Journal of Trends in Life Sciences
ISSN: 2960-0200
DOI: https://doi.org/10.61784/jtls3007

EVALUATION OF SOME HEMATOLOGICAL PARAMETERS AND BILIRUBIN IN MALARIA-INFECTED ADULTS ATTENDING FEDERAL UNIVERSITY TEACHING HOSPITAL, OWERRI, NIGERIA

Otti Chibuzor C.[1], Aloy-Amadi Oluchi C.[1*], Akogu Okechukwu[2], Eberendu I.F.[3], Dimeke Chioma[1], Uzodinma Janet A.[4]
[1]*Department of Medical Laboratory Science, Imo State University, Owerri, Nigeria.*
[2]*Department of Optometry, Imo State University, Owerri, Nigeria.*
[3]*Department of Public Health, Imo State University, Owerri, Nigeria.*
[4]*Department of Health Services, University of Agriculture and Environmental Sciences, Umuagwo, Owerri, Nigeria.*
Corresponding Author: Aloy-Amadi Oluchi C., Email: oluchialoy@yahoo.com

Abstract: Background: Malaria is a disease caused by a parasite called Plasmodium, which is transmitted by the bite of infected female anopheles mosquito. Plasmodium is a hemiparasite that affects red blood cells leading to their destruction (Hemolysis). In malaria disease, there is an increased bilirubin levels due to hemoglobin breakdown, decreased red blood cell count due to hemolysis and increased erythrocyte sedimentation rate value due to inflammation. Aim: This study was aimed at evaluating the effects of malaria parasitemia on erythrocyte sedimentation rate (ESR), red blood cell count (RBC), and bilirubin levels in affected adults attending federal university teaching hospital, Owerri, Nigeria. Method: A total of 90 subjects were recruited for the study. 50 were patients infected with malaria parasitemia, while 40 age - matched healthy subjects served as controls. Seven (7) milliliters of venous blood sample was collected at the antecubital vein aseptically. 2ml was dispensed into ethylenediaminetetraacetic acid containers for RBC estimation, 2ml was dispensed into sodium citrate bottles for ESR determination, while 3ml was dispensed into plain containers for bilirubin estimation. The EDTA, sodium citrate and plain containers were properly labeled with the subjects' names, sample numbers and date of collection. The blood dispensed into the EDTA, sodium citrate containers, were stored in a refrigerator at -4^{o}C while the serum was stored in a freezer at - 20^{0}C prior to use. ESR was analyzed using Westergren method, RBC count using hematology analyzer, and Bilirubin was analyzed using Jendrassik Grof's method. The procedure was carried out at the Federal University Teaching Hospital in Owerri. Results: The results of the tests were analyzed using SPSS version. The mean values of ESR (14.51±5.79) mm/hr, total bilirubin (1.15±0.35) mg/dl, conjugated bilirubin (0.19±0.08) mg/dl and unconjugated bilirubin (0.93±0.25) mg/dl were significantly increased in patients with malaria infection when compared to controls (7.7±5.79) mg/dl,(0.68±0.22) mg/dl, (0.15±0.06) mg/dl and (0.53±0.17) mg/dl (p=0.000, p=0.000, p=0.018 and p=0.000). The mean value of RBC (3.99±0.27) x10 ^{12}/L was significantly reduced in adult patients with malaria infection when compared to controls (4.56±0.47) x10^{12}/L (p=0.000). There was a significant positive correlation (r=0.38, p=0.007) of total bilirubin with ESR in adult patients with malaria infection. There was a significant negative correlation of total bilirubin with RBC in adult patients with malaria infection (r=-0.65, p=0.000). Conclusion: This study showed that levels of ESR, RBC count and bilirubin in malaria-infected adults are altered. Therefore, there is need to monitor these parameters in malaria infection to avoid complications. The results of these tests can help to determine the severity of the infection and to identify patients who are at risk for complications or death. Early identification of patients at high risk is essential for ensuring timely and appropriate treatment, which can improve outcomes and reduce mortality.
Keywords: Erythrocyte sedimentation rate; Red blood cell; Bilirubin; Malaria

1 INTRODUCTION

Malaria is a vector-borne infectious disease caused by the parasite Plasmodium. It is transmitted to humans through the bite of infected female Anopheles mosquitoes Malaria is a major global health concern, particularly in tropical and subtropical regions [1], affecting around 200 million people annually and causing approximately 400,000 deaths each year, mostly among children under the age of five. It remains a significant public health issue in many tropical and subtropical regions, leading to millions of infections and deaths annually. Nigeria, including the city of Owerri, is one of the countries heavily affected by malaria [2]. Malaria has been documented for thousands of years, with references dating back to ancient Greek and Roman times. It has had a significant impact on human health and socio-economic development throughout history. Malaria is caused by Plasmodium parasites, with five species being responsible for human malaria. The parasite is transmitted through the bite of infected female Anopheles mosquitoes. Different species of Plasmodium have varying degrees of severity and are prevalent in different regions. Symptoms of malaria typically include high fever, chills, headache, and muscle aches. Severe cases can lead to complications like anemia, organ failure, and cerebral malaria, which can be fatal if not promptly treated. There are several types of Plasmodium parasites that can cause malaria in humans, with Plasmodium falciparum being the most deadly and responsible for the majority of malaria cases and deaths. Other types include Plasmodium vivax, Plasmodium ovale, Plasmodium malaria, and

Plasmodium Knowles. Symptoms of malaria usually appear within 7-30 days after the infected mosquito bite Malaria can be diagnosed by examining blood smears under a microscope to identify the Plasmodium parasites or through rapid diagnostic tests that detect specific parasite antigens [3]. Molecular techniques such as PCR (polymerase chain reaction) can also be used for accurate and sensitive diagnosis. Antimalarial drugs are used for treating malaria, with the choice of medication depending on the species of Plasmodium and the severity of the infection. Prevention strategies include vector control measures (such as insecticide-treated bed nets and indoor residual spraying), chemoprophylaxis for travelers to endemic areas, and research towards the development of effective vaccines [1]. Malaria has a significant impact on public health, economic development, and healthcare systems worldwide.

Vaccines are also being developed, with the RTS, S/AS01 vaccine (Mosquirix) being the first and only licensed malaria vaccine so far. Efforts to control and eliminate malaria globally have shown significant progress in recent years, but the disease remains a major public health concern, especially in the areas with limited resources and infrastructure [4].

Hematological parameters play a crucial role in the diagnosis, management, and monitoring of malaria infections.

The ESR is a commonly used test to assess inflammation or infection in the body. It measures the rate at which red blood cells settle in a tube over time. Malaria infection can often cause inflammation, leading to an increase in ESR levels. Monitoring ESR can help assess the severity and progression of the disease and the response to treatment. ESR stands for erythrocyte sedimentation rate, which is a blood test used to measure inflammation in the body. The test measures how quickly red blood cells settle at the bottom of a tube over a specific period of time. When there is inflammation in the body, certain proteins called fibrinogen attach to the red blood cells, making them heavier and causing them to settle faster [5-6]. ESR is a non-specific test, meaning it does not diagnose a specific condition, but rather indicates the presence of inflammation and helps in monitoring the progression or response to treatment of an underlying condition. The test is typically performed by taking a blood sample from a vein in the arm. The blood is then placed in a specialized tube and allowed to settle for a certain period of time, usually one hour. The distance at which the red blood cells have fallen within that time is measured and reported as the ESR value, measured in millimeters per hour (mm/hr). A higher ESR value indicates increased inflammation in the body [6].

ESR can be influenced by various factors, including age, gender, anemia, pregnancy, certain medications, and chronic conditions such as arthritis, infections, or autoimmune diseases. Therefore, ESR results are usually interpreted in combination with other clinical findings and tests.

It is important to note that while ESR is a useful tool in assessing inflammation, it is not specific to any one condition and additional tests may be required to determine the underlying cause of inflammation [7].

The RBC count is a measure of the number of red blood cells present in a given volume of blood. Malaria affects red blood cells directly by infecting and destroying them, resulting in a decrease in RBC count. RBC stands for red blood cells. When it comes to malaria, red blood cells play a significant role in the disease cycle.

Once infected female anopheles' mosquito is inside the body, the Plasmodium invades the liver cells, where it multiplies and matures before infecting the red blood cells [1].

Within the red blood cells, the parasites continue to multiply, leading to their rupture and the release of more parasites into the bloodstream. This cycle causes episodes of fever, chills, and flu-like symptoms that are characteristic of malaria. Moreover, malaria affects the shape and functioning of red blood cells. The presence of the parasite causes the infected red blood cells to become sticky and adhere to the walls of blood vessels, leading to blockages and reduced blood flow. This can cause further complications in organs such as the brain, liver, and spleen [2].

In severe cases of malaria, the destruction of red blood cells can result in anemia, a condition where the body lacks enough healthy red blood cells to deliver oxygen to tissues and organs. Treatment for malaria aims to eliminate the parasite from the bloodstream and prevent further infection of the red blood cells. Antimalarial drugs are commonly used to target the parasite at different stages of its life cycle, both inside the liver cells and the red blood cells. Monitoring RBC count can provide insights into the extent of red blood cell damage and the severity of anemia associated with malaria infection [8].

Bilirubin is a yellow pigment produced during the breakdown of red blood cells. Malaria-induced hemolysis can lead to an increased release of bilirubin into the bloodstream. Elevated levels of bilirubin, specifically indirect bilirubin, can indicate liver and red blood cell dysfunction associated with malaria infection. It is primarily metabolized and excreted by the liver. In the context of malaria, bilirubin levels can be affected due to the destruction of red blood cells by the malaria parasite. When Plasmodium parasites infect red blood cells, they multiply and eventually cause the cells to burst, leading to the release of hemoglobin. The breakdown of hemoglobin results in the production of bilirubin [9].

Elevated levels of bilirubin in the bloodstream are observed during malaria infection, particularly in severe cases. This is often indicative of hemolysis, the destruction of red blood cells, which is a characteristic feature of malaria. The liver plays a crucial role in processing the excess bilirubin produced during hemolysis, leading to an increase in bilirubin levels in the blood. Bilirubin levels can be elevated in malaria due to increased red blood cell destruction and subsequent release of hemoglobin. However, it is important to note that bilirubin levels alone are not diagnostic for malaria and should be interpreted along with other clinical signs and laboratory tests [3].

Malaria remains a significant public health concern globally, particularly in developing countries. There are several reasons why it is important to evaluate some hematological parameters and bilirubin levels in malaria infected adults. The clinical manifestations of malaria are variable, and hematological parameters can help to confirm the diagnosis.

Hematological parameters can help to monitor the progress of the disease and assess the effectiveness of treatment. Bilirubin levels are a key indicator of the severity of malaria infection.

This information can help to identify people who are at risk for developing complications from malaria. The results of

this study will provide valuable information on the Characteristics of malaria infection in this population and may contribute to improved management of the disease.
In addition, the study will provide a baseline for future research on the topic. The study will help to fill a gap in the literature on some hematological parameters and Bilirubin. Furthermore, malaria has a significant economic impact on Nigeria, as it causes loss of productivity and increased healthcare costs. This study will provide valuable information that can be used to inform policies and programs aimed at reducing the burden of malaria. Evaluating ESR levels can help differentiate malaria from other febrile illnesses and assess disease severity. Malaria infection affects RBCs leading to anemia and other hematological abnormalities. Quantitative analysis of RBC count, hemoglobin concentration, and hematocrit levels can provide insights into disease severity and inform treatment decisions.
Elevated levels of total and indirect bilirubin may serve as markers of malaria infection and provide information about the degree of hemolysis, liver function and disease progression.
Malaria is a major public health concern in Nigeria, with a significant impact on the health and economy of the country. There is limited information on the effects of malaria on hematological parameters and bilirubin in adult patients [10].

2 MATERIALS AND METHODS

2.1 Study Area

This research study was carried out at the Federal University Teaching Hospital, Owerri, Imo state, from January to March, 2024.

2.2 Subjects

The subjects that were recruited for this study included fifty (50) malaria - infected patients of both sexes who were attending the medical outpatient clinic at federal university teaching hospital, Owerri. Forty (40) apparently healthy subjects of both sexes were also recruited to serve as controls. All the subjects were of ages 20-70 years.

2.3 Study Design

This study was a cross - sectional research study that involved individuals infected with malaria parasitemia. Malaria – infected adult patients at the out - patient department were recruited for this study. They were enrolled after providing their informed consents. Questionnaires were administered to obtain their medical history. A capillary blood sample (a drop of blood) was collected through finger prick with needle, a thick smear was made with the drop of blood and used for screening for malaria parasite through rapid field staining technique. Forty non- malaria infected adults were enrolled and used as controls.
Seven millimeters of venous blood were collected from each subject by means of a hypodermic needle and syringe at the antecubital vein aseptically. Two millimeters of blood were dispensed into sodium citrate containers and used to determine the erythrocyte sedimentation rate of each patient using westergren method. Three millimeters of the venous blood were collected aseptically and stored in a plane tube. The tube was centrifuged to obtain the serum, Total and direct Bilirubin levels were determined using Jendrassik Grof's spectrophotometric method. Two millimeters of the blood sample was dispensed into EDTA containers for RBC estimation using hematology analyzer which worked on the principle of flow cytometry. Results from malaria infected adults were compared with non-malaria infected controls.

2.4 Ethical Approval

The study was approved by the ethics committee of the Federal University Teaching Hospital, Owerri, and subjects who gave their informed consent were enrolled in the study.

2.5 Statistical Analysis

The results were analyzed using Software Package for the Social Sciences (SPSS). All values were expressed as mean ± standard deviation. The results were analyzed for statistical significance using the student t-test. Values with $P<0.05$ were considered statistically significant.

3 RESULTS

Table 1 Mean Values of ESR, RBC, Total, Conjugated and Unconjugated Bilirubin in Adult Patients with Malaria Infection and Healthy Controls

Parameter	Test	Control	t-value	p-value
ESR (mm/hr)	14.51±5.79	7.7±5.79	4.96	0.000
RBC ($x10^{12}$/L)	3.99±0.27	4.56±0.47	6.36	0.000
Total Bilirubin (mg/dl)	1.15±0.35	0.68±0.22	5.55	0.000
Conjugated Bilirubin (mg/dl)	0.19±0.08	0.15±0.06	2.43	0.018
Unconjugated Bilirubin	0.93±0.25	0.53±0.17	6.40	0.000

(mg/dl)				

The mean values of ESR (14.51±5.79) mm/hr, total bilirubin (1.15±0.35)mg/dl, conjugated bilirubin (0.19±0.08)mg/dl and unconjugated bilirubin (0.93±0.25)mg/dl were significantly increased in adult patients with malaria infection when compared to controls (7.7±5.79) mg/dl, (0.68±0.22) mg/dl, (0.15±0.06) mg/dl and (0.53±0.17) mg/dl(p= 0.000, p = 0.018 and p = 0.000). The mean value of RBC (3.99±0.27) $x10^{12}$/L was significantly reduced in adult patients with malaria infection when compared to controls (4.56±0.47) $x10^{12}$/L ;(p=0.000) (Table 1).

Table 2 Mean Values of ESR, RBC, Total Bilirubin, Conjugated Bilirubin and Unconjugated in Adult Male and Female Patients with Malaria Infection

Variable	Male	Female	t-value	p-value
ESR (mm/hr)	11..67±4.36	16.40±5.92	3.06	0.004
RBC ($x10^{12}$/L)	4.12±0.23	3.91±0.27	2.87	0.006
Total Bilirubin (mg/dl)	1.02±0.22	1.23±0.39	2.16	0.036
Conjugated Bilirubin (mg/dl)	0.19±0.07	0.21.±0.08	0.94	0.350
Unconjugated Bilirubin (mg/dl)	0.83±0.16	0.99±0.29	2.15	0.037

The mean values of ESR (11.67±4.36) mm/hr, total bilirubin (1.02±0.22) mg/dl, and unconjugated bilirubin (0.83±0.16)mg/dl were significantly reduced in adult male patients with malaria infection when compared to adult females. (16.40±5.92) mm/hr, (1.23±0.39)mg/dl and (0.99±0.29) mg/dl(p=0.004, p=0.036 and p=0.000). The mean value of RBC (4.12±0.23) x1012/L was significantly increased in adult male patients with malaria infection when compared to adult Females. (3.91±0.27) $x10^{12}$/L(p=0.006) (Table 2).

Table 3 Correlation of Total Bilirubin with ESR and RBC in Adult Patients with Malaria Infection

Variable	N	R	p-value
ESR	50	0.38	0.007
RBC	50	-0.65	0.000

There was a significant positive correlation of total bilirubin with ESR in adult Patients with malaria infection (r=0.38, p=0.007), and a significant negative correlation with RBC in adult Patients with malaria infection (r=-0.65, p=0.000) (Table 3).

Table 4 Correlation of Conjugated Bilirubin with ESR and RBC in Adult Patients with Malaria Infection

Variable	N	R	p-value
ESR	50	0.34	0.017
RBC	50	-0.59	0.000

There was a significant positive correlation of conjugated bilirubin with ESR (r=0.34, p=0.017) and a significant negative correlation (r = -0.59, p=0.000) with RBC in adult Patients with malaria infection (r = -0.59, p=0.000) (Table 4).

Table 5 Correlation of Unconjugated Bilirubin with ESR and RBC in Adult Patients with Malaria Infection

Variable	N	R	p-value
ESR	50	0.43	0.002
RBC	50	-0.59	0.000

There was a significant positive correlation of conjugated bilirubin with ESR (r=0.43, p=0.002), and a significant negative correlation of conjugated bilirubin with RBC in adult Patients with malaria infection (r=-0.59, p=0.000) (Table 5).

4 DISCUSSION

Malaria is a complex and multifactorial disease, with a number of different aspects to consider. The study investigated the evaluation of erythrocyte sedimentation rate (ESR), red blood cell count (RBC) and bilirubin in malaria infected adults at FUTH in Owerri, Imo State, Nigeria.

It was observed from the result of this study that the values of ESR and bilirubin was significantly increased in adult patients with malaria infection when compared to controls. The significant increase in the mean values of ESR and bilirubin might be due to the underlying liver damage caused by the malaria parasite. The malaria parasite, Plasmodium, can cause damage to the liver, leading to the release of bilirubin into the bloodstream. This explains why the liver is also responsible for producing proteins that are involved in blood clotting, so when it is damaged, the blood may become more clotting-prone, leading to an increase in the ESR [13].

The mean value of RBC count was significantly reduced in adult patients with malaria infection when compared to

controls. The reduction in RBC count, or anemia, is a common complication of malaria infection. Malaria parasites can cause the destruction of red blood cells, leading to anemia. In addition, the body's immune response to the infection can also damage the red blood cells. In severe cases of malaria, the body may also produce cytokines that further damage the red blood. The results of this study agrees with the study [14].

According to the study carried out by was significantly decreased in ESR and bilirubin in adults patients with malaria infection [15], which is in disagreement with this study.

There was a significant positive correlation of bilirubin and ESR in adult patients with malaria infection. The results of this study agrees with the findings of [16], who in their study reported similar findings. This is due to the common underlying cause of both conditions. As mentioned earlier, the malaria parasite can damage the liver, leading to an increase in both bilirubin and ESR. Therefore, when there is an increase in one of these markers, it is likely that the other marker will also increase [17]. There was a significant negative correlation of bilirubin, ESR and RBC in adult patients with malaria infection. This is due to the treatment of the infection. Treatment for malaria can reduce the level of bilirubin and ESR. In addition, the treatment can also increase the level of RBC, as the body can begin to produce more red blood cells once the infection is under control.

5 CONCLUSION

The evaluation of ESR, RBC count, and bilirubin in malaria infected adults at FUTH is a valuable tool for assessing the severity of the infection and the response to treatment. The positive correlation between ESR and bilirubin can indicate the presence of liver damage, while the negative correlation between ESR, bilirubin, and RBC can indicate a successful treatment response. It is important to note that these tests should be interpreted in the context of the individual patient's history and clinical presentation, and they should not be used in isolation to make a diagnosis or treatment decision.

It is important to understand that ESR, RBC count, and bilirubin are only a few of the many tests that can be used to evaluate a patient with malaria. Other tests that may be helpful include a complete blood count (CBC), a blood smear, and liver function tests. In addition, the patient's clinical presentation and history should be considered when interpreting the results of any test. For example, a patient with high bilirubin levels may also have jaundice, which can provide additional information about the severity of the infection.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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