platelet in the circulating blood. Normal reference interval of platelet count is 150,000-400,000cells/microliter 98000-337,000cells/microliter platelet is the normal reference interval for adult Ethiopian population (32).

# 1. Introduction

## 1.1. Background

Malaria is an infectious disease caused by protozoan parasites from the plasmodium family that can be transmitted by the bite of the anopheles mosquito or by a contaminated needle or transfusion. It remains one of the major diseases of the world, particularly in tropical countries. The World Health Organization (WHO) has reported a worldwide annual incidence of 247 million cases and malarial mortality of one million per year. Every 30 seconds, a child somewhere dies of malaria. Children are especially vulnerable to malaria. In Africa, where 80% of malaria cases are treated at home, the disease kills one child in twenty before the age of five. Pregnant women are also at high risk. They have an increased risk of disease and death, as well as adverse impacts for their developing babies including low birth weight, growth retardation, still births and death. Other high-risk groups include refugees, displaced persons, or labor forces entering into endemic areas (1, 2).

Malaria is also a major public health problem in Ethiopia. It has been consistently reported as one of the three leading causes of morbidity and mortality in the past years. It is caused by protozoan parasite of genus plasmodium. Five species of the plasmodium; *P. falciparum*, *P. vivax*, *P. ovale*, *P.malariae* & *P. knowlesi* cause malaria in humans. *Plasmodium falciparum* and *Plasmodium vivax* are the most dominant malaria parasites. They are prevalent in all malaria endemic areas in the country with *P. falciparum* representing about 77% of the total reported malaria cases. *Plasmodium malariae* accounts for less than 1% and *Plasmodium ovale* is rarely reported. Malaria transmission in Ethiopia mainly occurs up to the 2000 meters elevation but can also occasionally affect areas up to 2300 meter elevation. The parasite is principally transmitted by the major mosquito vector known as *Anopheles arabiensis*. In some areas *Anopheles pharoensis*, *Anopheles funestus* and *Anopheles nili* also transmit the disease. Infection is initiated when sporozoites from the salivary glands of a female anopheles mosquito are inoculated during a blood meal into the human blood stream (3)

The two laboratory diagnostic methods or tools most often used for confirming a diagnosis of malaria are Rapid Diagnostic Tests – RDTs that detect antigens (proteins produced by malaria parasite) in the blood of a patient with malaria and Light Microscopy which is the most acceptable method for detecting and identifying malaria parasites from the blood of a suspected patient. Automated hematology analyzers have been found to be useful in indicating diagnosis of malaria during routine blood counts. Although alternative malaria diagnostic methods exist, they are not as suitable for wide application in the field as microscopy or RDTs. Quantitative Buffy Coat (QBC) method, Thin film acridine orange technique/ Microscopy using Kawamoto's fluorochrome technique, immunological tests (Anti-malarial Antibody Test), Polymerase Chain Reaction (PCR) are Among them(5).

Changes in hematological parameters are likely to be influenced by any disease condition including endemic diseases, such as malaria, that can affects health of mankind with various clinical presentations. Malaria affects almost all blood components and is a true hematological infectious disease. Anemia and thrombocytopenia are the most frequent malaria-associated hematological complications and they play a major role in malaria pathogenesis, and have received more attention in the scientific literature due to their associated mortality. Hematological change involves the major cell types such as RBCs, WBC and platelets. The most common complication during malaria infection is thrombocytopenia. Persons with platelet counts < 150,000/μL were 12-15 times more likely to have malaria infection than persons with platelet counts > 150,000/μL. Hematological changes during malaria infection, such as thrombocytopenia and leukocytosis or leucopenia are well recognized. Diagnostic value of these hematological alterations may be easily obtained and useful in people living in malaria endemic areas (5).

A previous report indicates 60% sensitivity and 88% specificity of thrombocytopenia for malaria diagnosis in acute febrile patients. The sensitivity of thrombocytopenia together with the acute febrile syndrome was 100% for malaria diagnosis, with a specificity of 70%, a positive predictive value of 86% and a negative predictive value of 100% (6).

The hematopoietic response is, however, also somewhat blunted, and there is reduced platelet count and sometimes reduced WBC counts as well. Thrombocytopenia is a very common association of malaria. A number of observational studies have confirmed the association of thrombocytopenia to malaria but till date the cause of thrombocytopenia is poorly understood. The speculated mechanisms leading to thrombocytopenia are coagulation disturbances, splenomegaly, bone marrow alterations, antibody-mediated platelet destruction, oxidative stress, and the role of platelets as cofactors in triggering severe malaria. Abnormalities in platelet structure and function have been described as a consequence of malaria, and in rare instances, platelets can be invaded by malarial parasites themselves (7, 8).

There were two major changes of platelet during malarial infection, thrombocytopenia and platelet dysfunction. Thrombocytopenia has been observed in 60–80% of both *P. vivax* and *P. falciparum* infection. The severe degree and higher incidence of thrombocytopenia were observed predominantly in complicated *P. falciparum* infection. Maximum thrombocytopenia occurred on the fifth or sixth day of infection, and gradually returned to normal within 5–7 days after parasiteemia ceased. The mechanism of thrombocytopenia in malaria is due to peripheral destruction and consumption. During malarial infection, despite the presence of thrombocytopenia, the number of megakaryocytes in the bone marrow remained adequate or increased. Decreased survival of platelets has been also demonstrated (1).

The immune complexes consisted of malarial antigen and IgM or IgG antibodies with or without complement attached to platelets causing the sequestration of these injured platelets by macrophages in the spleen. Hyperactivity of macrophage also played a role on the destruction of platelets. The activated platelets lost sialic acid from their membrane, resulting in intravascular lysis and thrombocytopenia. Furthermore, consumption of platelets by the process of DIC was also another factor that contributed to thrombocytopenia in complicated *P. falciparum* malaria. Platelet hyperactivity followed by platelet hypo activity was also the two major changes in platelet function during malarial infection that causes decreased platelet count (1).

The presence of thrombocytopenia in acute febrile travellers returning from tropical areas has become a highly sensitive clinical marker for malaria diagnosis. Thrombocytopenia is quite frequently associated with malaria and has been reported by many workers. As thrombocytopenia is also seen in some other acute febrile illnesses therefore, a significant correlation between malaria and the presence of thrombocytopenia is mandatory before taking it as a hematological parameter of the disease (9).

## 1.2. Statement of the problem

Malaria is commonly associated with various degrees of hematological complications like anemia and thrombocytopenia. The anemia is usually due to varied reasons ranging from hemolysis to comorbidities like parasitic infections, folate, iron, and vitamin B12 deficiencies in endemic areas, antimalarial drug and further complicated by the coexistence of thalassemia and other hemoglobinopathies. Hematological abnormalities are considered a hallmark of malaria and statistical analyses have shown that many of these hematological values may lead to an increased Clinical suspicion for malaria, thus initiating a prompt specific therapy even in the absence of a positive smear report for malaria. Anemia and thrombocytopenia are the most frequent malaria-associated hematological complications (10).

The hematological abnormalities that have been reported to invariably accompany infection with malaria include anemia, thrombocytopenia, splenomegaly, mild-to-moderate atypical lymphocytosis and rarely Disseminated Intravascular Coagulation (DIC). There have also been reports of leucopenia and leukocytosis. Among this thrombocytopenia is very common. Prediction of the hematological changes enables the clinician to establish an effective and early therapeutic intervention in order to prevent the occurrence of major complications (10).

In the past it was thought that malaria is rarely associated with clinical features of thrombocytopenia like bleeding disorders and is usually an incidental finding on blood testing. Recent scientific evidences have invalidated this anecdote. It is pertinent that the finding of thrombocytopenia in patient may be an indication for a thorough lookout into the blood smear to rule out malaria as the cause. This fact is especially important in the workup for thrombocytopenia in febrile patients. Thrombocytopenia may be associated with bleeding tendency which is one of the important severe manifestations of *Falciparum* malaria (8).

Therefore this study was conducted to assess platelet count among malaria suspected patients and associated factors for patients of Arba Minch Health Center, Arba Minch, Ethiopia.

## 1.3. Literature review

A retrospective and descriptive Study was undertaken by Dhugat MP to evaluate incidence of thrombocytopenia in patients of malaria. The study correlate thrombocytopenia with the type of malaria and its clinical significance in bhausaheb sardessai talgaon rural hospital (bstrh) Maharashtra, India during a period of 1 year (Jan2011 - Dec2011). It suggests that thrombocytopenia is a common occurrence in *plasmodium falciparum, plasmodium Vivax* and in mixed infections. The study infers that it is a benign finding in mild and moderate cases of malaria and does not warrant platelet transfusion there by avoiding an unnecessary expenditure in the poor strata of patients. From A total number of 100 patients in the age group 15- 85 years, admitted in BSTRH for the treatment of malaria thrombocytopenia was detected in 88.5% of the patients suffering from *falciparum* malaria, 79.6% of patients with *vivax* malaria and 95% of patients with mixed infection. Only 15% of patients in the study had normal platelet count. The study has confirmed that thrombocytopenia is equally prevalent in *Plasmodium falciparum*, *Plasmodium vivax* and also in mixed infection. It has no bearing on the complications or disease process and it does not warrant any intervention (4).

Another study was conducted in 4,985 patients who admitted at phop phra hospital, Tak province, an area of malaria endemic transmission in Thailand during 2009 by Kotepui M. The effect of malarial infection on hematological parameters shows that Patients infected with malaria exhibited important changes in most of hematological parameters with low platelet, WBCs, and lymphocyte counts being the most important predictors of malaria infection. In malaria infected patients 352(50.1%) had *Plasmodium falciparum* infection whereas 351(49.9%) had *Plasmodium vivax* infection. RBCs, Hb, Platelet counts, WBCs, Neutrophils, Monocyte, Lymphocyte and Eosinophil counts were significantly lower in malaria infected patients, while MCV, MCH, MCHC, Neutrophil- Lymphocyte ratio (NLR), and Monocyte- Lymphocyte ratio (MLR) were higher in comparison to non-malaria infected patients. Patients with platelet counts <150,000/ μL were 31.8 times more likely to have a malaria infection. Thrombocytopenia was present in 84.9% of malaria infected patients and the study assured that when used in combination with other clinical and microscopy methods, these parameters could improve malaria diagnosis and treatment (5).

In a similar study conducted in a tertiary care hospital, Liaquat University of medical and health sciences Jamshoro, Pakistan over a 1 year period from April 2007- 2008 G.C by Ansari S. It evaluates thrombocytopenia in the patients suffering from acute *falciparum* malaria and indicates low platelet count helps in diagnosis of malaria. In the study a total of 370 patients with *Plasmodium falciparum* malaria were studied and among them 114(30.81%) had normal platelet counts and 256(69.18%) had thrombocytopenia. The mean platelet count was 170,000 ±56,500/ μL. The study concluded that mild to severe thrombocytopenia is of diagnostic help as it raises the suspicion of malaria (7).

Another clinical prospective observational study was conducted by Khan JS in Hayat Abad Medical Complex, Postgraduate Medical Institute; Peshawar, Pakistan from November 2008 to November 2010 G.C. It concluded that the presence of thrombocytopenia may be a predictor of malaria in adult population. Of the total 228 patients studied with fever and thrombocytopenia, 121 patients (53%) proved to be suffering from malaria. Of them 82 patients (68%) had *falciparum* malaria while 39 patients (32%) had *vivax* infection. The sensitivity and negative predictive value is high at low platelet counts (78% sensitivity and 67% negative predictive value 40% and 58% respectively) while the specificity and positive predictive value is high at relatively higher platelet counts (94% specificity and 89% positive predictive value against 50% and 64% respectively). The study finally concluded that malaria should be a consideration in all patients with low platelets and after excluding this common and easily treatable cause, further evaluation of thrombocytopenia should be undertaken (8).

An observational study, conducted by Jairajpuri SZ from January to December 2012 in total of 723 patients presenting with acute febrile illness at Hakeem Abdul Hameed Centenary Hospital, New Delhi, India showed that 172 out of 723 patients (24%) were diagnosed to have malaria by positive smear report. The study showed there was a statistically significant reduction in hemoglobin, platelet count and total leukocyte count levels in patients with malaria compared to patients without the disease. The presence of low platelet count (<100,000/mm3) was both 81% sensitive and 87% specific for the diagnosis of malaria with a likelihood ratio for a positive and negative test result of 6.55 and 0.2, respectively. Even if all hematological parameters are disturbed, thrombocytopenia emerged as a stronger predictor of malaria; an observation of many studies which this studies also confirms (10).

A clinical study conducted in A.J. Institute of Medical sciences Mangalore, Karnataka India, by Bhandary N 2011 showed that out of 102 patients who were hospitalize after confirmation of diagnosis for malaria by malarial parasite fluorescent technique (MPFT), 70.5% of the patients were thrombocytopenic. Of this patients 50(49.01%) had mixed malaria where 38 cases had platelet count less than 150,000 out of which 9( 18%) patients had platelet count less than 50,000. Out of 46 patients detected with *vivax* malaria 34 patients had platelet count less than 150,000 out of which (10.86%) patients had platelet count less than 50,000. A higher frequency of mild to severe thrombocytopenia was observed in the hospitalized patients, which should alert the possibility of malarial infection (11).

A similar retrospective study was conducted on correlation of thrombocytopenia in urban children with malaria by Joshi G at Surat Municipal Institute of Medical Education and Research, India from July 2010 to December 2010 G.C. Thrombocytopenia was more common with *Plasmodium falciparum* group than *Pasmodium vivax* group. Out of the 144 cases of *Plasmodium vivax* malaria, 103 (72%) patients had thrombocytopenia of which, 32 (31%) had severe thrombocytopenia. Out of 169 patients of *Plasmodium falciparum* malaria 130 (77%) cases had thrombocytopenia, of which 49 (38%) had severe thrombocytopenia (< 50,000 cells/µL). Overall Sensitivity of thrombocytopenia as a predictor of malaria was 74%. Sensitivity in *Plasmodium Vivax* and *Plasmodium falciparum* was 71.53% and 76.92%, whereas specificity was 66.67% for both respectively. The study showed Presence of thrombocytopenia in patients with acute fevers has a good diagnostic possibility of malaria not only in adults but also in pediatric patients and this is more with degree of severity of thrombocytopenia (12).

In one more similar prospective study with the previous one that uses a total of 120 patients of all ages to assess the presence and severity of thrombocytopenia in malaria thrombocytopenia was noted in 63.33% cases. The study was conducted by Gill MK in Department of Pathology, Adesh Institute of Medical Sciences and Research, Bhatinda, India. The mean platelet count in *Plasmodium vivax malaria* was 127,652/µl with a range of 8000-350,000/µl, as against *Plasmodium falciparum* malaria where the mean platelet count was 78,500/µl with a range of 9000-1, 90,000/µl. Platelet count < 50,000/µl was noted in only 17.4% cases of *Plasmodium vivax* malaria as against 33.3% cases of *Plasmodium falcipharum* malaria. The researcher concluded that Thrombocytopenia is common in the laboratory diagnosis of malaria. Its presence in patients, with acute febrile illness in the tropics, increases the probability of malaria. This may be used in addition to the clinical assessment, to heighten the suspicion of this disease. If thrombocytopenia is present, malaria has to be ruled out before performing expensive tests to rule out other febrile conditions, so that a prompt treatment can be initiated (13).

A different hospital based prospective study performed to evaluate the significance of the platelet count in the diagnosis of malaria from January 2010 to July 2010 by Faseela TS in India also signifies similar result with the previous studies. Among 1101 patients with febrile illness who participated in the study, 267 were positive for malaria and out of these patients 51.7% of patients had *Plasmodium vivax* infection, 1.12% of patients had *Plasmodium falciparum* infection and 47.2% of patients had mixed infection. Thrombocytopenia is observed in 221 from 267(82.8%) malarial infected patients. Most of these patients (43%) had platelet counts between 51 to $100x10^9$/L, 14% of the patients had platelet counts between 101 to $150x10^9$/L and 17% of the patients had platelet counts below $50x10^9$/L (14).

Likewise, a study was carried out by Igbeneghu C in south western Nigeria on impact of acute malaria on some hematological parameters. 671 adults with acute malaria infection and 523 apparently healthy subjects were examined for full blood count. Participants with acute malaria had significantly lower mean values of hematocrit, leucocyte, platelet, hemoglobin concentration and erythrocyte count compared to the apparently healthy subjects. Although, anemia, leucopenia and thrombocytopenia were all significantly associated with acute malaria and thrombocytopenia showed the strongest association. Thrombocytopenia was the strongest predictor of acute malaria infection among the study population (15).

Alternative study done at Narayan Medical College and Hospital, Sasaram District, Bihar, India by Kumar J showed that thrombocytopenia is a common occurrence in malaria, so that one can use this as a sign to support malarial presence especially of patients negative for malarial parasite. Out of 200 patients that participated in the study only 20% of cases have normal platelet count and the remaining 80% shows reduced number of platelet count. The study also signifies thrombocytopenia was present in both types of malaria (16).

A prospective study was carried out at PRL Malaria control program Fatima Jinnah General and Chest Hospital, tertiary care Hospital, Quetta, Baluchistan, Pakistan by Kakar N. Among 389 identified cases of thrombocytopenia 1 in every 5 patients had underlying malaria i.e. around in 21.85% of the patients. *Plasmodium vivax* were found in 36.5% of the patients and 63.5% of cases were *Plasmodium falciparum*. So the study finding pressure patients with thrombocytopenia in endemic regions should be screened for possible malaria and low platelet count can aid in the diagnosis of malaria (17).

In a similar study conducted in S.P. Medical College and from associated Group of Hospitals, Bikanaer, India by Kochar KD from January 2007- July 2008 Shows that out of 1064 cases of malaria 49.34% had *Plasmodium falciparum* mono infection, 43.23% had *Plasmodium vivax* infection and 7.22% had mixed malaria infection. Platelet count <150,000 per microliter of blood was observed in 16.9%, 31.09%, and 3.04% of *Plasmodium falciparum, Plasmodium vivax*, and mixed infection respectively. From the observation the study was evident that thrombocytopenia is a common hematological finding in patients with plasmodium infection and it notifies the association of severe thrombocytopenia was strongest with *Plasmodium vivax* infection as compared to *Plasmodium falciparum* and mixed infection (18).

A study from February to December 2010 done in Pakistan by Shaikh MA on platelet count in malaria patients also confirms that malaria is associated with different degrees of low platelet count with rarely increased bleeding tendency. The study was conducted on 200 diagnosed cases of malaria. 52.5% of cases were *Plasmodium falciparum*, 46.5% were *Plasmodium vivax* and 1% was *Plasmodium malaria*. The result showed that 85.5% patients were having low platelet count meaning that 70.5% had mild, 10.5% moderate and 4.5% had severe thrombocytopenia (19).

Another Study was done by Koltas IS to evaluate hemoglobin, leukocyte, platelet and red cell distribution width value during acute *vivax* malaria from May 2002 to December 2004 G.C in Adana, Cukurova region, Turkey. 90 symptomatic *vivax* malaria patients were compared with 52 healthy controls. The average value for the mentioned parameters above was found to be significantly lower in the *vivax* malaria patients. The finding suggests that routinely used laboratory finding such as low hemoglobin, leukocyte or platelet counts provide more supportive information in the diagnosis of *vivax* malaria in endemic areas (20).

Sever malarial thrombocytopenia were studied in papua, Indonesia between April 2004 and December 2012 G.C by Lampah DA. The study compared the platelet counts and clinical correlates of patients with and those without malaria. Among 215,479 patients 66,421 had clinical malaria. Lowest platelet count and greatest risk of severe thrombocytopenia were seen in patients with *Plasmodium falciparum* mono infection. Sever malarial thrombocytopenia had an adjusted OR of 2.77 (95% CI, 2.20-3.48) and it identifies severe thrombocytopenia ascertain both children and adults at increased risk of death from *falciparum* or *vivax* malaria (21).

### 1.4. Significance of the study

Hematological changes are among the most common complications encountered in malaria. Of these complications, as different literatures presents, thrombocytopenia is a very common one. Persons with platelet counts < 150,000/μL (normal RI 150,000-450,000/ μL) were 12-15 times more likely to have malaria infection than persons with platelet counts > 150,000/μL (15).

In the routine process of malaria microscopy if there are low paracetemia professionals may report a false negative result to the clients even if they scan the recommended amounts of fields.

Therefore the proposed study is significant in that it shows the implication of Thrombocytopenia as another important suspicion conditions in parallel to the clinical manifestation during malaria infection Diagnosis. The study can also serve as a base line for other future studies.

## 2. Objectives

### 2.1. General objective

- To assess platelet count among malaria suspected patients and associated factors for patients that come to Arba Minch Health Center, Arba Minch, Ethiopia; February to April, 2015.

### 2.2. Specific objectives

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- To determine the prevalence of low platelet count in malaria positive patients.

- To compare platelet count in malaria negative and positive patients alongside with the presence and absence associated factors.

- To determine malarial species specific effect on platelet count in malaria positive patients.

## 3. Hypothesis

The null hypothesis, that was stated as ``low Platelet count in malaria positive patients cannot predict presence of malaria`` is rejected and the alternative Hypothesis is accepted.

# 4. Materials and methods

### 4.1. Study design

An Institutional based Quantitative cross sectional study was conducted in Arba Minch Health Center.

### 4.2. Study area

The study was conducted at Arba Minch, a town and separate woreda in southern Ethiopia, located in the Gamo Gofa Zone of the Southern Nations, Nationalities, and Peoples Region about 500 kilometers south of Addis Ababa, at an elevation of 1285 meters above sea level. It is the largest town in Gamo Gofa Zone and the second town in SNNPR next to Awassa and surrounded by Arba Minch Zuria woreda. Malaria infection is endemic in the area with a prevalence of 7 %( 28 malaria positive patients out of 400 suspects) with the predominant plasmodium specious detected was *plasmodium falciparum* 18(64.3%) followed by *plasmodium vivax* 7(25%) (32). that was why this study area was selected.

In Arba Minch Town there are Two Health Centers. The present study was conducted in one of them which is located near the center of the town and known to be established in 1953 E.C. The Health Center serves approximately 40,000 peoples of Arba Minch town. There are 5 pharmacists, 13 Nurses, 6 Midwiferies, 7 Health Officers and 5 Laboratory professionals in the Health center. Different Laboratory sections are available for different laboratory examinations. The Health Center has a quarterly EQA program in regional laboratory for different laboratory tests.

### 4.3. Study period

The study was conducted from February 1 to April 30, 2015.

### 4.4. Population

#### 4.4.1. Source population

The source populations were all patients that present in Arba Minch Health Center.

### 4.4.2 Study population

Patients suspected of malaria and who attended the laboratory within the study time period were study populations.

## 4.5. Sample size and Sampling method

### 4.5.1. Sample Size

The required sample size was calculated using single population proportion formula by taking the following assumption.

- $n = \frac{(Z_{\alpha/2})^2 pq}{d2} =$ (1.96*1.96) 0.5*0.5/0.052= 384
- When 10% nonresponse rate is considered = 38.4 ≈ 39
- The final sample size is by adding 10% none response rate it becomes = 423

Where n = sample size, Z = Z statistic for a level of confidence (95%), P = expected prevalence or proportion (P = 0.5), q=1-p=0.5 and d = precision or degree of error (d = 0.05) Z = Z statistic: For the level of confidence of 95%, which is conventional, Z value is 1.96.

### 4.5.2. Sampling method

By using sequential sampling technique all malaria suspected patients were included until the required sample size was reached.

## 4.6. Inclusion and Exclusion criteria

### 4.6.1. Inclusion criteria

- Malaria suspected patients of all age that present to Arba Minch Health Center and willing to participate in the study.
- Blood samples that passed acceptable criteria by the laboratory Standard Operational Procedure were included in the study.

### 4.6.2. Exclusion criteria

Blood specimen with:

- Improper specimen collection,
- Incorrect amount of blood to anticoagulant ratio,

- Hemolyzed sample,
- Clotted sample were excluded from the study.

## 4.7. Study Variables

### 4.7.1. Dependent variable

- platelet count

### 4.7.2. Independent variables

- Socio demographic characteristics (age, sex, occupation)
- Malaria status, Chloramphenicol, Alcohol intake, pregnancy, anti-malarial drug, bacterial infection, infectivity status.

## 4.8. Measurement and Data collection

### 4.8.1. Data collection Procedure

Four medical laboratory technologists together with the principal investigator were involved in the data collection. One of the medical laboratory technologists together with principal investigator acted as supervisor. Both the data collectors and supervisor were trained for two days with the objective of uniformity of the data collection instrument and with basic skill of collecting blood specimen, processing and analysis. Before the actual data collection, a pre-test of the instruments and the procedure was conducted and corrective measures were taken. The structured pre-tested questionnaire was used to study the associated factors that affect the current platelet count of malaria suspected patients

### 4.8.2. Sample Processing

From the study participants 3-5mls of venous blood was collected into ethylene diamine tetra-acetic acid (EDTA) tube and promptly analyzed for hematological parameters by routinely qualified automated hematology analyzer (Cell Dyn 1800) in Arba Minch general Hospital Laboratory. Both thin and thick blood films were prepared for malaria parasite examination and examined by trained laboratory professionals.

### 4.8.3. Data quality control

✓ **Pre-analytical quality assurance:**

- Blood samples were collected by well trained and experienced phlebotomist according to the Standard Operating Procedures (SOPs).
- All reagents were stored properly at 2-8$^{0}$C and reagents that were beyond expiry date were avoided.
- Samples were checked whether they are in the acceptable criteria like; hemolysis, clotting, volume and collection time.
- All blood samples collected were labeled properly.
- Prior to analysis, samples were homogenized and inverted 10-15 times.
- Cold box was used for transportation of the blood samples that were collected from the Health Center.

✓ **Analytical quality assurance:-**

- Daily Internal Quality control for the automated hematology analyzer was done by quality control reagents to keep reliability of the data collected from the machine.
- Quality of Giemsa stain was assured by using positive and negative control blood films.

✓ **Post-analytical quality assurance**:-

- All data recording was checked for completeness.
- The results were recorded with the patients' identification number.

### 4.9. Data Analysis and interpretation

Data entry and Statistical calculations were done using SPSS version 20 soft wares to see the current platelet count among malaria negative and positive patients and associated factors with 95% confidence interval. Binary and multivariable logistic regressions were calculated to see the association between independent and dependent variables. Percentages, tables and graphs were used for depiction of the data. P values less than 0.05 was considered statistically significant.

### 4.10. Ethical Considerations

Before starting the study, ethical clearance was obtained from the Departmental Research and Ethics Review Committee (DRERC) of, Department of Laboratory Sciences, College of Health Sciences of Addis Abba University and the acquiescence was also obtained from the study institutions (Arba Minch health Center and Arba Minch Hospital). Full clarification about the purpose of the study was made to the Authorized person of the health facilities. A formal letter of cooperation was obtained from Arba Minch Health Center and Arba Minch Hospital. The respondents were informed of their right to refuse or settle to participate in the study, or discontinue their participation whenever they feel the need as it is present on the informed consent for each participant. Privacy was maintained during data collection, and confidentiality of the data was assured.

### 4.11. Dissemination of results

This study on completion could serve as a reference material to researchers, experts and policy makers for intervention. To reach these bodies the completed paper will be submitted to College of Health Sciences, Department of Laboratory Sciences. In addition, a copy of this material will be given to Arba Minch Health Bureau and Arba Minch Health Center. The result will also be disseminated through publication in peer reviewed local and international journals and through presenting it in related workshops and seminars.

# 5. Results

## 5.1. Socio demographic characteristics

In this study 424 malaria suspected patients were included. Majority of the participants were between the age group of 20 – 45. 231(54.5%) of the respondents were females. Among the study populations 205(48.3%) were students and 41(9.6%) were Private workers. About 335 (79%) belonging to the Gamo ethnic group (Table 1).

Table1. Socio demographic distribution in the study group SNNPR, Gamo Gofa Zone Arba Minch Health Center, Arba Minch, Ethiopia from February to April 2015.

| Variables | Frequency | Percent (%) |
|---|---|---|
| **Age** | | |
| **<20** | 170 | 40.1 |
| **20-45** | 212 | 50 |
| **≥45** | 42 | 9.9 |
| **Sex** | | |
| **Male** | 193 | 45.5 |
| **Female** | 231 | 54.5 |
| **Occupation** | | |
| **Student** | 205 | 48.3 |
| **House wife** | 100 | 23.6 |
| **Gov't worker** | 78 | 18.4 |
| **Private business** | 41 | 9.7 |
| **Ethinicity** | | |
| **Gamo** | 335 | 79 |
| **Gofa** | 23 | 5.4 |
| **Welayta** | 26 | 6.1 |
| **Amhara** | 40 | 9.5 |

## 5.2. Status of Platelet count among malaria suspected study groups

From a total of 424 study groups 117(27.6%, n=424) of the subjects had malaria infection confirmed by microscopy. Majority of the malaria infected patients were thrombocytopenic (Platelet count less than 150,000/µl). Platelet count less than 150,000/µl was noted in

153(36.1%, n=424) respondents and 84 (54.9%, n=153) were positive for malaria and 69(45%, n=153) of the thrombocytopenic patients were negative for malaria (Table 2).

**Table.2**. Frequency of Thrombocytopenia in malaria positive and negative patients SNNPR, Gamo Gofa Zone Arba Minch Health Center, Arba Minch, Ethiopia from February to April 2015.

| Malaria status | Thrombocytopenic (%) | Non-Thrombocytopenic (%) | Total |
|---|---|---|---|
| **Positive for malaria** | 84(19.8%) | 33(7.8%) | 117(27.6%) |
| **Negative for malaria** | 69(16.3%) | 238(56.1%) | 307(72.43%) |
| **Total** | 153(36.1%) | 271(63.93%) | 424(100%) |

### 5.3. Thrombocytopenia status in the presence of different associated factors

Among malaria infected patients, 59 (50.4%, n=117) had *Plasmodium falciparum* infection whereas 56 (47.7%, n=117) had *Plasmodium vivax* infection and only 2(0.02%, n=117) had mixed infection. Thrombocytopenia risk is higher in malaria positive patients than that of non-malarial patients. The mean platelet count in *Plasmodium vivax* was 163,000/µl (SD 115,000) with a range of 18,000-403,000/µl as against *Plasmodium falciparum* malaria 128,000(SD72, 324/µl) with a range of 33,000-311,000/µl).

In this study among the malaria positive thrombocytopenic patients 44(52.4%, n=84) were positive for malaria for the first time and the rest 40(47.6%, n=84) were had repeated malaria infection. This shows thrombocytopenia probability is high in a newly malaria infected patients than that of the repeated one.

Of the 117 patients that are malaria positive patients platelet count ranged 18,000/µl-403,000/µl with a mean value of 146,931/µl and median of 115,000/µl. Likewise of the 307 malaria negative individuals the count ranged between 43,000/µl-444,000/µl with a mean value of 229,719/µl and median of 221,000/µl(Table 3 & 4).

**Table3**. Prevalence of malaria species among the study groups and their respective mean platelet count, SNNPR, Gamo Gofa Zone Arba Minch Health Center, Arba Minch, Ethiopia from February to April 2015.

| Malaria Positive | Frequency (%) | Mean platelet count |
|---|---|---|
| *P. falciparum* | 59(50.4) | 128,000(SD±72, 324/µl) |
| *P. vivax* | 56(47.7) | 163,000/µl (SD± 115,000) |
| Mixed | 2(0.02) | |
| Total | 117(100) | |

In this study the prevalence of new malaria infection status was 62(53%, n=117) in both the thrombocytopenic and non-thrombocytopenic groups which was greater than the repeated 55(47%, n=117) infection.

**Table 4** Prevalence of Thrombocytopenia in different Malaria infection status among the study groups SNNPR, Gamo Gofa Zone Arba Minch Health Center, Arba Minch, Ethiopia from February to April 2015.

| Malaria Infection status | Frequency of Thrombocytopenia (%) | Frequency of Non-Thrombocytopenia (%) | Total(%) |
|---|---|---|---|
| **New infection** | 44 (37.6) | 18 (15.4) | 62 (53) |
| **Repeated infection** | 40 (34.2) | 15 (12.8) | 55 (47) |
| **Total (%)** | 84 (71.8) | 33 (28.2) | 117 (100) |

Among 231(n=424) female respondents 163(70.6%, n=231) were aged between 15-45 years. Totally 31(19%, n=163) pregnancy were seen. Among this 28(90.3%, n=31) were thrombocytopenic regardless of malaria infection and the rest 3(9.7%, n=31) were positive for malaria.

Here in this study 69(82.1%, n=84) were malaria positive and thrombocytopenic with the absence of any associated factors described here. The rest 33(39.3%, n=84) were positive for one or more associated factors. (Table5).

**Table 5**. Thrombocytopenia status in the presence and absence of different associated factors in malaria positive and malaria negative patients SNNPR,Gamo Gofa Zone Arba Minch Health Center Arba Minch Ethiopia from February to April 2015.

| **Associated Factors** | | **Malaria infection status** | | | | |
|---|---|---|---|---|---|---|
| | | **Malaria Positive** | | **Malaria Negative** | | **Total n (%)** |
| | | Thrombocytopenic | Non - thrombocytopenic | Thrombocytopenic | Non-thrombocytopenic | |
| **. Diuretics** | **With Diuretics medicine** | --- | --- | 2(0.5%) | --- | 2(0.5%) |
| | **Without Diuretics Medi** | 84 (19.8%) | 33(7.8%) | 67(15.8%) | 238(56.1%) | 422(99.5%) |
| | **Total** | 84(19.8%) | 33 (7.8%) | 69 (16.3%) | 238(56.1%) | 424(100%) |
| **Anti Malarial drug** | **With Anti-malarial drug** | 18(4.2%) | 12(2.8%) | 24 (5.7%) | 56(13.2%) | 110(25.9%) |
| | **Without anti-malarial drug** | 66(15.6%) | 21(5%) | 45(10.6%) | 182(42.9%) | 314(74.1%) |
| | **Total** | 84(19.8%) | 33(7.8%) | 69(16.3%) | 238(56.1%) | 424(100%) |
| **Chloramphenicol** | **With Chloramphenicol** | -- | -- | 8(1.9%) | -- | 8(1.9%) |
| | **Without chloramphenicol** | 84(19.8%) | 33(7.8%) | 61(14.4%) | 238(56.1%) | 416(98.1%) |
| | **Total** | 84(19.8%) | 33(7.8%) | 69(16.3%) | 238(56.1%) | 424(100%) |
| **Alcohol Intake** | **Enduring consumer of alcohol** | --- | ---- | 7(1.7%) | 6 (1.4%) | 13(3.1% |
| | **Without alcohol** | 84(19.8%) | 33 (7.8%) | 62(14.6%) | 232(54.7%) | 411(96.9%) |
| | **Total** | 84(19.8%) | 33(7.8%) | 69(16.3%) | 238(56.1%) | 424(100%) |
| **Bacterial Infection** | **Bacterial infection** | 12 (2.8%) | 8 (1.9%) | 44 (10.4%) | 92 (21.7%) | 156(36.8%) |
| | **Without Bacterial infection** | 72 (17%) | 25 (5.9%) | 25 (5.9%) | 146 (34.4%) | 268(63.2%) |
| | **Total** | 84(19.8%) | 33(7.8%) | 69(16.3%) | 238(56.1%) | 424(100%) |
| **Pregnancy** | **Pregnant** | 3(0.7%) | - | 28(6.6%) | - | 31(7.3%) |
| | **Non pregnant(females 15-45 age)** | 16(3.8%) | 13(3.1%) | 25(5.9%) | 78(18.4%) | 132(31.1%) |
| | **Others** | 65(15.3%) | 20(4.7%) | 16(3.8%) | 160(37.7%) | 261(61.6%) |
| | **Total** | 84(19.8%) | 33(7.8%) | 69(16.3%) | 238(56.1%) | 424(100%) |

**Table6** Grade of Thrombocytopenia in patients with malaria and without malaria SNNPR,Gamo Gofa Zone Arba Minch Health Center, Arba Minch Ethiopia from February to April 2015.

| | Malaria diagnosis | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Grade of Thrombocytopenia** | **Malaria positive** | | | | | **Malaria** | | **Total (%)** |
| | ***Pf* (%)** | C.frq % | ***Pv* (%)** | C.frq % | **Mixed (%)** | **Negative (%)** | C.frq% | |
| **Severe (plt< 50,000/µl)** | 10(2.4) | 4.6 | 4(0.9) | 2.2 | - | 3(0.7) | 1.3 | 17(4.0) |
| **Moderate(plt≥50,000 &<100,000/µl)** | 11(2.6) | 7.2 | 20(4.7) | 22.5 | - | 23(5.4) | 27.9 | 54(12.7) |
| **Mild (plt≥100,000&<150,000/µl)** | 25(5.9) | 33.8 | 14(3.3) | 13.6 | - | 43(10.1) | 43.9 | 82(19.3) |
| **Normal(plt≥150,000/µl)** | 13(3.1) | 10.3 | 18(4.2) | 17.8 | 2 (0.6) | 238(56.1) | 100 | 271(64.0) |
| **Total** | **59(14%)** | | **56(13.1%)** | | **2(0.6%)** | **307(72.3%)** | | **424(100%)** |

The risk of thrombocytopenia was more in *Plasmodium falcipharum* 46(77.9%, n=59) than *Plasmodium vivax* 38(67.8%, n=56). Prevalence of severe thrombocytopenia in *Plasmodium falciparum* 10(71.4%, n=14) infection was higher than *Plasmodium vivax* infection 4(28.6%, n=14). In this study moderate thrombocytopenia was seen to be higher in *Plasmodium vivax* 20(64.5%, n=31) than *Plasmodium falciparum* infection 11(35.5%, n=31). [Figure 1] shows the graphic representation of the platelet count range in correlation with the type of malaria.

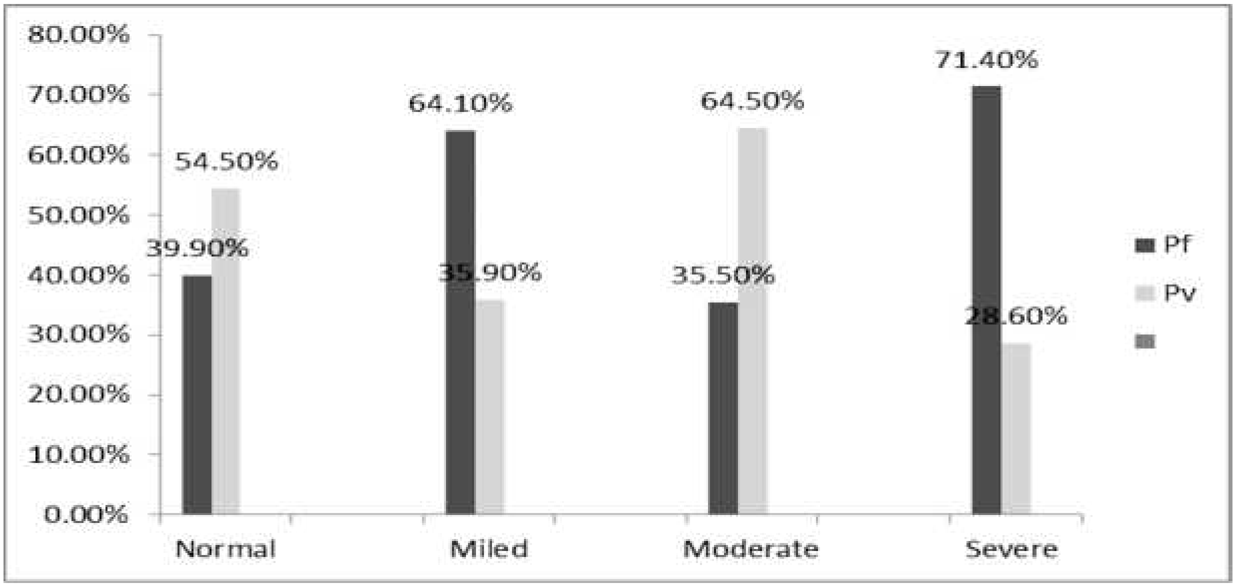


**Figure 1**: Graphic presentation of platelet count in *Plasmodium vivax* and *Plasmodium falciparum* malaria cases SNNPR,Gamo Gofa Zone Arba Minch Health Center, Arba Minch, Ethiopia 2015.

In Multivariate analysis, when 95% CI for the adjusted odds ratios were calculated among these variables, thrombocytopenia status on the malaria suspected cases showed statistically significant association with Occupation of the participant (p=0.002), Chloramphenicol treatment (p<0.0001), Bacterial Infection (p=0.008), and Malaria status (p=0.001) of the study groups. Those study subjects, who were private workers were 0.27 times more likely to have thrombocytopenia than those who were house wife, government worker and student (AOR= 0.27; 95%CI 0.09-0.78).

Clients who were suspected of malaria showed different thrombocytopenia status when compared with those having normal thrombocyte count. Those taking chloramphenicol treatment are 32 times more likely to develop thrombocytopenia (AOR=32.2; CI 5.67-183.28), those with bacterial infection were 2 times more likely to develop thrombocytopenia (AOR=2.14; CI 1.22-3.76), and those who were positive for malaria are 15 times more likely to develop thrombocytopenia (AOR = 15.79; CI 8.70-28.66) than non-infected subjects. However, there was no significant difference in overall thrombocyte count of the study participants by difference in sex, age group, anti-malarial drug, and Infectivity status (Table 8).

**Table 7.**Association between platelet count with each explanatory variable (Crude & adjusted OR) among malaria suspected patients,SNNPR,Gamo Gofa Zone Arba Minch Health Center, Arba Minch, Ethiopia from February to April 2015.

| Factors | Thrombocytopenic | Normal Platelet | Crude OR (95%CI) | AdjustedOR (95% CI) | P-Value |
|---|---|---|---|---|---|
| **Sex** | | | | | |
| **Male** | 79 | 114 | 1 | 1 | |
| **Female** | 74 | 157 | 0.68(0.46-1.01) | 0.64(0.36-1.16) | 0.14 |
| **Age** | | | | | |
| **<20 years** | 68 | 102 | 1 | 1 | |
| **20-45 years** | 73 | 139 | 0.78(0.52-1.19) | 0.83(0.44-1.58) | 0.58 |
| **≥45** | 12 | 30 | 0.68(0.28-1.25) | 0.88(0.29-2.61) | 0.82 |
| **Occupation** | | | | | |
| **House wife** | 35 | 66 | 0.85(0.52-1.39) | 1.06(0.46-2.48) | 0.89 |
| **Private worker** | 11 | 30 | 0.59(0.28-1.23) | 0.27(0.09-0.78) | 0.02** |
| **Gov't worker** | 28 | 49 | 0.91(0.53-1.57) | 0.54(0.22-1.33) | 0.18 |
| **Student** | 79 | 126 | 1 | 1 | |
| **Anti-mal drug** | | | | | |
| **Yes** | 42 | 68 | 1.13 (0.72-1.77) | 0.70(0.37-1.33) | 0.28 |
| **No** | 111 | 203 | 1 | 1 | |
| **Chloramphenicol treatment** | | | | | |
| **Yes** | 8 | 2 | 7.42 (1.56-35.41) | 32.2(5.67-183.28) | 0.001** |
| **No** | 145 | 269 | 1 | 1 | |
| **Consumer of alcohol** | | | | | |
| **Yes** | 7 | 6 | 2.12(0.69-6.42) | 3.15(0.79-12.41) | 0.101 |
| **No** | 146 | 265 | 1 | 1 | |
| **Pregnancy** | | | | | |
| **Yes** | 14 | 17 | 1.51(0.72-3.15) | 1.59(0.59-4.25) | 0.36 |
| **No** | 139 | 254 | 1 | 1 | |
| **Bacterial Infection** | | | | | |
| **Yes** | 56 | 100 | 0.99(0.65-1.49) | 2.14(1.22-3.76) | 0.008** |
| **No** | 97 | 171 | 1 | 1 | |
| **Malaria status** | | | | | |
| **Positive** | 84 | 33 | 8.8(7-11) | 15.79(8.70-28.66) | 0.0001** |
| **Negative** | 69 | 238 | 1 | 1 | |
| **Infection status** | | | | | |
| **New** | 44 | 18 | 15.19(6.92-33.37) | 0.696(0.27-1.82) | 0.46 |
| **Repeated** | 40 | 15 | 16.57(7.31-37.59 ) | 2.05(0.96-4.37) | 0.06 |
| **Non-infected** | 69 | 238 | 1 | 1 | |

**P< 0.05 (statistically significant association) for the Adjusted Odds Ratio

## 6. Discussion

In this Institutional based cross sectional study a total of 424 study subjects were included out of which 117(27.6%) of the subjects had malaria infection confirmed by microscopy. The prevalence of thrombocytopenia from the total study subjects was 153(36.1%) and among this 84(54.9%) were positive for malaria (p=0.001) and the rest 69(45.1%) were negative. This study showed that 71.8% of malaria infected patients had thrombocytopenia than non-infected ones, which is in close approximation to the study conducted in Thailand by Kotepui M et al 2013 where the prevalence was 84.9%, and study conducted in India by Faseela TS et al 2010 (82.8%) where more than half of malaria positive patients were known to be thrombocytopenic (14).

Although the mechanism of thrombocytopenia is not fully understood, it is thought to be due to peripheral destruction and consumption. Immune complexes generated by malaria antigen lead to sequestration of the injured platelets by macrophages in the spleen and platelet consumption in disseminated intravascular coagulation together with platelet dysfunction resulting in hyper aggregation are thought to contribute to thrombocytopenia in malaria (15).

Thrombocytopenia was present in both the *P. vivax* 38(67.9%, n=56) and the *P. falciparum* 46(77.9%, n=59) infections. Similar finding was seen in study conducted by Saleem AK et al from Lahore 2008 where prevalence of thrombocytopenia in *P. vivax* (82.5%) was known to be in good approximation with the prevalence of thrombocytopenia in *P. falciparum* (86.4%) infection (33).

This study classified thrombocytopenia into three groups, mild, moderate and severe (13). There were 82(53.6%, n=153) cases of mild thrombocytopenia, 54(35.3%, n=153) moderate thrombocytopenia, and 17(11.1%, n=153) severe degree of thrombocytopenia. Majority of the patients in this study were positive for *P. falciparum* infection 59(50.4%, n=117) and it was in this group higher amounts of low platelet count 46 (77.9%, n=59) registered than *P. vivax* 38(67.9%, n=56).

This result was in agreement to the study conducted by Dhugat MP et al in Maharashtra India 2011, Joshi G et al in Surat Gujarat and Kumar J et al in Bihar India 2014, where the majority of thrombocytopenia is observed in *P. falciparum* infection followed by *P.vivax* (4, 13, and 17). This may be because of decreased AT (Anti Thrombin) - III activity and elevated plasma concentrations of Thrombin-Antihrombin III complexes, confirming activation of the coagulation cascade in *Falciparum* malaria.

In the current study majority of malaria negative suspects 238(77.5%, n=307) had normal platelet count. In both *P. falciparum* and *P. vivax* infected patients mild thrombocytopenia 39(46.4%, n=84) was high followed by Moderate 31(36.9%, n=84) and Severe thrombocytopenia 14(16.7%, n=84). This finding is comparative with the study conducted by Kakar N et al in Pakistan 2014 in that 84.1% were mild, 59.1% moderate and 44.3% of patients were severely thrombocytopenic. In contrast to this finding a result of study conducted by Faseela TS et al in India 2010, showed that most of malaria positive patients (43%) had there platelet count between 50,000-100,000/µl followed by severe thrombocytopenia (plt<50,000/µl) (17%), This might be due to majority of their study participants had mixed infection with *Plasmodium falciparum* and *Plasmodium vivax* for that with multiple genotype and specious infection the prevalence of more severe thrombocytopenia was increased.

Among the associated factors studied being a private worker (Farmer, petty trader) were 0.27 times more likely to have thrombocytopenia than those who were house wife, government worker and student (AOR= 0.27; 95%CI 0.09-0.78) (p=0.02). Those taking a chloramphenicol treatment were 32 times more likely to develop thrombocytopenia [AOR= 32.2, 95%CI 5.67-183.28] (p<0.001) and being positive for bacterial infection have a risk of 2 times more likely to develop thrombocytopenia [AOR= 2.14, 95%CI 1.22-3.76] (p=0.008) than the negative ones. The level significance of being malaria positive was also high with 15 times more likely to develop thrombocytopenia than non-infected subjects [AOR=15.8, 95% CI 8.7-28.7] (p<0.0001).

In the current study 67.9% of patients with *P. vivax,* infection had platelet count less than 150,000/µl which is in close approximation to other study reported by Bhandary N et al in Karnataka, India 2011; where 73.9% of patients detected with *P. vivax* malaria had platelet count less than 150,000/µl (11).

In similar with the study conducted by Gill MK et al 2013 in Adesh Institute of Medical Sciences and Research, Bhatinda, India, the mean platelet count in *Plasmodium vivax* (163,000/µl) infection was seen to be higher in this study than the mean platelet count in *Plasmodium falciparum* malaria (128,000/µl) infection, where in their study 127,652/µl was mean platelet count for *P. vivax* and 78,500/µl was mean platelet count in *P.falciparum* infection (13).

Likewise to the study conducted by Jairajpuri SZ et al at Hakeem Abdul Hameed Centenary Hospital, New Delhi, India, 2014; there was statistically significant reduction of platelet count ($p<0.001$) in patients with malaria compared to patients without malaria which was similar to this study (10).

Here in this study among the 84 malaria positive and thrombocytopenic patients more than half were malaria infected for the first time 44(52.3%) and the rest 40(47.6%) were repeatedly infected. This result shows prevalence of thrombocytopenia was high in first time infected patients than that of repeated infection. A study from Nigeria by Igbeneghu C et al, 2013; also signifies that 78% of the study groups were infected for the first time which shows the prevalence is high in firstly infected patients, similar to this study (16). Platelet phagocytosis may contribute to this condition.

Another study from here Ethiopia conducted by Desta Kassa et al on parasito-hematological features of acute *P. falciparum* and *P.vivax* malaria patients showed that Hgb, Hct, and platelet values decreased in *falciparum*( $p<0.0001$)and *vivax* malaria patients. In their study they do not found a significant hematological variation between the two malaria groups (33).

Apparently, platelet activation by plasmodium can potentially lead to the formation of micro-aggregates of infected red blood cells and platelets which can occlude blood vessels and it also leads to binding to and activation of the endothelium. Increased Von Willebrand Factor concentrations is also thought to play a role; along with oxidative stress, alterations in splenic function and immune mechanisms (28, 29, and 30).

# 7. Strength and Limitation of the study

## 7.1. Strength of the study

- To our knowledge this is the first study in Ethiopia that specifically assesses thrombocytopenia as an indicator of Malaria infection and can be used as a baseline study for future researches.

## 7.2. Limitation of the study

- A large scale prospective study could not be conducted because of time constraint which may give a better figure.
- A clinical History of the participants was taken and not laboratory examinations were conducted for the investigation of the associated factors which if performed can clearly show the association with a minimized confounder's effect.

# 8. Conclusion and recommendations

## 8.1. Conclusion

From the finding of the study even if other Hematological parameters were altered in malaria infection reduced number of platelet count is a significant marker for the malaria infection and is associated with both *Plasmodium vivax* and *Plasmodium falciparum* infection. Newly malaria infected patients had a greater probability of getting low platelet count than that of a repeated infection. Identification of thrombocytopenia is important not only as a screening tool for identification of malaria in endemic regions but also has important prognostic significance. In an endemic area the platelet count has to be checked in because the presence of thrombocytopenia may indicate malaria infection.

In addition bacterial infection, occupational status and chloramphenicol treatment had a significant association to low platelet count. In diagnosis of malaria infection obtaining mild to severe thrombocytopenia gives a clue to determine the species of malaria. Mild thrombocytopenia was the most common finding in malaria infection with severe thrombocytopenia was higher in *Plasmodium falciparum* infection.

## 8.2. Recommendations

- A physician that suspects a patient for malaria diagnosis using clinical manifestations only can use platelet parameter as another supporting marker.
- If laboratory technologists' register severe thrombocytopenia in parallel with marked anemia in patients that live around malaria endemic area, malaria diagnosis should be considered.
- Since finding of low platelet count in patients significantly indicates the presence of malaria, laboratory technologists are highly recommended to consciously scan, detect and identify malaria parasite before reporting it as negative for hemo parasite.
- In similar with malaria infection, since bacterial infection, chloramphenicol treatment and occupational status can cause a significant reduction of platelet count, Health professionals are recommended to treat the underlying cause of low platelet count before prescribing platelet transfusion.

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# Annex I: Tests protocol

**SOP for venous Blood Collection**

1. Place a tourniquet above the venipuncture site.

2. Palpate and locate the vein. Disinfect the venipuncture site meticulously with alcohol by swabbing the skin concentrically from the center of the venipuncture site outwards. Let the disinfectant evaporate. Do not repalpate the vein again.

3. Perform venipuncture.

4. If withdrawing with conventional disposable syringes, withdraw 5-10 ml of whole blood from adults, 2-5ml from children and 0.5-2ml from infants

5. Remove the tourniquet. Apply pressure to site until bleeding stops.

6. Using aseptic techniques transfer the blood specimen to appropriate tubes (if using syringe). Secure tube caps tightly.

7. Label the tube with the patient's unique identifier (name and/or number), date and time of collection, using a marker pen.

8. Do not recap used sharps. Discard directly into the sharps disposal container.

9. Complete the patient laboratory request forms using the same identifier.

10. Proceed to the preparation of malaria blood films and CBC.

**Transportation of whole blood from the collection site to the Laboratory**

1. After collection blood should be cooled to between $+2^0$C and $+10^0$C during transport in the designed blood transport cold box.
2. Wet ice in leak-proof container, such as plastic bags is recommended.
3. Supper cooled cubed ice, canned ice or dry ice should not be used for shipping or storage of whole blood. (*WHO, manual on the management, maintenance and use of blood cold chain equipment.)*

**Testing Protocol for Complete Blood Count (CBC)**

**Principle of the Test**

Automated Hematology Cell Counters counts the cells based on the Coulter principle, i.e. electrical resistance principle, which depends on the fact that blood cells are nonconductive to electricity, so when they pass through an electrical field they will increase the electrical impedance (resistance).

CELDYN 1800 Hematology analyzer uses two independent measurement methods; they are:

- ✓ Electrical impedance method is used for determining WBC, RBC, and PLT count.
- ✓ Modified Met hemoglobin Method for determining HGB through photometric measurement.

**Procedure Quality Control**

- From the MAIN MENU screen, press [RUN]
- From the RUN menu, press [SPECIMEN TYPE] followed by [QC TYPE]
- Select the desired level of control (Low, Normal, High, or Replicates)
- Using the [ ] and [ ] arrows keys, select the desired control file.
- Press [RETURN]. Remove the cap from a well - mixed control specimen tube and place the open tube under the sample Aspiration probe. Raise the tube so that the end of the probe is deeply immersed in the specimen.
- Press the Touch Plate to activate the run. When the well-mixed control has been aspirated from the tube and the probe moves up through the Wash Block, remove the specimen tube and replace the cap
- Verify that control results are within your laboratory’s acceptable limits. If the control results fall within acceptable limits, review the data for shifts or trends, record the results, and begin to process patient specimens
- If one or more result falls outside the laboratory’s acceptable limits, review section 10: Troubleshooting and Diagnostics. If the problem persists, contact Abbott Diagnostics Customer Service. Do not process patient specimens.

**Procedure for Sample run**

- Prior to running patient specimens, perform DAILY Start –up procedure Detailed earlier in this section.
- When the READY message is displayed on the RUN Screen, the instrument is ready to run specimens. To run patient specimens, proceed as follows:
- With the cap tightly secured on the specimen tube, slowly invert the tube 10 to 15 times
- Remove the cap from the pre-mixed specimen tube
- Place the tube under the aspiration probe and raise the tube so that the end of probe is deeply immersed in the specimen
- Press the Touch Plate to activate the run.
- When the sample has been aspirated from the tube, the probe will move up through the Wash Block. Remove the specimen tube and Recap the tube
- After the cycle is completed, run results are displayed on screen and the aspiration probe moves into position to accept a new specimen. The current run data is saved to the data log.
- If Automatic Graphics Printout has been specified in the SETUP menu, a report is Printed according to the parameters selected during the setup procedure
- If Automatic Graphics printout has not been specified in the SETUP menu, press [PRINT REPORT] to obtain a copy of the results. The print report format is the only method to be used for reporting patient results
- For an explanation of results that fall outside of acceptable ranges, see Section 3: Principles of operation, Subsection: System –Initiated data flags and messages

**Procedure daily shutdown**

- The daily shutdown procedure consists of rinsing the flow system with one of the reagents (detergent). On a daily basis depend on instrument usage and the laboratory’s procedures. It may not be necessary to perform this procedure every day because the instrument goes in to A STANDBY state automatically if it has been idle for hours or some other duration specified by the operator (see Automatic Start-up and Shutdown in user manual).Before the instrument enters the STANDBY state, the flow panel is automatically rinsed.

If desired, the operator may place the instrument in STANDBY, by pressing [DAILY SHUTDOWN] in the SPECIAL PROTOCOLS menu. When this key is pressed or when Automatic shutdown is initiated, the following occurs:

- The Flow system is rinsed.
- The timer control, which periodically opens all of the solenoid valves to prevent pinched tubing, is set.
- When the instrument enters the STANDBY state, the LCD back light will turn off after 15 minutes of noon-use**.**
- The operator may turn the backlight on by pressing any key on the PC keyboard. However, the instrument must be initialized to run patient specimens.
- The daily shutdown cycle takes approximately 3-4 minutes
- When the power is required to be OFF, the operator must perform the same procedures described in Daily Shutdown within this section.
- From the MAIN MENU screen, press [SPECIAL PROTOCOLS].
- Press [DAILY SHUTDOWN].
- When the daily Shutdown cycle is completed, turn the instrument power switch off
- Cover the machine with appropriate cover cloth for dust protection

**Result Interpretation:**

Hematology results are printed on the host computer processing sheets

Before reporting any patient results, the following checks are required:

- ✓ That the number printed with the results matches the number of samples runs.
- ✓ That the patient ID number matches the patient sample order
- ✓ That the sequence and cassette number matches the patient sample order.
- ✓ In addition, each CBC parameter is automatically checked against a set of Abnormal hematologic criteria

**Expected Values**

| Analyte | Reference Interval | Units |
|---|---|---|
| Platelet | 150-450x109 | cells per Lite |

## SOP FOR BLOOD FILM PREPARATION

**Principle**: The thick film is used as a screening test to establish the presence of malaria, and the thin film is used to identify the species of the organism. Examination of malaria blood films by microscopy is a basic technique, which remains the gold standard for the diagnosis of malaria. Good quality blood films are essential to establish accurate diagnosis.

**Using venous blood**:

1.Using a micropipette, place a 2µl drop of blood in the smaller circle and 6 µl in the bigger circle of the slide (pre-labeled) placed over the template. Do not delay between applying and spreading the drop.

**Preparation of the thin film**

2. Working quickly, obtain a second clean and polished slide (spreader) and place it front of the small drop blood at a 30º - 45º angle. Pull back the slide and hold until the blood is evenly spread along the edge of the slide. Do not delay between applying and spreading the drop.

3. Rapidly push the slide forward in a single, smooth, continuous motion. Avoid hesitation or jerky motions when spreading the blood. (A feathered end of the film should have red blood cells that are lying individually without overlapping and relatively evenly distributed).

**Preparation of thick blood film**

1**.** With one corner of the spreader slide, in a circular motion, spread the blood out to make a circle with approximately **1cm (1/3 inch) in diameter,** finishing off at the center.

2. The ideal thickness of the smear should allow for printed text to be readable when it is placed on it.

3. Discard the spreader into an appropriate slide container and **DON'T** re-use it for another patient's blood sample.

4. Allow both the blood films to air dry in a horizontal position on a slide tray or folder. If EDTA blood is used, drying should be between 24–72 hours. Slow drying prevents cracking. Avoid using a fan or blow dryer to dry these slides.

**SOP for Staining:** Good quality staining of blood films is essential to establish accurate diagnosis.

**Procedure:** **Fixing the thin film**

1. When the films are completely dry, fix ONLY the thin film by dipping if in absolute methanol for approximately 30seconds. Care must be taken not to fix any portion of the thick film.
2. Allow the film to dry.

**Staining the thick and thin films**

1. Gently pour 3% or 10% Giemsa working solution in to the staining jar.
2. Put the slides in a rack inside the staining jar; the slides should be fully submerged/covered with the stain.
3. Stain for 30-45minutes and 10-15minutes for 3% and 10% Giemsa working solutions, respectively.
4. Pour clean water gently in to the jar to float off the iridescent scum on the surface of the stain. Alternatively, gently immerse the whole jar in a vessel filled with clean water.
5. Gently pour of the remaining stain, and rinse slides again in clean water for a few seconds. Pour the water off.
6. Wipe the back of each slide with paper towels.
7. Dry the slides in a vertical position with the thin film down wards.

### Evaluation of a well-stained thin film

1. The background should be clean and free from debris; the color of erythrocytes is a pale green pink.
2. Neutrophil leukocytes have deep purple nuclei and well-defined granules.
3. The chromatin of malaria parasites is a deep purplish red and cytoplasm a clear purplish blue.
4. Stippling should show up as Schuffner's dots in erythrocytes containing *P. vivax* or *P. ovale*, and Maurer's spots in erythrocytes containing thelarger ring forms of *P. falciparum*.

### Evaluation of a well-stained thick film

1. The background should be clean and free from debris, with a pale mottled-grey color derived from the lysed erythrocytes.
2. Leukocytes nuclei are a deep, rich purple.
3. Malaria parasites are well defined with deep-red chromatin and pale purplish blue cytoplasm. In *P. vivax* and *P. ovale* infections the presence of Schuffner's stippling in the "ghost" of the host erythrocyte can be seen especially at the edge of the film.

### Evaluation of staining quality

**1.** A MBF that is too pinkish suggests low pH or over-staining.

**2.** A MBF that is too bluish or purplish suggests high pH or under-staining.

## SOP FOR EXAMINATION OF MALARIA BLOOD FILMS

**Focusing and scanning the blood film**

**Procedure:**

1. Place the MBF (Malaria Blood Film) on the microscope stage, switch on the light and adjust the light source optimally by looking through the ocular and the 40X objective.
2. Place a drop of immersion oil on the dry stained slide. To avoid cross contamination, ensure that the immersion applicator never touches the slide.
3. Slowly change to the oil immersion objective, and a thin film of oil will form between the slide and the lens.

4. Adjust the light source optimally by looking through the 10x ocular (eyepiece) and the100X objective and use the fine adjustment knob to focus the field; the lens should not be allowed to touch the slide.

5. Examine the slide in a systematic fashion. Start at the left end of the thick film and begin reading at the periphery of the field and finish at the other end. When the field is read, move the slide right to examine adjacent fields.

**Examining the thick blood film**

1.Scan the thick film under oil immersion objective (x100) and ascertain whether a smear is positive or negative.

2.Use the **"WHO Bench Aids in the Diagnosis of Plasmodium Infections"**

3.If positive, determine all species and stages present.

4.Read a minimum of 200 oil immersion fields before declaring a slide negative. If time permits, scan the whole thick film.

**Examining the thin blood film**

1. When species is doubtful on the thick film, or mixed infections are suspected, a careful examination of the parasite morphology should continue on the thin smear for verification.

2. If different species are observed, this should also be recorded.

**Result Interpretation**

- Report" Parasite species, stage If it is negative, report "No haemoparasite seen'.

- One asexual parasite form that is 100% typical must be observed for a slide to be called POSITIVE. A slide is positive if both chromatin and perfect cytoplasm OR chromatin and pigment are observed.

-Parasites are considered atypical (<100%) if classical features are distorted i.e. cytoplasm is not well defined, ring is broken, ring is not the correct color or shape etc…

-If atypical parasites are observed, both thick and thin films should be examined carefully. Alternately a follow-up slide can be prepared.

## Annex II: English and Amharic Version Questionnaire Consent

### Informed Consent Form

**Name of the Principal Investigator:** Kidist Mikre (BSc)

**Name of Advisors:** Melaku Tamene (MSc, Phd candidate) and Mintwab Hussein (Lecturer).

**Name of the organization:** Addis Ababa University, College of Health Science, School of allied health sciences, Department of medical laboratory science.

Hello, my name is ____________________________________ I am currently a data collector on behalf of a research going to be conducted by___________________________ on assessment of current platelet count and associated factors for malaria suspected patients that comes to Arba Minch Health Center. So to help me/us investigate this study your cooperation is very helpful. The purpose of the study is to evaluate the current platelet count both in malaria positive and negative patients and the associated factors that affect the current platelet count. So we include all malaria suspected/requested patients like you.

If you agree to participate in the study, about 3-5 ml of blood will be collected from you or you will allow us to use the sample that you will give for your medical examination and you will be interviewed for the associated factors that affect your platelet count. During collection of blood, you may feel some discomfort, but this does not produce serious pain. All the data obtained will be kept strictly confidential by using only code numbers and locking the data, only study personnel will have access to the files. You may not get a personal benefit or any payment by participating in this study and you are not asked to pay for the laboratory examination. The sample that you give will not be used for other purpose other than this study. Since I am going to perform a test that may or may not requested for you a significant test result will be send to the physician and clinical treatment will be forwarded to you. Participating in the study is entirely your choice and you cannot participate. Participating and not participating has no influence on the service you seek to get.

**Participant's response:** I am free to decline to be in this study. My decision as to whether or not to participate in this study will have no influence on my present or future medical service. My signature below indicates that I agree to participate in this study.

Name and Signature of Person Obtaining Consent ______________

Date of signature________________________

Subject's signature___________________ date of signature_______________

Supervisor's name _________________________ Signature __________________

**በአዲስ አበባ ዩንቨርሲቲ የጤና ሳይንስ ኮሌጅ እና ተዛማጅ ሳይንስ ትምህርት ቤት የህክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል**

**በአርባምንጭ ጤና ጣቢያ የወባ በሽታ ምርመራ በታዘዘላቸዉ ህመምተኞች ላይ ስለ ፕሌትሌት የደም ህዋስ አጠቃላይ ሁኔታ እና የፕሌትሌትን የደም ህዋስ ቁጥር ስለሚቀንሱ ተያያዥነት ስላላቸዉ ሁኔታዎች ለማወቅ ሲባል ለሚደረገው ጥናት የተዘጋጀ የእጩ ተሳታፊዎች የፍቃደኝነት መጠየቂያ ውል**

የጤና ድርጅት ስም---------------------------------- መለያ(ኮድ)--------------

የመረጃ ሰብሳቢው ስም----------------------------

የተከበሩ ተሳታፊ

እንደምን አሉ፣ ስሜ--------------------------------------- ይባላል፡፡ በአሁኑ ሰአት በአዲስ አበባ ዩንቨርሲቲ የጤና ሳይንስ ኮሌጅ ህክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል የማስትሬት ዲግሪ ትምህርቴን በመከታተል ላይ የምገኝ ስሆን በዚህ በአርባ ምንጭ ጤና ጣቢያ ጥናቴን ለማካሄድ አስቤአለሁ፡፡ የጥናቱ መነሻ ርዕስ የሚያተኩረው በአርባምንጭ ጤና ጣቢያ የወባ በሽታ ምርመራ በታዘዘላቸዉ ህመምተኞች ላይ ስለ ፕሌትሌት የደም ህዋስ አጠቃላይ ሁኔታ እና የፕሌትሌትን የደም ህዋስ ቁጥር ስለሚቀንሱ ተያያዥነት ስላላቸዉ ሁኔታዎች ስለማወቅ ይሆናል፡፡

ስለሆነም በጥናቱ ላይ ለመሳተፍ ፍቃድኛ ከሆኑ ለጥናቱ አስፈላጊ የሚሆን ከ3-5ሚ.ሊ. የሚሆን የደም ናሙና ከእርስዎ እንወስዳለን፡፡ይህ የደም ናሙና በሚወሰድበት ወቅት መጠነኛ ህመም ሊሰማዎት ይችላል፡፡ ይሁን እንጂ ህመሙ ከፍተኛ ችግር አያመጣም፡፡የሚሰጡት የደም ናሙና ከታቀደለት አላማ ውጪ አይውልም፤በሚሰጡት የደም ናሙና የሚሰራው የላቦራቶሪ ስራ በሚስጥር ኮድ (መለያ) ሲሆን ስም እንደመለያ አንጠቀምም፡፡ ውጤቱም ከመረጃ ሰብሳቢዎቹ እና ከጥናት ባለሙያው ውጪ ለማንም ግልጽ አይሆንም፡፡ በዚህ ጥናት በመሣተፍዎ የሚያገኙት ግላዊ ጥቅም ወይም ክፍያ አይኖርም፤ በደም ናሙናዎ ላይ በሚሰራው የላቦራቶሪ ስራም ክፍያ አይጠየቁም፡፡

በተጉአዳኝነት የነዚህን የፕሌትሌት ህዋሳትን የሚቀንሱ በሽታዎች አሉ፡፡ ስለነሱም ለማወቅ የተዘጋጀ መጠይቅ አለ፡፡ ለዚህ ጥናት አላማ ስኬት እነዚህን ጥያቄዎች መጠየቅ እና መሙላት አስፈላጊ ይሆናል፡፡

በዚህ ጥናት ላይ የመሳተፍ ወይም ያለመሳተፍ ሙሉ መብት አለዎት፤ በዚህ ጥናት ባለመሳተፍዎ አሁን እያገኙ ባለዉ የህክምና አገልግሎት ወይም ወደፊት ሊያገኙ በሚፈልጉት አገልግሎት ላይ ምንም አይነት ተጽዕኖ አይኖረዉም፡፡

**የተሳታፊ ምላሽ፡** በዚህ ጥናት ለመሳተፍ ወይም ላለመሳተፍ ወይም ባልፈለኩ ሰአት ለማቋረጥ ነጻ ነኝ፤ በዚህ ጥናት ላይ በመሳተፌ ወይም ባለመሳተፌ በዚህ ተቋም በወቅቱ ወይም ለወደፊቱ በማገኘዉ አገልግሎት ላይ ምንም አይነት ተጽእኖ አያሳድርም፡፡ ከዚህ በታች ያለዉ ፊርማዬ በዚህ ጥናት ለመሳተፍ ፍቃደኛ መሆኔን ያረጋግጣል፡

የተሳታፊ ፊርማ--------------------------------

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የፍቃድ አግኚዉ ስም እና ፊርማ-------------------------------------------------

ቀን-------------------------

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የተቆጣጣሪ ስም እና ፊርማ-----------------------------------------------------------

ቀን--------------------------

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ስላደረጉልን ትብብር እናመሰግናለን!

**Questionnaire on the associated factors that can affect platelet count**

**Addis Ababa University College of health Science School of allied health sciences Department of medical laboratory science.**

SOCIO DEMOGRAPHIC CHARACTERISTICS. **CODE ________**

1. Sex of the patient 1.Male 2.Female .

2.Age of the patient ________

3. Ethnicity 1. Gamo 2. Gofa 3. Amhara 4. Welayta 5. Tigray 6. Others

4. Occupation 1. Government worker 2. House Wife 3. Farmer 4. Student

1. History of thrombocytopenia?

Yes ☐ No ☐

2. Do you ever take a treatment of cancer/radiation/chemotherapy?

Yes ☐ No ☐

3. Do you have a history of Anemia?

Yes ☐ No ☐

4. Do you have a history of exposure to toxic chemicals/pesticides/Arsenic/Benzene…?

Yes ☐ No ☐

5. Are you taking diuretics medicines?

Yes ☐ No ☐

6. Are you taking Chloramphenicol?

Yes ☐ No ☐

7.Are you enduring consumer of alcoholic beverage?

Yes ☐ No ☐

8.Do you have a history of parvovirus /mumps/rubella/EBV/chickenpox/HIV infection?

Yes ☐ No ☐

9.Are you pregnant? ( for females' age group 15-49)

Yes ☐ No ☐

10. If yes, are you close to delivery?

Yes ☐ No ☐

11.Do you have a history of bleeding disorder/ITP/TTP/DIC?

Yes ☐ No ☐

12.Is there a history of Lupus Erythematous/ Arthritis?

Yes ☐ No ☐

13.Are you taking medicine for seizures like Dilantin/Vancomycin/Rifampin/Quinidine/interferon/anti-convulsants?

Yes ☐ No ☐

14.Are you taking anti-malarial drug currently /quinine/chlorquine?

Yes ☐ No ☐

15.If yes How money times you take these madicine or tablate ?

--------------------------times

16.Are you on the treatment of Heparin?

Yes ☐ No ☐

17.If yes, is there any formation of blood clot in/around your leg (deep vein thrombosis) or pulmonary embolism?

Yes ☐ No ☐

18.Do you have a history of infection by mononucleosis/CMV/Bacterial infection?

Yes ☐ No ☐

18.Do you have a history of heart valve surgery?

Yes ☐ No ☐

19.Do you have a history of liver disease/ BM dysfunction/splenomegaly diagnosed currently?

Yes ☐ No ☐

20. Do you have a history of meningitis? Yes ☐ No☐

21. Do you encounter tissue damage currentely like burns, fracture?

Yes☐ No☐

22. Any hemorhage? Yes ☐ No☐

23.Are you taking a drug terapy like Corticosteroids, Aderenalin, thrombopoietien?

Yes ☐ No☐

24. Do you have history of spleenectomy? Yes ☐ No☐

**Reporting format**

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**T= Tropzoite G=Gametocyte S=Shizonts PF=plasmodium falciparum**

**PV=plasmdiume Vivax Mx= Mixed Dt= Date**

## Annex III: Declaration

I, the undersigned, declare that this MSc thesis is my original work, has not been presented for a degree in Addis Ababa University or any other universities. I also declare that all sources of materials used for the thesis have been duly acknowledged.

Name of the candidate: Kidist Mikre (BSc)

Signature______________________

Place: Addis Ababa University School of Medical Laboratory Sciences, Ethiopia

Date of submission_____/_______/__________

This Thesis has been submitted with my approval as university advisor.

Name of advisor: Mr. Melaku Tamene (MSc, Phd candidate)

Signature ____________________

Place: Addis Ababa University School of Medical Laboratory Sciences, Ethiopia

Date of submission______/______/________

Name of advisor: Mintewab Hussein (Lecturer)

Signature ____________________

Place: Addis Ababa University School of Medical Laboratory Sciences, Ethiopia

Date of submission______/______/________