IMPACT OF MALARIA INFECTION ON HEMORRHEOLOGICAL FACTORS AMONG UNDERGRADUATES IN THE UNIVERSITY OF BENIN

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CERTIFICATION

This is to certify that this project work titled “THE IMPACT OF MALARIA INFECTION ON HEMORRHEOLOGICAL FACTORS AMONG UNDERGRADUATES IN THE UNIVERSITY OF BENIN” was carried out by EVIDENCE EHIGIE EHIMARE with matriculation number BMS1702078 under my supervision in the Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Benin City, Edo state, Nigeria as part of the requirements for the award of a Bachelor of Science degree in Medical Laboratory Science.

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DEDICATION

I dedicate this seminar work to God almighty for making this seminar work a huge success and also to my Parents for their unwavering support.

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I give thanks to God almighty for His grace upon my life and for seeing me through this seminar work. My profound gratitude goes to my supervisor **Dr. Mrs. C. Otikor** for her concern, constructive and supportive idea which has aided this seminar work. Special thanks to the Head of Department, Medical Laboratory Science, **Dr. B.I.G Adejumo** and also to the entire staff of the department for investing so much in my academic development. I am grateful to my lovely Parents, Mr. and Mrs. Uadiale, for their immense support towards my academic development. My profound gratitude goes to my Pastors and Leaders of BLW Campus Ministry. My Special thanks also goes to Pastor Nelson Iheagwam,Pastor Emmanuel Iren, Pastor Freedom Utoyo, Bro Miracle Ebeh,Peace Aisosa Osarenren, Alex Hormozi,Mr Albert Omondan, Mrs Mabel Omondan, Fjero Ododofor for their words of advice and encouragement through the Years

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ABSTRACT

The persistent burden of malaria in sub-Sahara Africa despite different interventions spanning decades makes it a major public health concern, especially in this region. The study was aimed at investigating how malaria infection affects these hemorheologic factors (haematocrit, fibrinogen and whole blood viscosity) and understand their implications in the severity of the disease. This research was conducted at the University of Benin, Benin City, on a a total of 35 students within the age range of 18-25 years. Blood samples were collected from the participant and tested for haematocrit, fibrinogen and whole blood viscosity using a micro hematocrit centrifuge, the clauss method, reid and ugwu method respectively. Twenty five students who had no traces of malaria infection were used as control. Results showed a non-significant decrease in Packed Cell Volume (PCV) in the test subjects when compared with the control subjects. There was also no significant increase in fibrinogen level and whole blood viscosity between the test subjects and the control subjects. However, on the basis of sex, the malaria-infected males showed marked difference in their haemorrhegic factors when compared to the non-infected control male subjects. While the haemorrhaegic factors of the malaria-infected female subjects did not differ significantly from the haemorrhaegic factors of the female control subjects. Thus, implying that the male subjects were responsible for the significant difference observed in the overall number of malaria-infected subjects in this study, due to slight changes in PCV Furthermore, there was no significant difference in the haemorrhaegic factors between the groups of control subjects overall and on the basis of sex. It can thus, be concluded that malaria infection does have an impact on haemorrhegic factors. In view of the attempts at eliminating malaria in sub-sahara Africa, everyone in the region must contribute their quota to its elimination.

CHAPTER ONE

1.0 Introduction

1.1 Background of Study

Malaria is caused by hemoprotozoan parasites from the Plasmodium genus. Despite extensive investigation and public health endeavors, malaria persists as the most severe and prevalent parasitic ailment among humans. Approximately 500 million individuals contract malaria annually, leading to up to 3 million fatalities due to the affliction(Baskurt *et al.,*2007).Four species of Plasmodium infect humans but P. falciparum and P. vivax together cause the vast majority of the disease worldwide(Baskurt *et al.,*2007).Almost all malaria related deaths, however, are due to P. falciparum because of its apparently unique ability to cause severe clinical syndromes such as cerebral malaria and multi-organ failure that, even if treated early, are frequently fatal(Baskurt *et al.,*2007).Malaria is one of the oldest recorded diseases known to mankind. The term "Malaria" came into use in the 18th century from Italy where people associated malaria with bad air (Pam *et al*.,2015).The mosquito genus, Anopheles, includes over 400 species which are widely distributed. The most important malaria transmitting species in Nigeria and other parts of Africa include Anopheles gambiae, Anopheles funestus, Anopheles arabiensis and Anopheles mellas (Okhuebor and Izevbuwa, 2021).In addition Okpu *et al*., (2019) reported that mlaria transmission is initiated when the sporozoites of the Plasmodium is inoculated by female anopheles' mosquitoes into the human blood stream, sporozoites disappear and invade hepatic cells to establish the liver stage. During the next red blood stage, the gametocytes are taken up by mosquito to ensure the survival of the species. There is no denying that malaria portends a serious present and future global concern; it is present in over one hundred countries worldwide, responsible for over 100 million clinical cases and an estimated 1-

2 million deaths annually; however, the burden of mortality and morbidity is worse in poor countries (Adefemi *et al*.,2015).Malaria has become the most important vector borne disease in Africa (especially south of the Sahara) parts of Asia and Latin America.With over 300, 000 malaria related deaths annually, especially in children under 5years, Nigeria with three other countries, suffer about 50% of global malaria mortality (Kazeem *et al*.,2015). The genus of the Plasmodium that causes malaria has four major species including Plasmodium ovale, P. vivax, P. falciparuum, P. malariae and are mostly found in the sub-Saharan Africa (Alaba and Alaba 2009 ; Bassey *et al* .,2017).Hematological changes in the course of a malaria infection, such as anemia, thrombocytopenia and leukocytosis or leucopoenia are well recongnozed and established(Kotepui *et al*.,2015).However, little is known about the impact of malaria infection on hemorrheological factors particularly hematocrit,whole blood viscosity and fibrinogen as this have not been extensively studied. These factors, such as PCV, fibrinogen levels, and whole blood viscosity, play essential roles in the physiology of blood circulation and are indicative of the overall health of the circulatory system. HCT (hematocrit)measures the volume of packed red blood cells (RBC) relative to whole blood. Hence, it is also known and reported as a packed cell volume (Mondal and Lotfollahzadeh, 2023).Hematocrit is the packed spun volume of whole blood that is made up of RBCs and is expressed as a percentage of total blood volume. It can be measured or calculated as Hct=(RBC*MCV)/10 (Kundrapu and Noguez, 2017).It is a simple test to identify conditions like anemia or polycythemia and also to monitor response to the treatment.Fibrinogen is a soluble 340-kDa glycoprotein synthesized by hepatocytes in the liver(Davalos 2011). Majorly known as factor I for its role in secondary haemostasis with a life span of four days.however under pathological conditions, such as following trauma or illnesses associated with vascular disruption, infection, or inflammation, there is a significant rise in the

concentration of fibrinogen in the bloodstream, often increasing several times over the normal levels(Adams *et al*., 2004).it is termed an acute -phase reactant. Whole blood viscosity is the thickness and stickiness of blood, and it is a direct measure of the resistance of blood to flow through vessels (Trayman 2017).In conclusion, this study aims to shed light on the relationship between malaria infection and hemorrheological factors in undergraduate students at the University of Benin. The results of this research could have significant implications for both the healthcare of students and in identifying changes in hemorrheological factors in response to malaria which can contribute to early detection and timely treatment of the infection, reducing the risk of severe complications.

1.2 Statement of Problem

Despite the extensive research on malaria infection, there is a lack of comprehensive investigation focusing specifically on alterations in hemorheological factors, including packed cell volume (PCV), fibrinogen levels, and whole blood viscosity, in students with malaria. While it is known that malaria infection affects hematological parameters, there is limited knowledge about the specific changes in these hemorheological factors and their clinical significance in undergraduate populations .By analyzing the variations in PCV, fibrinogen levels, and whole blood viscosity, researchers can potentially identify biomarkers that indicate the impact and severity of malaria infection in children, as well as predict the likelihood of complications. Furthermore, investigating these alterations may aid in developing targeted interventions and therapies that specifically address the hematological abnormalities associated with malaria infection in children.

1.3 Justification of Study

Although reports have shown that Malaria parasites cause significant hemalogical changes characterised with high frequency of thrombocytopenia, anemia (Kotepui *et al*.,2015).Further studies needs to be carried out to investigate malaria's effects on clotting factors such as fibrinogen and to assess if it (malaria) increases the resistance of blood to flow with could lead to hyperviscosity syndrome.Furthermore these hemorrheological factors have the potential to serve as diagnostic and prognostic markers for malaria infection in children. Identifying specific changes in PCV, fibrinogen levels, and whole blood viscosity associated with the disease can contribute to the development of non-invasive, cost-effective tools for diagnosis and monitoring.

1.4 Aim of Study

The study aims to investigate how malaria infection affects hemorheologic factors (heamatocrit, fibrinogen and whole blood viscosity) and understand their implications in the severity of the disease.

1.5 Specific Objectives of Study

- To measure and compare the packed cell volume (PCV) in individuals infected with malaria and those without the infection, in order to assess the impact of malaria on PCV levels.
- To evaluate and compare fibrinogen levels in individuals with malaria infection and those without the infection, aiming to understand the influence of malaria on fibrinogen levels.
- To measure and analyze whole blood viscosity in individuals infected with malaria and those without the infection, in order to determine the effect of malaria on blood viscosity.

- To investigate the association between malaria infection severity and changes in packed cell volume, fibrinogen levels, and whole blood viscosity, aiming to understand how disease severity affects hemorheologic factors.
- To explore potential correlations between hemorheologic factors (PCV, fibrinogen levels, and blood viscosity) and clinical parameters of malaria infection, such as parasitemia levels or disease duration.

1.6 Research Questions

- To determine the differences in PCV levels between individuals with malaria infection and those without, and how does malaria infection affect PCV.
- How do fibrinogen levels differ between individuals with malaria and those without, and what insights can we gain into the impact of malaria on fibrinogen.
- What are the variations in whole blood viscosity between malaria-infected individuals and uninfected individuals, and how does malaria infection affect blood viscosity.
- How does the severity of malaria infection relate to alterations in PCV, fibrinogen levels, and blood viscosity, and what insights can be gained into the impact of disease severity on hemorheologic factors.
- Are there significant correlations between hemorheologic factors (PCV, fibrinogen levels, and blood viscosity) and clinical parameters of malaria infection, such as parasitemia levels or the duration of the disease.

1.7 Research Hypothesis

i. **Null Hypothesis**

There is no significant impact of malaria infection on hemorrheological factors, specifically packed cell volume (PCV), fibrinogen levels, and whole blood viscosity among undergraduates in the University of Benin

CHAPTER TWO

LITERATURE REVIEW

2.1 HEMORRHEOLOGY

The word hemorheology was introduced by A.L. Copley in a survey on rheology of blood in 1952(Stolz *et al.*,1999).He defined the term as follows: Hemorheology is concerned with the deformation and flow properties of cellular and plasmatic components of blood in macroscopic, microscopic and submicroscopic dimensions, and with the rheological properties of the vessel structure which directly comes in contact with blood. This definition was adopted at the Foundation Meeting of the International Society of Hemorheology in Reykjavik in 1966. Additionally, A.L. Copley and G. Seaman widened this definition in the sense that: Hemorheology is also the study of the interaction of blood or its components and the vascular system with added foreign materials, such as drugs, plasma expanders, or prosthetic devices. Thus, hemorheology is the study of how the blood and the blood vessels can function and interact as parts of the living organisms (Stolz *et al.,* 1999).

2.2 History of Haemorrheology

The discovery by William Harvey, in the early seventeenth century, of the circulation of the blood was one of the greatest physiological discoveries of all time . Prior to Harvey's insight, it had been believed that blood ebbed and flowed in the veins and arteries spreading "vital spirit" to all the tissues, and the massive flow from the heart was considered to be involved in the replenishment of the blood that was consumed in the process(McMullen 1995) .His insight was based primarily on the observations of the one-way valves in the veins and in and around the heart, the different pressures in the veins and arteries and the effects of ligations on blood flow. They enabled him to hypothesize the idea of blood circulating from the heart through the arteries

and returning to the heart via the veins. This was a huge physiological insight and is obviously of great hemorheological significance as, for the first time, the importance of flowing blood became exposed and so the rheological properties of blood became relevant. Harvey's discovery stands as one of the pivotal points in the history of hemorheology (Baskurt *et al*.,2007).Harvey's insight can be seen to have been especially acute as he did not have access to microscopy and so, though he could see the arteries and the veins, he could not see the microcirculatory vessels. It was left to Malpighi in 1660 to be the first to see the microcirculation (Malpighius and Epistel, 1941). And to prove microscopically the connection of the network of small vessels between the arterial and venous sides of the circulation. A major problem at this time was that the true nature of blood was still very poorly understood (Basket *et al*., 2007). It was largely looked on as a simple liquid, but that view began to change after Malpighi had seen the red cells, even though he mistook them for fat globules. It was left to Anthoni van Leeuwenhoek to give the first accurate description of the red cell in 1674 (Baskurt *et al*., 2007). He was able to see them flowing in the microcirculation so was the first to confirm Harvey's postulate about the circulation. He observed the deformation of red cells as they negotiated the capillaries, and the extent of their deformability seems to have amazed him, as he commented in one of his letters that they could elongate "up to three times their original dimensions without break-up", but he realized that such deformability was necessary for them to negotiate the minute blood capillaries (Baskurt *et al*.,2007). Following Harvey's groundbreaking revelation of blood circulation, medical practitioners embarked on a quest to comprehend the underlying determinants guiding blood flow variation within diverse organs. This intellectual pursuit spawned divergent theories attributing blood flow regulation to factors such as neural influences, intrinsic attributes of blood vessel walls, and hemodynamic properties of the blood itself. A notable proponent of the latter

perspective, substantiated by an array of meticulous microscopic examinations of blood circulation in diverse animal species, emerged in the form of Jean Leonard Marie Poiseuille (Baskurt *et al* .,2007).After completing his doctoral dissertation on heart and pulse wave dynamics, Poiseuille directed his focus towards understanding hemodynamics in microcirculation. His examination of the mesenteric microcirculation in frogs uncovered noteworthy findings. Notably, blood flow in arterioles and venules exhibited a plasma layer near the vessel wall, low red cell concentration, and observed "plasma-skimming" at vessel bifurcations. Additionally, he noted the tendency of white blood cells to adhere to vessel walls.

Furthermore Jean Leonard Marie Poiseuille conducted extensive inquiries into the genesis of the marginal plasma area devoid of cells. This phenomenon arises from the accumulation of erythrocytes in the axial region within arteries, veins, and capillaries across a spectrum of living organisms.(Skolz *et al*.,1999).Recognizing the limitations of uncontrolled in vivo studies, Poiseuille embarked on meticulous investigations involving liquid flow in narrow glass capillaries to establish clearer laws governing microcirculatory blood flow(Sutera and Skalak,1993).When he tried to apply his law holding well for ideally viscous fluids to the flow of blood in glass tubes, his law was not applicable. The same conclusion was reported by Duncan and Gamgee in 1871 who measured blood viscosity in glass tubes. Hess noticed in 1915 , that the blood obeys the law for ideally viscous fluids only at the high intensity flow and shear rates(Skolz *et al*.,2007).Robin Fahraeus was the scientist who delved into the intricacies of the connection between red blood cell aggregation and the stability of blood suspension. His research played a pivotal role in establishing the correlation between the aggregation process and the erythrocyte sedimentation rate (ESR), which had been previously elucidated by Biernacki. Since the early 20th century, ESR has stood as one of the most widely employed clinical

laboratory examinations as a non-specific gauge of inflammation and the acute phase response (Baskurt and Meiselman, 2013)

2.3 Principles of Rheology

Rheology is the study of the flow and deformation of matter (gases, liquid- and solid-like matter) under the influence of a mechanical force(Ramli *et al.,*2002)

Deformation can be defined as the relative displacement of material points within the body (Baskurt and Meiselman, 2003).When subjected to an applied force, solids exhibit a response by undergoing a specific degree of deformation. In the case of elastic solids, this deformation maintains a direct proportionality with the magnitude of the applied force. As long as the deformation remains within certain limits, the solid regains its initial shape upon removal of the force (Baskurt and Meiselman, 2003). If a lasting deformation is observed even after the force is removed, we classify the material as plastic. In the case of fluids, they undergo continuous deformation or flow due to applied forces. It's worth noting that certain substances display a viscoelastic nature, which involves a blend of characteristics from both fluids and solids(Baskurt and Meiselman, 2003). In the realm of rheology, liquids can be classified into two primary categories, as illustrated in Figure 1. Firstly, we have Newtonian liquids where viscosity remains consistent regardless of changes in shear rate or shear stress. In such fluids, the slope of the shear stress-shear rate relationship remains constant across a specified range.(Baskurt and Meiselman,2003).For non-newtonian fluids in this case Blood,the viscosity changes as shear rate and stress changes(Hamlin and Benedik,2014).As the shear rate is raised, the apparent viscosity of a non-Newtonian fluid may either decrease (shear-thinning behavior) or increase (shear-thickening behavior).Non-Newtonian liquids' flow characteristics may also be time-dependent; for example, a thixotropic liquid's viscosity reduces over time at a constant shear rate. The

viscosity of a liquid relies on its temperature for both kinds of fluids, and for the majority of fluids, viscosity decreases as temperature rises. The most popular units for measuring viscosity are millipascals per second (mPa/sec). (Baskurt and Meiselman, 2003).

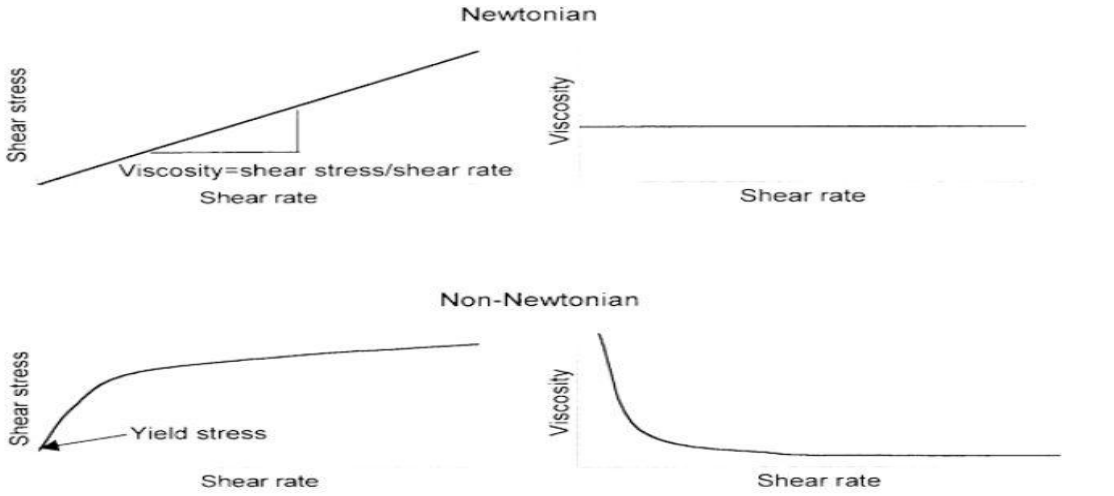

Figure 1 showing the relations between viscosity and shear rate and shear stress for Newtonian and non-Newtonian liquids(Baskurt and Meiselman, 2003).

2.4 Compositional Properties of Blood

Blood constitutes a biphasic fluid system characterized by cellular components, namely erythrocytes (red blood cells), leukocytes (white blood cells), and platelets, suspended within a liquid phase known as plasma(Hamlin and Benedik

,2014).The plasma constitutes a intricate solution of diverse substances (e.g., proteins, lipoproteins, and metabolites), constituting roughly 9% of plasma by mass, with the remainder being water. In typical circumstances, plasma viscosity is generally believed to have a limited impact on flow resistance in the macrovascular or microvascular context. Nevertheless, during

pathological situations like acute-phase responses (e.g., infection, postoperative trauma), the rise in plasma components, particularly fibrinogen, will significantly contribute to the indiscriminate elevation of plasma viscosity, leading to adverse effects on blood circulation in all blood vessels, particularly those within the microcirculatory system.(Hamlin and Benedik, 2014)

Red blood cells (RBCs) are of utmost importance in facilitating blood flow throughout the entire circulatory system. This holds true whether in larger blood vessels where bulk flow occurs, or in microcirculation where the vessels are as small as RBCs themselves(Barvitenko *et al.,*2013).To persist within the rigorous setting of the circulatory system, erythrocytes must maintain their ability to promptly undergo deformability and display utmost membrane stability when exposed to hemodynamic shear(Baskurt *et al.,*2007).During its usual 120-day lifespan, a single red blood cell will journey through the circulatory system over 1000 times (Baskurt *et al.,*2007).Deformability has historically been linked predominantly to the distinctive cells' geometric and substance attributes. The double-concave, disc-shaped form enables a 250% linear expansion of RBCs without substantial adjustments to the membrane's surface area(Barvitenko *et al.,*2013)

Engaged in safeguarding against infection, white blood cells (leukocytes), conversely, consist of multiple distinct types (i.e., monocytes, granulocytes, and lymphocytes), all harboring intricate interiors of organelles and a nucleus suspended within a thick cytoplasm. Leukocytes assume a minor function in ascertaining the thickness of entire blood (i.e., macrocirculation) since their quantity and concentration in volume are significantly less than those of erythrocytes. However, leukocytes play a significant role in governing the movement within the microcirculatory system for two reasons,(1) Their inner substance displays superior stickiness and flexibility compared to that of the red blood cell, and (2) all variations of white blood cells are more sizable and less

pliable, with reduced deformability than the red blood cell. White blood cells are sluggish and exhibit erratic motion, which has the potential to postpone and alter the capillary passage of the red blood cell and affect microvascular resistance and tissue perfusion (Hamlin and Benedik, 2014) Finally, platelets are essentially uniform in their composition; they lack a nucleus and contain relatively intricate components of vacuoles and fibers suspended in a dense cytoplasm. Their function pertains to hemostasis. Although platelets possess a complicated internal content with substantial thickness, they do not affect overall blood thickness or microvascular resistance due to their much smaller dimensions (diameter 2–3 mm) in contrast to red blood cells or white blood cells. Additionally, their total volume is even less than that of white blood cells.(Baskurt *et al .,*2007)

2.4 Whole Blood Viscosity

Blood viscosity is the thickness and stickiness of blood, and it is a direct measure of the resistance of blood to flow through vessels. The primary determinants of blood viscosity are hematocrit, red cell deformability, red blood cell aggregation, and plasma viscosity(Traystman 2017) Blood is a shear thinning non-Newtonian fluid, meaning its viscosity decreases as the shear rate increases. In Newtonian fluids (for example, water), viscosity remains unaltered despite fluctuations in shear stress, remaining consistent. In non-Newtonian fluids like blood, viscosity hinges on the magnitude of either shear stress or shear rate, and it's computed as a ratio of the two. At moderate to high shear rates, an increase of 4% in blood viscosity occurs with each unit rise in hematocrit (e.g., 45%–46%). For both fluid categories, viscosity and temperature share an inverse relationship.(Hamlin and Benedik, 2014).Under normal circumstances, blood viscosity fluctuates based on the hematocrit ratio (volume of red blood cells to total blood volume) and the diameter of the vessel through which it circulates. On the

contrary, plasma serves as the suspending agent for blood's cellular constituents; thus, any shift in plasma viscosity directly impacts blood viscosity, irrespective of hematocrit levels(Hamlin and Benedik, 2014).

2.5 Effect of shear rate on Blood Viscosity

Because plasma functions as a Newtonian liquid, the non-Newtonian nature of blood arises from the inclusion of cells within this substance. Blood comprises clusters that result from the interplay between erythrocytes and plasma proteins like fibrinogen and globulin. In a stationary blood state, all erythrocytes create substantial clusters, resulting in its viscoelastic properties (Stolz *et al.,*1999).The established configuration in repose is exceedingly responsive to fluid circumstances. As the rate of shearing amplifies, blood clusters have a propensity to disintegrate, culminating in a decline in blood viscosity. Upon additional escalation of the shearing rate, the clusters are entirely disintegrated. Consequently, the input attributed to the amalgamation process is diminished to a bare minimum. Subsequently, with a further augmentation in the shearing rate, individual erythrocytes undergo distortion until they are wholly aligned with the direction of flow. Consequently, presenting minimal opposition to fluid motion. Under these particular conditions, the apparent viscosity of the blood is decreased to the lowest level (Stolz *et al* .,1999)In simple terms ,viscosity of blood decreases as the shear rate increases, and vice versa ,this property of blood is known as shear thinning (Dintenfass 1980).In the spectrum of shear rates, blood viscosity exhibits significant fluctuations. In the proximity of negligible shear rates, the thickness of whole blood can range from 100 to 1000-fold greater compared to that of water. Conversely, at elevated shear rates, it is only 3 to 6 times more viscous than water(Dintenfass 1980).The relatively high viscosity of blood at low shear rates is due mainly to aggregation of red cells(Dintenfass 1980).The shear rate is determined by the velocity of blood

flow and by the size of the blood vessel. High shear rates are typically present in large arteries with high blood flow velocity, whereas low shear rates are typically present in the microcirculation where blood flow velocity is low (Eckmann *et al.,*2000)

2.6 Red Blood Cell Aggregation

Human crimson blood cells (RBC) possess the capability to clump together to create both two- and three-dimensional arrangements when placed in watery solutions containing substantial plasma proteins (like fibrinogen) or polymers (such as 70 kDa dextran) (Baskurt and Meiselman, 2003).Two-dimensional arrangements are created when cells come into direct contact in linear formations resembling a pile of discs; these configurations are commonly referred to as "rouleau" for the singular form or "rouleaux" for the plural(Baskurt and Meiselman, 2003).The process of aggregation is dependent upon the magnitude of shear forces exerted upon the cells, wherein an elevated shear milieu (such as rapid fluid motion within diminutive microvessels) will deter aggregation, consequently mitigating the viscosity of blood(Hamlin and Benedik,2014) .Alterations in erythrocyte aggregation predominate under conditions of reduced flow, with heightened viscosity resulting in diminished blood perfusion within the microcirculation. These changes in erythrocyte aggregation have been observed in numerous clinical scenarios, including sepsis, cardiac ischemia, and myocardial infarction(Hamlin and Benedik, 2014).

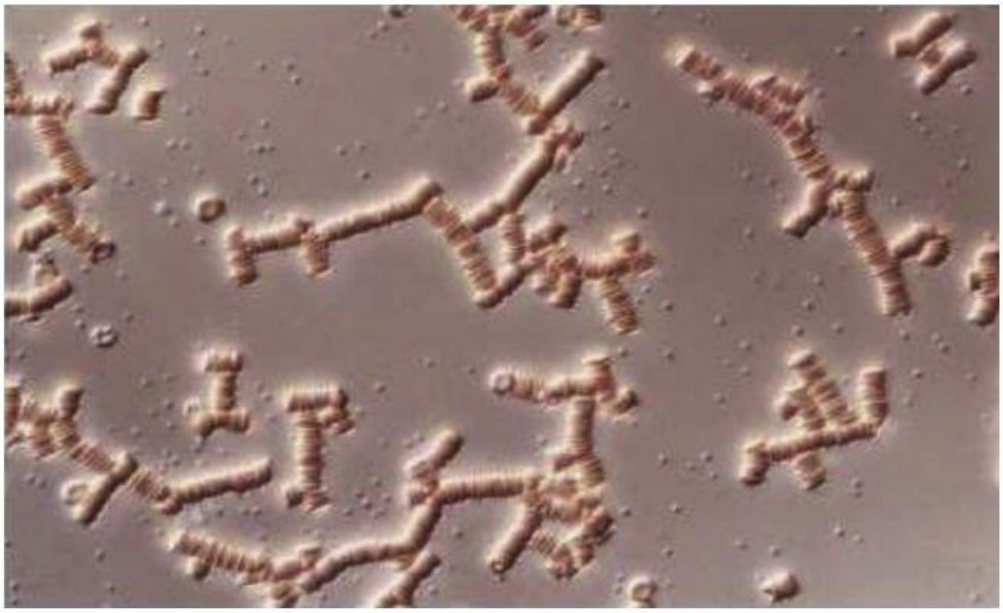

Figure 2 showing Erythrocyte aggregates forming stacked-coin appearance(Hamlin and Benedik,2014).

2.6.1Hematocrit

The term "hematocrit (HCT)" originated from English “hemato-“ and Greek “krites.” HCT measures the volume of packed red blood cells (RBC) relative to whole blood. Hence, it is also known and reported as a packed cell volume(Mondal and Lotfollahzadeh, 2023).Hematocrit is the packed spun volume of whole blood that is made up of RBCs and is expressed as a percentage of total blood volume. It can be measured or calculated as Hct=(RBCxMCV)/10 (Kundrapu and Noguez, 2017). Abnormal calculated hematocrit values may occur as a result of interferences that may cause erroneous RBC and MCV measurements which include very high WBC count, high concentration of platelets, or agglutinated RBC (Kundrapu and Noguez, 2017).It is a simple test to identify conditions like anemia or polycythemia and also to monitor response to the treatment. A glass tube and a centrifuge machine are sufficient to measure HCT. After centrifugation, the component of blood separates into three distinct parts. From below upwards, the layers are - a layer of red blood cells (RBC), a layer of white blood cells(WBC) and

platelets, and a layer of plasma at the top. This method of determining HCT by Wintrobe hematocrit tube is known as the “macro-hematocrit” method (Mondal and Lotfollahzadeh, 2023).An increased hematocrit may be due to dehydration or polycythemia. Decreased values may be due to anemia, over hydration, kidney failure, or chronic inflammatory conditions. Pregnancy may also cause slightly decreased hematocrit due to an increase in blood volume (Kundrapu and Noguez, 2018).This evaluation of the hematocrit hinges upon the count and dimensions of red blood cells, Conventionally, it registers between 40.7–50.3% for males and 36.1–44.3% for females(Medlineplus 2019).The Haematocrit test constitutes a segment of an individual's comprehensive blood count outcomes, in conjunction with hemoglobin concentration, white blood cell quantity, and platelet quantity(Medlineplus 2019)

2.6.2 Effects of Hematocrit on Blood Viscosity

The proportion of particles in a suspension significantly influences its rheological characteristics(Baskurt *et al.,*2007).In the context of typical blood, this proportion is denoted by the hematocrit, representing the volume fraction of RBCs. Platelets, unless clumped together, are too scarce in quantity and size to impact viscosity, and the viscosity is affected by white blood cells only when their volume fraction is exceptionally elevated (Baskurt *et al .,*2007)The ease of blood flow in major vessels relies on the hematocrit and diminishes as the hematocrit rises, yet remarkably never reaches zero, even when the hematocrit exceeds 95% and the cells are tightly packed(Skolz *et al.,*1999)

2.6.3 Fibrinogen

Fibrinogen is a soluble 340-kDa glycoprotein synthesized by hepatocytes in the liver(Davalos and Akassoglou, 2012).They are elongated structures of 45 nm, consisting of two D domains connected by a coiled-coil segment to a central E domain. They are formed by two sets of three polypeptide chains (Aa, Bb, and c) joined by disulfide bridges in the E domain, including an asymmetrical disulfide ring(Mosesson 2005).This process yields the homodimeric fibrinogen molecule, which circulates within the bloodstream(Davalos and Akassoglou 2012).

The mature molecule(fibrinogen) is constitutively secreted into the circulation, where it exhibits a half-life of 4 days and a fractional catabolic rate of 25% per day(Cassini *et al.,* 2013).In addition to the circulating fibrinogen found in the plasma component of blood, there exists an intracellular reservoir of fibrinogen enclosed within platelet α-granules. This internalization of plasma fibrinogen is achieved by both megakaryocytes and platelets, employing the fibrinogen glycoprotein IIb/IIIa (GpIIb-IIIa; αIIbβ3) receptor as the key molecular mechanism for this process (Cassini *et al.,*2013).The transformation of fibrinogen into a fibrin clot is a process that unfolds in three well-defined phases:

1. Thrombin, an enzyme, first breaks down fibrinogen into fibrin monomers.
2. These fibrin monomers then join together on their own to build a structured, polymeric arrangement.
3. Factor XIIIa comes into play by creating covalent connections between the fibrin molecules(Cassini *et al.,* 2013)

Thrombin connects with its target, fibrinogen, through a specific site on thrombin called "exosite 1," which acts like a docking station for this interaction(Cassini *et al.,*2013).When fibrinogen is exposed to thrombin, the conversion of fibrinogen to fibrin results in the α-subunit releasing two

fibrinopeptides denoted as fibrinopeptide A(FPA). Following the release of FPA, theβ-subunit also releases two subunits denoted accordingly as fibrinopeptide B (FPB)(Stang and Mitchell 2013).However, under pathological conditions, such as following trauma or illnesses associated with vascular disruption, infection, or inflammation, there is a significant rise in the concentration of fibrinogen in the bloodstream, often increasing several times over the normal levels(Adams *et al.,* 2004).Consequently, fibrinogen is recognized as an acute-phase reactant. When the coagulation cascade is triggered, thrombin enzymatically cleaves fibrinopeptides A and B from the fibrinogen molecule. This process exposes multiple sites for polymerization, enabling the spontaneous assembly of fibrin fibrils(Davalos and Akassoglou 2012)

A series of crosslinking events that involve factor XIIIa, giving rise to an insoluble fibrin clot which gains resilience against mechanical, chemical, and proteolytic challenges(Davalos and Akassoglou 2012).The γ-chain's C terminus in fibrinogen binds to a specific location on the platelet surface's αIIbβ3 integrin receptor. This interaction facilitates the establishment of connections between platelets, promoting their aggregation (Davalos and Akassoglou 2012)

2.6.4 Role of Fibrinogen in Blood Viscosity

Bell in his experiment on the defibrinogenation by arvin in thrombosis demonstrated that after the administration of a defibrinating agent (Ancrod),whole blood viscosity decreased,commensurate with the plasma fibrinogen level (Kwaan 2010).Furthermore, Fibrinogen plays a significant role in influencing the aggregation of red blood cells within plasma. Specifically, fibrinogen enhances the size of these aggregates, augments their yield stress, and contributes to the alteration of the low-shear viscosity characteristics of red cell suspensions (Kwaan 2010). Fibrinogen impacts whole blood viscosity by causing roleaux formation of red blood cells which leads to red blood cell aggreation(Chien and Jan 1972).

Fibrinogen causes roleaux formation by interacting with the sialic acid of red blood cells thereby coating them and causing the red cells to stick to each other (Piwowar *et al.,*2015).

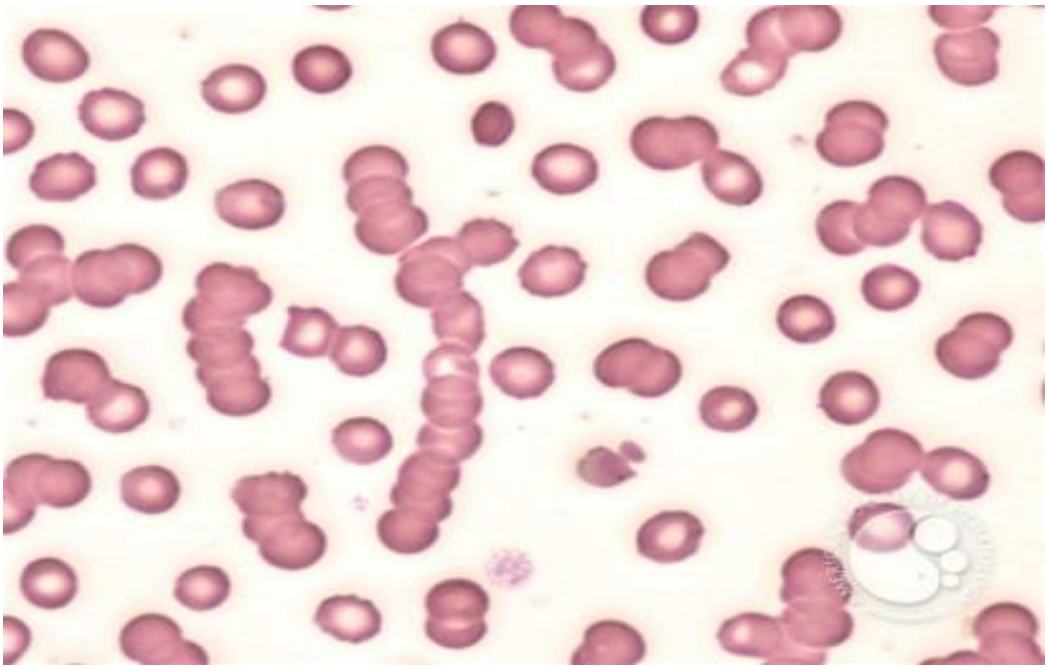

Figure 3 showing roleaux formation of red blood cells (American society of Hematology 2021).

2.6.5 Malaria

Malaria stands as the globe's most lethal parasitic ailment (Okonko *et al*., 2009). It unquestionably ranks as the world's most pivotal tropical parasitic affliction and claims more lives than any other contagious ailment apart from tuberculosis (Ani 2004). Malaria ensues from an infection caused by solitary-celled organisms within the Plasmodium genus, which are part of the apicomplexan phylum. Female Anopheles mosquitoes transfer these parasites from one individual to another via their stings (Okonko *et al*., 2009; Okoroiwu *et al*., 2021). Malaria holds the distinction of being one of the most anciently documented illnesses known to humankind. The term "Malaria" was adopted in the 18th century, originating from Italy, where people linked

malaria to noxious air (Pam *et al*., 2015). The mosquito group Anopheles encompasses more than 400 species with extensive distribution. The most crucial malaria-conveying species in Nigeria and various other regions of Africa encompass Anopheles gambiae, Anopheles funestus, Anopheles arabiensis, and Anopheles mellas (Okhuebor and Izevbuwa, 2021). French military surgeon Alphonse Laveran (1880) holds the credit for initially identifying the responsible agent Plasmodium, within the red blood cell (RBC) of a patient in Algeria (Sastry and Bhak ,2014).

2.6.6 Epidemiology of Malaria

According to Okeke *et al .,*(2016), malaria stands as one of the most pivotal causes of morbidity across the globe. Malaria is roughly estimated to be the cause of approximately two million deaths each year (Bassey *et al.,*2017).Malaria occurrences are more prevalent in Africa, with a prevalence rate ranging from 81% to 93% of the total global cases(Okpu *et al.*, 2019). As per the World Malaria Report 2018, in 2017, there were 203-262 million cases and 435,000 deaths recorded, reflecting a decrease of 18% in malaria cases and 28% in malaria-related deaths compared to 2010. Globally, nearly 50% of the world's population, estimated to be between 3.28 billion and 3.40 billion people, resides in areas susceptible to malaria infections (Okoroiwu *et al.,*2021).

Reports indicate that approximately half of the worldwide malaria-related fatalities occur in Nigeria, the Democratic Republic of the Congo, Uganda, and Ethiopia (Okpu *et al.,* 2019). Furthermore, the prevalence of malaria in children aged 6 to 59 months in Nigeria, based on geographical coverage, ranges from 41% to 50% in the North-West, North-Central, and South-West regions; 31% to 40% in the North-East and South-South regions; and 21% to 30% in the South-East region (Nigeria Malaria Fact Sheet, 2011).Malaria deaths in 2019 were estimated to be highest in Nigeria at 43%, followed by the Democratic Republic of the Congo (23%),

Tanzania (5%), and Mozambique (4%). In the same year, Nigeria accounted for the highest number of global malaria cases (27%) and deaths (23%). It was estimated that 33 million pregnancies occurred in the WHO African Region in 2019, out of which 36% (12 million) were afflicted with malaria during pregnancy. The East and Southern Africa regions exhibited the lowest prevalence of malaria exposure during pregnancy, at 24% (Agunbiade *et al.,* 2023).

Life Cycle of Plasmodium Falciparum

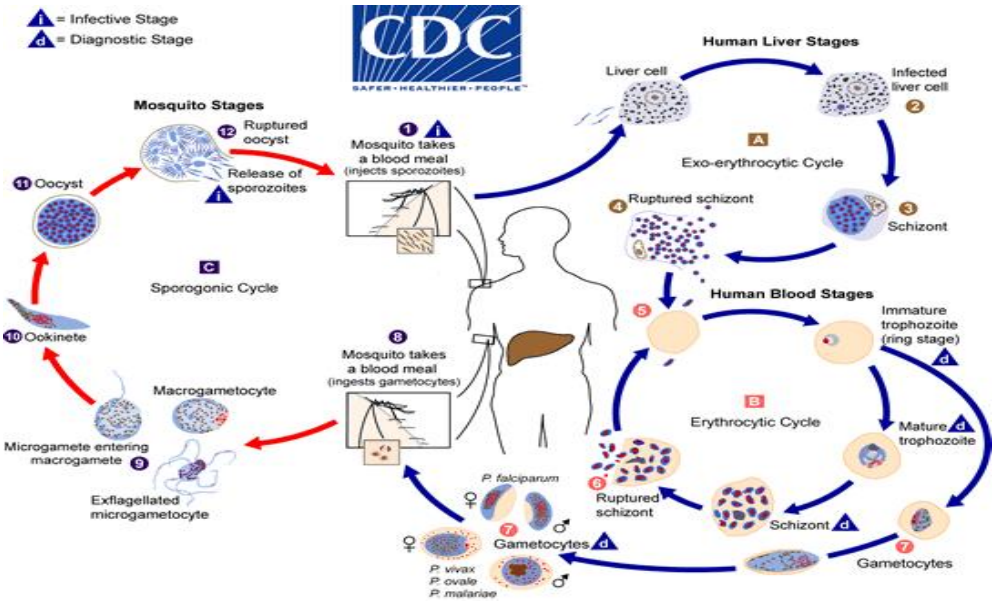

Figure 4 showing the life cycle of Plasmodium Falciparum (CDC 2020)

In the context of human malaria infections, Plasmodium falciparum emerges as the most potent, displaying the greatest virulence among the four Plasmodium species that afflict humans. This particular species accounts for roughly 85% of all reported malaria cases, and is the primary cause of nearly all malaria-related deaths(Rich *et al.,* 2009).

The life cycle of the malaria parasite comprises a duo of hosts. While feeding on blood, a malaria-infected female Anopheles mosquito introduces sporozoites into the human

recipient(CDC 2022).Rarely, it can also be transmitted by blood transfusion and Transplacental transmission.(Sastry and Bhat 2014).Sporozoites invade liver cells and develop into schizonts, subsequently bursting to liberate merozoites. Worth mentioning, in P. vivax and P. ovale, a quiescent phase (hypnozoites) can endure in the liver and induce recurrent infections by infiltrating the bloodstream weeks or even years afterward(CDC 2022).

In humans, the asexual cycle takes place through the following stages:

- Pre-erythrocytic schizogony
- Erythrocytic schizogony
- Gametogony (Sastry and Bhak 2014)

a. Pre-erythrocytic schizogony

Prior to invading red blood cells (RBCs), malaria parasites go through an exoerythrocytic stage, also known as the intrahepatic or tissue stage.

- Motile sporozoites exit the bloodstream and enter the liver within 30 minutes.
- The circumsporozoite protein on sporozoite surfaces binds to hepatocyte receptors, facilitating their entry into liver cells.
- Once inside hepatocytes, the spindle-shaped sporozoites transform into trophozoites, the feeding stage.
- Trophozoites undergo nuclear divisions (schizogony) and become pre-erythrocytic schizonts, containing multiple merozoites which are later released (Sastry and Bhak 2014)

b. Erythrocytic Schizogony

- Merozoites infect red blood cells,by establishing a 'tight junction' with the host cell membrane for entry, powered by the parasite's actin-myosin motor.Notably, the

merozoite's surface coating is shed through a proteolytic process involving SUB2, a serine protease in apical microneme organelles(Cowman and Crabb 2016)

- The ring stage trophozoites mature into schizonts, which rupture releasing merozoites
- Some parasites differentiate into sexual erythrocytic stages (gametocytes)
- Blood stage parasites are responsible for the clinical manifestations of the disease (CDC 2022)

Ring forms are the first asexual form that can be demonstrated in the peripheral blood. The time interval between the entry of the parasite into man and demonstration of the parasite in the peripheral blood is called as prepatent period. It varies between the species:

- P. vivax—8 days
- P. falciparum—5 days
- P. malariae—13 days
- P. ovale—9 days (Sashtry and Bhak 2014)

c. Gametogony

- The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an Anopheles mosquito during a blood meal.
- The parasites' multiplication in the mosquito is known as the sporogonic cycle.
- While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes .
- The zygotes in turn become motile and elongated (ookinetes) The ookineted invade the midgut wall of the mosquito where they develop into oocysts .
- The oocysts grow, rupture, and release sporozoites .
- The Oocysts their way to the mosquito's salivary glands.

- Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle (CDC 2022)

Structure of Plasmodium Falciparum

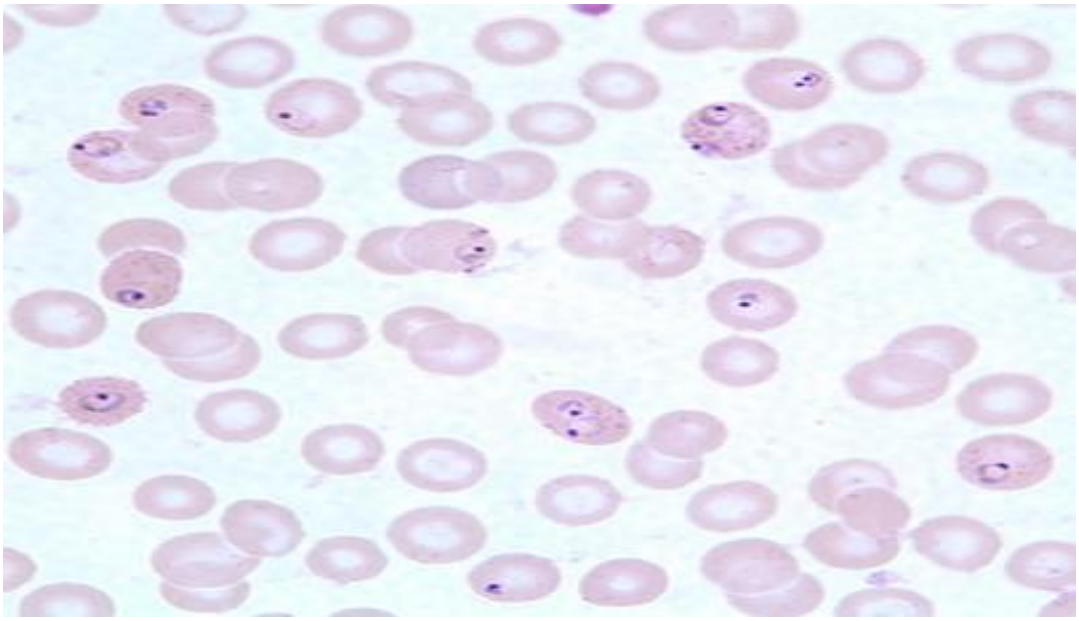

Figure 5 showing the Ring form(Trophozoites) of Plasmodium falciparum in infected erythrocytes (CDC 2022) Plasmodium belongs to the phylum Sporozoa(Apicomplexa) which is distinguished morphologically by the presence of a specialized complex of apical organelles (micronemes, rhoptries, polar ring, conoids and dense granules) which help in invasion into the host cell(Sastry and Bhak 2014).A sporozoite form of Plasmodium has a spindle-like shape and measures approximately 10–15 μm in length. Within the liver, it develops into an oval-shaped schizont ranging from 30–70 μm in diameter(Lucius and Roberts 2017).Each schizont generates merozoites, each measuring approximately 1.5 μm in length and 1 μm in diameter. Within the

erythrocyte, these merozoites arrange into a ring-shaped structure, transitioning into trophozoites(Lucius and Roberts, 2017).Distinct from the gametocytes of other Plasmodium types, P. falciparum's gametocytes exhibit an elongated and crescent-like morphology, often used for recognition. A fully developed gametocyte measures 8–12 μm in length and 3–6 μm in width. The ookinete, likewise elongated, has dimensions of approximately 18–24 μm. The oocyst adopts a spherical shape and can expand up to a diameter of 80 μm(Lucius and Roberts, 2017).In P. falciparum infections irregular darker-stained masses called Maurer's clefts appear in the infected erythrocytes under the light microscope. they appear as a clear narrow circle bounded inside and out by two membranes(Trager and Bradbury, 1966).

2.6.7 Pathogenicity of Malaria

Malaria transmission initiates when an individual is attacked by a contaminated female anopheles mosquito, introducing Plasmodium spp (species) parasites as sporozoites into the bloodstream. These sporozoites migrate to the liver, undergoing asexual reproduction for approximately 7–10 days, during which no symptoms manifest (Mawson 2013).The parasites, now as merozoites, exit the liver cells within vesicles and journey through the heart to lung capillaries. The vesicles eventually break down, liberating the merozoites into the bloodstream, where they invade and multiply within red blood cells. Symptoms, such as fever, coincide with the bursting of infected red blood cells, releasing cell and parasite remnants, including malarial pigment (hemozoin) and glycophosphatidylinositol, the suspected 'malaria toxin.' (Mawson 2013).In some contaminated blood cells, instead of reproducing asexually, the merozoites transform into sexual forms (gametocytes), which flow in the bloodstream and get consumed during mosquito bites. The ingested gametocytes mature in the mosquito into mature sex cells (gametes) that turn into ookinetes, actively penetrating the mosquito's mid-gut wall to create

oocysts. These oocysts eventually burst, releasing thousands of active sporozoites. These sporozoites travel to the mosquito's salivary glands. The cycle of human infection begins anew when the mosquito bites another person(Mawson 2013).All the clinical symptoms associated with malaria are caused by the asexual erythrocytic or blood stage parasites. When the parasite develops in the erythrocyte, numerous known and unknown waste substances such as hemozoin pigment and other toxic factors accumulate in the infected red blood cell (CDC 2020).These substances are discharged into the bloodstream when the infected cells burst and release infiltrating merozoites. The hemozoin and additional harmful components, like glucose phosphate isomerase (GPI), incite macrophages and other cells to generate cytokines and additional soluble agents that trigger fever and shivering and likely impact other severe physiological responses linked to malaria (CDC 2020).Symptoms of Severe Malaria Anaemia includes the breakdown of both infected and uninfected red blood cells, the retention of red blood cells in the spleen(Splenic sequestration), abnormal red blood cell formation, bone marrow inhibition, concurrent infections with bacteremia, HIV-1, and hookworm, and persistent malaria transmission in highly endemic areas(Perkins *et al.,*2011).Plasmodium falciparum-infected red blood cells, especially those with mature trophozoites, stick to the inner lining of small blood vessels and don't flow freely in the bloodstream. When this attachment happens in the brain's vessels, it's thought to contribute to the deadly condition called cerebral malaria, linked to high mortality (CDC 2020).

2.7 Ecology of Mosquitoes

Insects play a significant role as primary vectors for the transmission of various diseases, particularly in tropical regions (Bassey *et al.,* 2017). Mosquitoes, in particular, serve as vectors for numerous infectious pathogens, including arboviruses, filariae, and protozoans, responsible

for common and emerging diseases. These diseases encompass malaria, dengue, Zika, chikungunya, and Japanese encephalitis, with transmission occurring among humans or from other creatures to humans (Huynh *et al .,*2022). Furthermore, as highlighted by Huynh *et al.,* in 2022, mosquito-borne diseases are globally distributed, but they tend to be concentrated predominantly in tropical and subtropical regions characterized by warm and humid climates.According to Bassey *et al.,* (2017) Culex, Aedes, Anopheles, and Mansonia mosquitoes are key transmitters of diseases affecting both humans and animals. Among these, the major vectors responsible for human malaria include Anopheles gambiae, Anopheles funestus, Anopheles arabiensis, and Anopheles melas. A. arabiensis predominates in savannah areas and urban environments, while A. gambiae thrives in densely forested regions. A. funestus exhibits an uneven distribution, and A. melas is adapted to saltwater environments (Nmadu *et al.,* 2015). Furthermore, Nmadu *et al.,* (2015) also reported that Anopheles mosquitoes can adapt to urban breeding sites over time. For instance, in India, Anopheles Stephensi has evolved into an urban species, found in much greater numbers within many cities compared to rural areas. Mosquito vectors from the Anopheles genus can transmit both malaria and filariasis, while the Aedes genus can transmit diseases such as dengue virus (DENV), chikungunya virus (CHIKV), Zika virus (ZIKV), yellow fever virus (YFV). The Culex genus, on the other hand, is responsible for transmitting filariasis, Japanese encephalitis virus (JEV), West Nile virus (WNV), and Rift Valley Fever (Huynh *et al.,* 2022).The acquisition of malaria infection is typically linked to areas where human hosts carrying Plasmodium parasites coexist with an adequate population of anopheline mosquitoes and suitable environmental conditions, particularly temperature and humidity (Bassey *et al.,* 2017). According to Okonko *et al.,* (2019), research has indicated that the breeding of Anopheles mosquitoes decreases as one moves closer to the center of urban areas.

It's worth noting that malaria can be categorized as tertian when the periodicity of erythrocyte schizogony (rupturing of red blood cells) occurs every 48 hours, which is common in Plasmodium vivax, Plasmodium ovale, and Plasmodium falciparum. Conversely, malaria is termed quartan when the erythrocyte cycle's periodicity is 72 hours, a characteristic unique to Plasmodium malariae (Pam *et al.,*2015). Additionally, Sunday *et al.,* (2017) emphasized the connection between mosquito ecology and poor sanitation, which contributes to the severity of malaria. Malaria parasites also play a significant role in causing anemia, particularly in children under 59 months, who are at a higher risk due to the substantial iron requirements for their development (Egbon2021). The transmission of malaria parasites is closely tied to the socio-cultural and economic statuses of communities (Obasohan *et al.,*2021).

2.7.1 Malaria Adhesins

The malaria parasite Plasmodium employs distinct proteins to attach to cellular receptors in its mosquito vector and human host. This attachment is vital for parasite growth, traversal of host cells, invasion, and defense against immune responses from the vector and host (Malpede and Tolia ,2014).The mosquito's blood feast kickstarts the parasite's development within its vector, enabling gametes to access the suitable conditions for fertilization. Surface coat adhesives become instantly necessary, and during fertilization, gametes employ members from the universally preserved apicomplexan 6-cysteine group for identification and bonding (Malpede and Tolia, 2014).Following fertilization, zygotes can stick together via tube projections covered in p25 protein, which features four successive evolutionarily preserved epidermal growth factor-like (EGF) sections(Rupp *et al* .,2011)In the ensuing stages, proteins p25 and its counterpart, p28, are subsequently manifested on the exterior of the mobile ookinete, which is the resultant form stemming from the maturation of the zygote (Saxena et al., 2007). As surface constituents of the

ookinete, it is postulated that p25 and p28 serve the dual purposes of safeguarding against enzymatic mosquito defenses and promoting attachment to the midgut membrane(Malpede and Tolia, 2014).In the human body,the circumsporozoite protein on sporozoite surfaces binds to hepatocyte receptors, facilitating their entry into liver cells (Sastry and Bhak, 2014).Plasmodium sporozoites target the highly sulfated heparan sulfate of the liver, and binding to these heparan sulfate chains is required for attachment to hepatocytes (Sinnis *et al*.,2007). This binding is mediated by the circumsporozoite protein, the major surface protein of the sporozoite, which is probably also involved in sporozoite

attachment to mosquito salivary glands(Sinnis *et al.,*2007).CSP has a unique domain structure: an initial domain, an area labeled as Region I, a series of tetra-peptide repeats, a C-terminal region consisting of Regions II and III, and a conserved thrombospondin type-I repeat (TSR) domain (Malpede and Tolia, 2014).A glycosylphosphatidylinositol (GPI) anchor connects CSP to the membrane(Malpede and Tolia, 2014).CSP functions in two separate phases of the sporozoite's life cycle. Initially, the CSP's N-terminal region/Region I binds to heparan sulfate on the mosquito's salivary glands. When the mosquito feeds on blood, sporozoites are released from the salivary glands into the human host and move to the liver. In the liver, the second phase of CSP host cell recognition relies on the proteolytic removal of the N-terminal region and the timed exposure of the C-terminal TSR domain(Malpede and Tolia, 2014).After release from the liver into the bloodstream, the merozoite recognizes RBCs and must form a tight link with the host cell membrane. Two distinct protein families are involved at this stage: the erythrocyte binding like (EBL) and the reticulocyte binding like protein homologue (RH) (Malpede and Tolia 2014 ;Adams *et al., 1972)*.MAEBL,a distinct member among EBL ligands, plays a crucial role in sporozoite entry into mosquito salivary glands, as opposed to its primary function in the

blood stage. Sporozoites without MAEBL cannot adhere to salivary glands but maintain regular motility, emphasizing its unique role in host cell attachment(Malpede and Tolia, 2014).In addition Plasmodium falciparum, the primary cause of human malaria, can enter both Duffy-negative and Duffy-positive red blood cells with equal proficiency. Instead, it selectively adheres to erythrocyte glycophorin, binding specifically to neuraminidase-sensitive sialic acids(Adams *et al*.,1992).The P. falciparum protein with a molecular weight of 175 kDa, initially named Erythrocyte Binding Antigen 175 (EBA175), has been renamed the P. falciparum Sialic Acid Binding Protein due to its specific binding to sialic acid (Adams *et al*.,1992).

CHAPTER THREE

MATERIALS AND METHOD

3.1 Study Location

This research was conducted at the University of Benin between August to September, 2023. University of Benin is located in Ovia north east local government area, Edo state. Edo state is situated in the equatorial rainforest. The wet season extends from March to October, while the dry season lasts from November to February. In the rainy season, Edo state experiences temperatures ranging from 20 degrees Celsius to 36.5 degrees Celsius, and during the dry season, temperatures vary between 27 degrees Celsius and 36.5 degrees Celsius. Benin city has a population of approximately 1,147,188 as per the 2006 nationwide census. Edo State shares its borders with Kogi state in the Northeast, Anambra in the East, Delta in the Southeast, while to the South, it's adjacent to Ondo, and to the West and Northwest. Geographically, it is situated at Latitude 6.6342°N and Longitude 5.9304°E.The climate conditions and the surrounding flora at specific times of the year create favorable breeding grounds for Anopheles mosquitoes, which act as carriers for Plasmodium parasites. The University of Benin has two campuses, well-planned in the Ekenwan and Ugbowo areas of Benin City. While the student housing is not densely clustered, there is an abundance of natural vegetation surrounding the residential halls and the university grounds. Most students live off-campus in areas like Ekosodin, Osasogie, Building Development Property Authority, the University of Benin Teaching Hospital, and the University staff quarters. Many of the roads are in poor condition, with potholes and stagnant

water accumulation. Similarly, the drainage system is inadequate, providing ideal breeding sites for mosquitoes.

3.2 Study Design

This is a case-control study of students diagnosed and showed symptoms of malaria infection and apparently healthy non-exposed participants in the same environment. The study participants consisted of students who were recently diagnosed with malaria infection, within the age range of 18-25 years ,were recruited for this study. While the non-exposed (control) within the appropriate age range were matched with exposed participants.

3.3 Study Population

Ten (10) malaria infected students (age range ;18-25 years) and another twenty-five(25) non-exposed participants of the same age group were matched and enrolled in the study. The me Structured questionnaire was used to obtain the socio-demographic information from the participants and were all recruited for this study . They were all recruited from the hostels in the University of Benin and environs particularly, Hall 1 ,Hall 2 ,Hall 3 and Ekosodin .

3.4 Sample Size determination

Sample size was determined using the formula.

The sample size was calculated using the formula of $n = \frac{z^2 \times P(1-P)}{d^2}$ (Obeagu *et al.*,2022*).*Using the formula above at a prevalence of 75% or 0.75(Adeyemo *et al.,* 2013) ,a sample size of was obtained

$n = z^2 \times = z^2 \times P(1-P)$

d^2

Where

n = Sample size

p = prevalence rate 75%

z = confidence interval 95% - 1.96

d = Degree of accuracy- 0.05

$N = 1.96^2 \times 0.75(1-0.75)/0.05^2 = 288$

Under the n=288

Due to difficulties in obtaining samples from study subjects and financial constraints, 35 samples was considered for this project work.

3.5 Inclusion Criteria

Individuals having the following conditions were used for this experiment

1) Individuals having Malaria Only

2) Individuals having malaria or on anti-malaria medications

3.5.1Exclusion Criteria

Individuals with bleeding disorders,on anticoagulant drug therapy,or individuals with other health related issues asides malaria are excluded from this study.

3.5.2 Experimental Design

The subjects were divided into 2 groups consisting of people infected with malaria ,and people without malaria

Group 1: People without any trace of malaria infection

Group 2: People with malaria infection or just started anti -malaria treatment

3.5.3 Ethical Approval

Ethical approval was sought from the Health Research Ethics Committee, College of Medical Sciences ,University of Benin, Benin City .

3.5.4 Collection of Blood Samples

Five(5) ml of blood was collected from the upper arm of the patients by using a tourniquet to tie the arm ,and using a wet swab to sterilize the area of collection to prevent asepsis.Aliquot samples (1.8ml) of blood was dispensed into a sodium citrate container for Fibrinogen assay, and 3.2 ml of blood into an Edta container for Malaria parasite testing, packed cell volume and whole blood viscosity.

3.6 Methods of Analysis

The samples collected was analysed for hemorheological parameters such as Fibrinogen,whole blood viscosity and packed cell volume

3.6.1 Packed Cell Volume

It measures the proportion of blood that is made up of cells.

3.6.2 Principle of Packed Cell Volume

The principle of packed cell volume (PCV) measurement involves determining the proportion of whole blood occupied by red blood cells. This is done by centrifuging anticoagulated blood in a specific glass capillary under controlled conditions (RCF 12,000-15,000 xg) for 3-5 minutes, ensuring a consistent packing of red cells. A small amount of plasma remains trapped among the packed red cells. The PCV value is obtained by reading the scale of a microhematocrit reader or calculating it as the ratio of the height of the red cell column to the total height of the blood column in the capillary(Monica Cheesbrough 2006).

3.6.3 Procedure

1. Fill a plain capillary with well-mixed EDTA anticoagulated blood, leaving 10-15 mm unfilled.

2. Seal the unfilled end using an appropriate sealant material, avoiding open flames.

3. Place the filled capillary in a numbered slot of the microhematocrit rotor with the sealed end against the rim gasket. Note the slot number on the patient's form.

4. Carefully position the inner lid to prevent tube dislodgement.

5. Centrifuge for 5 minutes at 12,000-15,000 xg. If PCV > 0.50, centrifuge an additional 3 minutes.

6. Immediately after centrifuging, check for blood leakage or breakage.

7. Read the PCV using a hand-held microhaematocrit reader. Align the base of the red cell column with the 0 line and the top of the plasma column with the 100 line. Read the PCV from the scale at the top of the red cell column, just below the buffy coat layer (WBCs and platelets) (Monica Cheesbrough 2006).

3.7 Fibrinogen Assay

It assesses the capacity of fibrinogen to create fibrin following exposure to an elevated concentration of purified thrombin.

3.7.1 Method

Clauss Method

3.7.2Procedure

1. Plasma samples are pre-diluted to minimize assay interference from substances like heparin and FDPs.

2. Diluted plasma is incubated at 37°C before adding the pre-warmed (37°C) thrombin reagent.

3. Time to clot is measured from the moment thrombin is added.

4. Clotting time is measured in seconds and interpolated from a standard curve.

5. The standard curve is constructed using various dilutions of assayed standard plasma.(Stang and Mitchell 2013).

3.7.3Whole Blood Viscosity

Measures the resistance of blood to flow

3.7.4 Materials

1ml syringe, 21g needle, retort stand

3.8 Method

Reid and Ugwu method

3.8.1 Procedure

1. Measure whole blood viscosity using a 1.0ml graduated syringe.

2. Attach a vertical hypodermic needle (21.6 x 0.8 x 4mm) to the syringe.

3. Draw whole blood into the syringe, ensuring there are no air bubbles, exceeding the 1.0ml mark.

4. Hold the composite syringe with its plunger and needle vertically using a retort stand.

5. Completely withdraw the plunger, and as soon as the lower meniscus of plasma/blood reaches the 1.0ml mark, start a stopwatch.

6. Repeat this process twice for each sample.

7. Note the time required for 1.0ml of whole blood/plasma to flow down the syringe.

8. Express the plasma/whole blood viscosity as Relative Plasma Viscosity (RPV) and Relative Whole Blood Viscosity (RWBV), which is the ratio of the flow time for 1.0ml of plasma/whole blood to the flow time for 1.0ml of distilled water(Ifeanyichukwu *et al*.,2015).

3.8.2 Malaria Test

This test is done to detect the presence of malaria in the blood film.

3.8.3 Materials

Blood sample, slide, immersion oil,giemsa stain

3.8.4 Procedure

1. A thick film is made on the glass slide and stained with giemsa stain for 30 mins .it is then viewed microscopically with a 100x objective lens(Monica Cheesbrough 2006).

3.9 Methods of Analysis

Data generated was analyzed with the IBM statistical software SPSS 20. the Mean, Standard Deviation, Probability Value (p value), and a level of significance was gotten using independent sample T- test,one way analysis of variance will be used to analyze the variation in the mean samples, Statistical significance was set at $p \leq 0.05$,highly significant at $p<0.001$ and not significant at $p>0.05$.

CHAPTER FOUR

RESULTS

In Table 4.1, the Packed Cell Volume (PCV) values was significantly reduced ($p>0.05$) when comparison between test subjects and control subjects were made. However, there was no significant difference ($p>0.05$) between the control subjects. Also, fibrinogen levels did not significantly increase ($p>0.05$) when compared between test subjects and control subjects (control A and control B), while there was no significant difference between the control subjects. There was also no significant decreased ($p>0.05$) in relative whole blood viscosity when test subjects was compared with control subjects and when the control subjects were compared.

Table 4.1 **Mean ± S.D of parameters compared among malaria infected subjects, non-malaria infected subjects (Control A) and non-infected control subjects (Control B)**

Subjects	Parameters	PCV (%)	Fibrinogen (g/dl)	Whole blood viscosity
Subject 1	Malaria infected subject (n =12)	41.3±4.8	192.1±45.1	125.7±13.0
Subject 2	Non-malaria Infected Control A (n = 14)	49.5±12.2	150.4±74.5	125.2±17.2
Subject 3	Non Infected Subject control B (n = 10)	54.4±8.8	198.3±55.4	124.9±7.6

F(P) Value	8.38 (0.996)	1.771 (1.000)	6.59 (0.999)
Subject 1 vs Subject 2 (P – value)	0.040*	0.144	0.943
Subject 1 vs Subject 3 (P – value)	0.000*	0.791	0.867
Subject 2 vs Subject 3 (P – value)	0.293	0.085	0.963

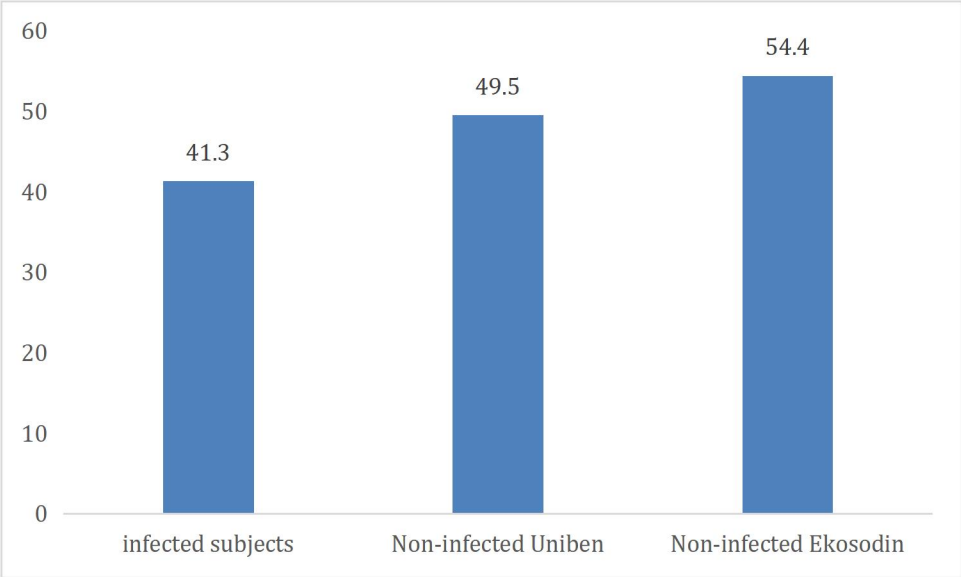

Figure 4.1: PCV of subjects (Infected, Non-infected Uniben and Uninfected Ekosodin)

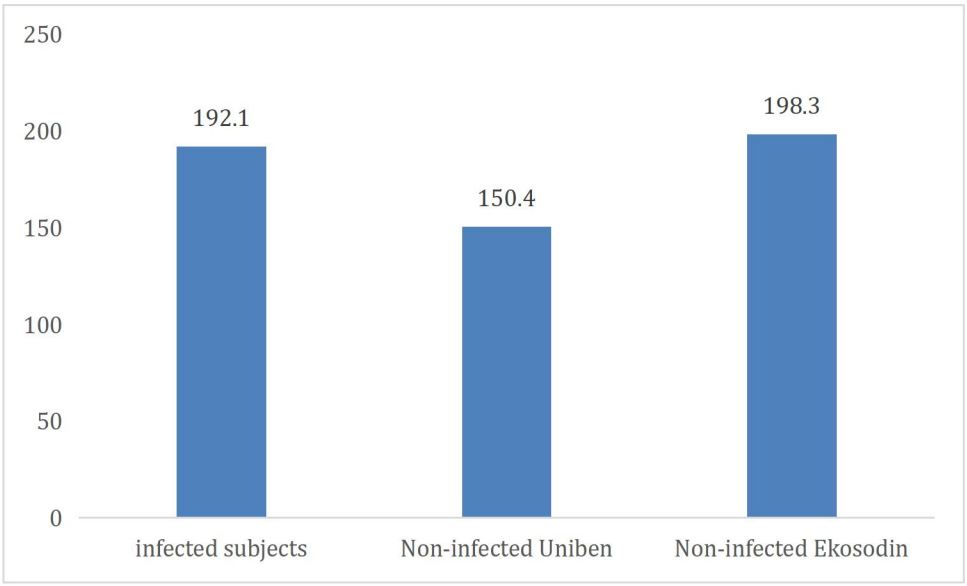

Figure 4.2: Fibrinogen of subjects (Infected, Non-infected Uniben and Uninfected Ekosodin)

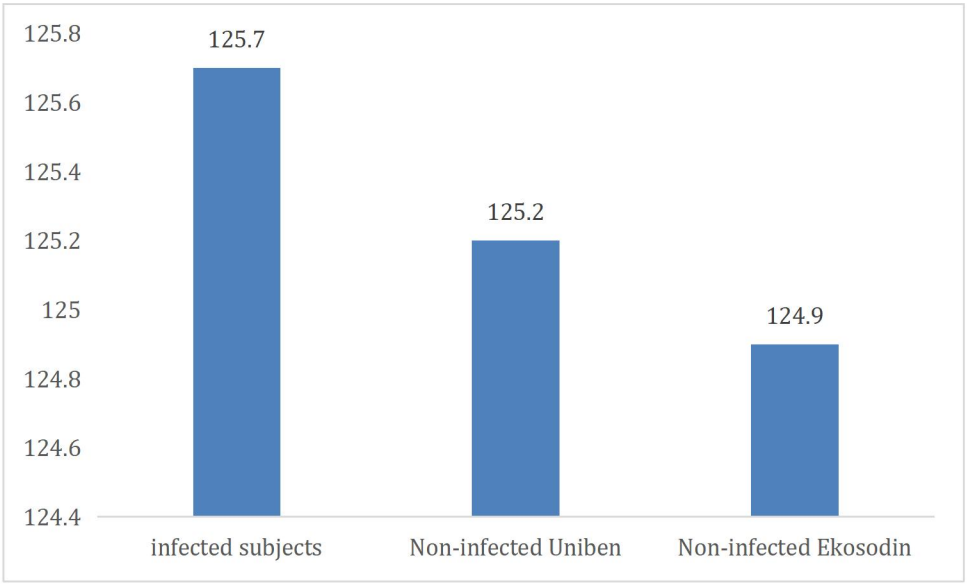

Figure 4.3: Whole blood viscosity of subjects (Infected, Non-infected Uniben and Uninfected Ekosodin)

In Table 4.2, the Packed Cell Volume (PCV) values was significantly reduced ($p<0.05$) when comparison between test subjects and control subject A in the males, but there was no significant difference between the test subjects and the control subjects B for the males. Also, the PCV did not differ significantly between the control subjects (control subject A and B) in the males ($p>0.05$). However, there was no significant difference ($p>0.05$) in the PCV between the test subjects and the control subjects in the females ($p>0.05$). Also, there was no significant difference ($p>0.05$) between the control subjects for the females.

Table 4.2 **Mean ± S.D of parameters compared among malaria infected subjects, non-malaria infected subjects (Control A) and non-infected control subjects (Control B) according to sex**

Subjects	**Parameters**	**Male PCV (%)**	**Female PCV (%)**
Subject 1	Malaria infected subject (n =12)	44.4±2.8	41.3±4.8
Subject 2	Non-malaria Infected Control A (n = 14)	53.5±10.8	49.5±12.2
Subject 3	Non-infected Subject Control B (n = 10)	54.6±8.2	54.4±8.8
	F(P) Value	5.68 (0.995)	0.86 (0.931)
	Subject 1 vs Subject 2 (P – value)	0.049*	0.272
	Subject 1 vs Subject 3 (P – value)	0.002*	0.878
	Subject 2 vs Subject 3 (P – value)	0.829	0.075

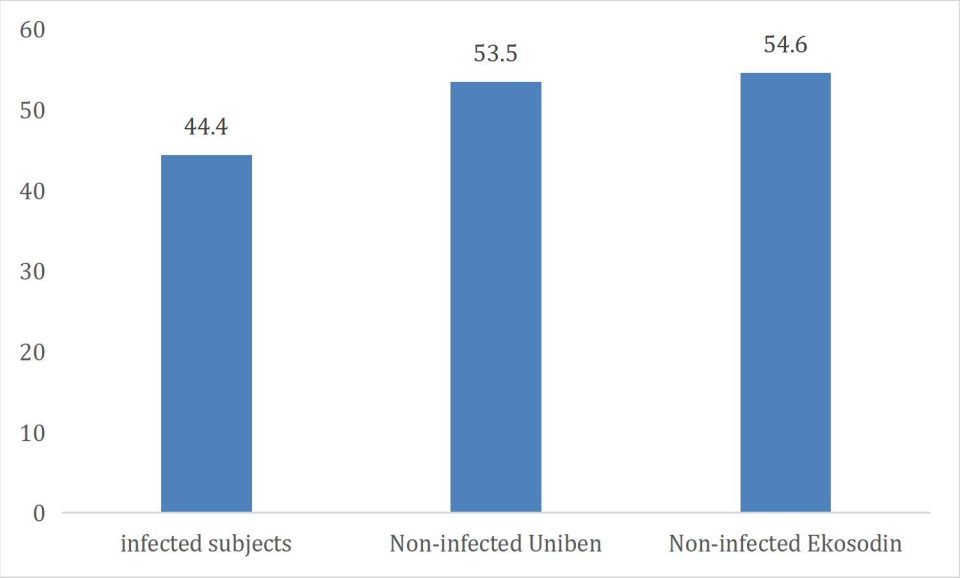

Figure 4.1: PCV of male subjects (Infected, Non-infected Uniben and Uninfected Ekosodin)

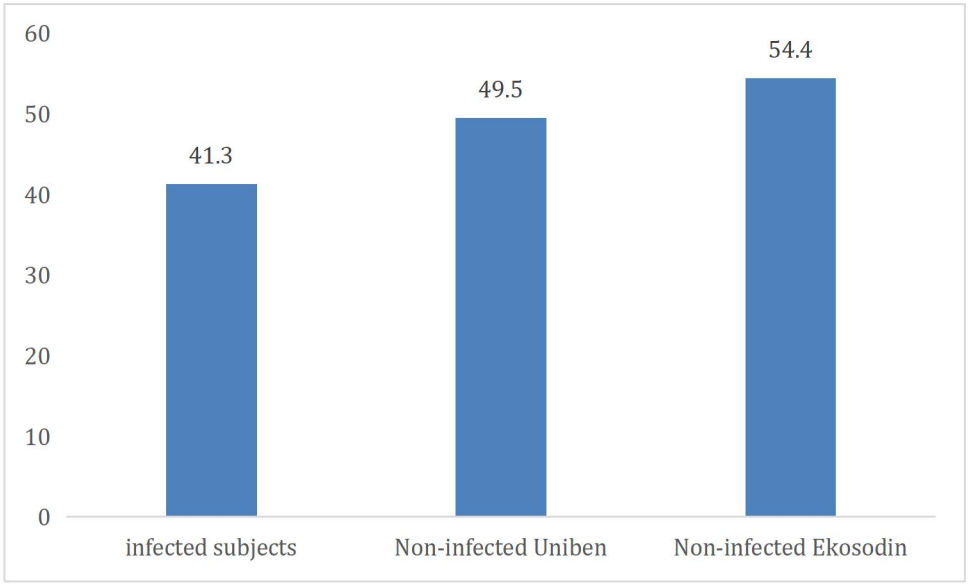

Figure 4.1: PCV of female subjects (Infected, Non-infected Uniben and Uninfected Ekosodin)

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

Discussion

PCV, fibrinogen, and whole blood viscosity were among the haematological system changes that were found in prior research (Salawu and Durosinmi, 2001; Desai et al., 2007). This was supported by the findings of this study, which showed that PCV was statistically significantly lower in malaria-infected subjects (32.0–49.0%) compared to non–malaria-infected subjects (Control A) (33.0–71.0%) and control subjects (Control B) (40.0–64.0%). According to earlier studies (Egushi *et al*., 1993; Imoru and Emeribe, 2008), this was the case. This decrease could be caused by the physiological anaemia that the subjects are predisposed to (due to a marked increase in plasma volume without a corresponding increase in RBC) and by the malaria parasites further destroying the subjects' PCV, resulting in severe anaemia (Menedez *et al*., 2000; van den Broe and Letsky, 2001). Anaemia could be caused by malaria and pregnancy working together to cause severe anaemia.

Also, there was an insignificant decrease in fibrinogen in this study, which is in agreement with the report of Ifeanyichukwu *et al*. (2015). However, Egushi *et al*. (1993) reported that an increase in fibrinogen in spite of malaria infection in pregnant women could be as a result of increase protein synthesis to cope with the increase protein needs of the mother and foetus or hormonal changes as levels of oestrogen and progesterone has been reported to increase throughout pregnancy.

Similarly, there was an insignificant decrease in whole blood viscosity between the test subjects and the control subjects and between the control subjects in this study. This is also in agreement with the findings of Ifeanyichukwu *et al*. (2015).

It was also observed from this study that changes in haemorrheological factors was more evident in males than in females, with significant differences observed between test subjects and control subjects, while there was no significant difference observed in the females. However, there is a need to protect women from malaria because of pregnancy, which leads to a reduction in the immunity of women against infections, thus, making them more prone to malaria infection. Additionally, malaria infection has been reported to cause causes anemia with decreased uterine activity and low birth weight among others (Steketee *et al*., 1996).

Contribution to Knowledge

Due to the proximity of students to anti-malaria drugs,there was no significant impact of malaria on fibrinogen and whole blood viscosity.This is due to the fact that fibrinogen is an acute phase reactant, which means fibrinogen will only increase dramatically in the blood when there's an acute inflammation in the body.This acute inflammation causes roleaux formation which in turn leads to RBC aggregation and an increase in whole blood viscosity.

This effect is only seen in acute cases of malaria.

Conclusion

When test subjects were compared to controls, the results showed a significant decrease in PCV and an insignificant increase in fibrinogen and whole blood viscosity. When compared to the controls, the male malaria subjects' haemorrheological characteristics were noticeably different from those of the females. These results demonstrate that the haemoheological profile of the subjects changes as a result of the severe anaemia to which these subjects are predisposed due to

malaria. Therefore, it is crucial that subjects in malaria-endemic areas undergo a hemorheological examination.

Recommendations

An all-encompassing strategy that incorporates community involvement, prevention, diagnosis, and treatment is necessary to manage malaria in endemic areas. Because malaria is a complicated illness, many parties are involved in its management, including local communities, governments, and non-governmental organisations. Public education and awareness campaigns, indoor residual spraying (IRS) with insecticides to reduce the density of mosquitoes that transmit malaria, community meetings, radio, television, and local health workers are some examples of prevention measures. There are also chemoprevention programmes, early diagnosis, prompt and effective treatment, provision of healthcare infrastructure, and robust surveillance and data management systems.

An ongoing, multifaceted strategy that addresses both the immediate difficulties of diagnosis and treatment and the underlying causes of transmission is needed to control malaria in endemic areas. To reduce malaria-related morbidity and mortality in a sustainable manner, ongoing monitoring and strategy adaptation are crucial.

Limitations of Study

The following were difficulties encountered during this study

1) Lack of funding for proper execution of study

2) Lack of available malaria samples

3) Storage related issues leading to spoilage of some samples.

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