

Diagnosis and Treatment of Malaria in Nigeria

Hongzhe Chen^{1, †}, Siyue Fang^{2, *}, †, Linzhuo Li^{3, †}, Chunze Zou^{4, †}

¹University of Southern California, Los Angeles, CA, US

²University of North Carolina at Chapel Hill, North Carolina, Chapel Hill, United States

³Rutgers University, New Jersey, New Brunswick

⁴Qingdao Grand High School, Qingdao, Shandong Province, China

*Corresponding author: fsiyue@ad.unc.edu

†These authors contributed equally.

Abstract. There are 241 million malaria cases in 2020 globally based on WHO's "World Malaria Report 2021", Nigeria is the hardest hit region. The mechanism of the parasite caused many complications and the two hosts of the transmission. The malaria parasite is transmitted from the salivary glands of the mosquito to the human blood. Once the parasite was transmitted to the human, it can cause a human to human transmission through the contacting with the infected blood. Mainstream diagnostic techniques of Malaria includes Malaria Rapid Diagnostic Testing (RDT) / Histidine-rich Protein 2(HRP2) MRDT/Paracheck-Pf RDT; microscopy; nested PCR (nPCR) ; quantitative PCR (qPCR) . There are also advanced diagnostic technologies such as Loop-mediated Isothermal Amplification (LAMP) and CRISPR-based Diagnostic-using the nucleic acid detection platform Sherlock (specific high-sensitivity enzymatic reporter unlocking) . Nowadays in Nigeria, despite the decreased drug sensitivity against the disease, new methods and drugs have emerged in a bid to control this parasitic infection. The treatment continues to change and more discoveries have been made on how to treat the disease. This paper summarizes the status quo of malaria in Nigeria and provides possible and effective ways for Nigeria to eliminate malaria.

Keywords: Malaria infection, Malaria diagnosis, Malaria treatment, Malaria prevention.

1. Introduction

Malaria is a mosquito-borne disease caused by a parasite. Its symptoms include high fever, shaking chill, vomiting and flu-like illness. Malaria is a serious disease and could be fatal to humans. There are at least 627,000 deaths caused by malaria in 2020. The WHO African Region suffers the most from malaria since it has 95% malaria cases. Moreover, The WHO African Region has 96% of malaria deaths. More than 80% of malaria deaths are actually children under 5. These data depict a horrible image and serious status quo in this area. And Nigeria has the worst situation in the WHO African Region. Nigeria's total malaria deaths are 31.9% of world total malaria deaths [1]. This is almost one third of the total malaria deaths in the world. So Nigeria was chosen for analysis.

This paper consists of mechanisms of malaria, current diagnosis techniques, treatments for malaria and conclusion based on analysis.

2. Mechanism of Malaria

The mechanism of malaria includes parasite invasion, parasite biology and host defense.

Human malaria is transmitted through the saliva gland from the infected female Anopheline mosquito that contains plasmodium sporozoites. The mosquitoes are the first host and the human and other vertebrates are secondary hosts [2].The mosquito from its saliva gland which had developed with the parasite, to the blood of the individual and the virus will soon invade the liver. The transmission of malaria can also happen through the exposure of the infected blood from the patient. This can be transferred from mother to child, blood transfusions and sharing the same needles used in injected drugs.

The blood parasite Plasmodium have 156 variations, however, only 4 species of Plasmodium can truly effect human and that is P.faliparum , P. Viax, P.ovale , P.malalae, P. Malariae [3].

The prevalent symptom of malaria has a similar symptom with other epidemiology which includes a high fever. The other symptoms of malaria may include headache, vomiting, joint pain, hematuria and retinal damage. Malaria may also cause many complications to the infected individuals and anemia is the most common one. More than 25% of adults and 40% [4]of children can develop anemia as the complications of malaria. The anemia is caused by the destruction of red blood cells by the malaria parasite. This makes the red blood cell unable to carry enough oxygen to the organs and cells, leaving the patient weak, drowsy and faint.[5] There is 5-25% in adults and 29% [4]. in pregnant women that the Malaria will develop acute respiratory distress syndrome as complications. Malaria may also cause kidney failure when the malaria parasite causes hemolysis and heme to enter the urine, making the urine turn red and black.

The parasite is formed inside the mosquitoes when the gametocytes is ingested by an Anopheles mosquito during a blood meal plan. The parasite multiplication inside the mosquito is known as the sporogonic cycle [3]. The zygotes form when the microgametes penetrate the macrogametes inside the mosquitoes' stomach. After the female gametes was fertilized they will develop the oocysts. When the oocysts bursts inside the mosquito, the sporozoites will be released into the mosquitoes' saliva gland. This is why the parasite is transferred from the salivary gland of the mosquitoes to the blood of humans [6]. The parasites named sporozoites will migrate into the salivary glands of mosquitoes and will mature under the 25 degrees and moist environment within 10-18 days. The asymptomatic period after the infection of disease can last from 8-30 days [7].

Inside the human body, there will be two phases developed after the invasion. When sporozoites enter the bloodstream of the individual after mosquito bite, the parasite will invade the hepatocytes cell in the liver [8]. The parasite stays inside the red blood cell and Hepatocyte cell which makes it evade the detection of immune system. Once the sporozoites cells invade the liver, it can divide up to 1000 folds until mature tissue schizonts develop. This is known as the exoerythrocytic phase .The schizonts require about 6 to 30 days to rupture. This process may cause the invasion of red blood cells. The parasite will digest the protein of the red blood cell known as the hemoglobin. Once the hemoglobin is digested, the toxic metabolite is formed. This process where a red blood cell is invaded is known as the Erythrocytic phase [9]. The infected red blood cell can be structurally vulnerable, making it prone to be damaged by the splenic organ. The malaria parasite will coherent its surface protein to the red blood cell to make the red blood cell stick to the walls of small blood vessels which stops the cycle going to the splenic organ. Due to this mechanism, malaria can lead to blood vessels congestion. Furthermore, the infected red blood cell can damage the blood- brain barrier and cause brain microvascular hemorrhage. The microvascular hemorrhage will cause Cerebral Malaria [4].

Biological factors may protect people from malaria. To be more specific, people who have sickle cell trait in the red blood cells are more protected from the P. Falciparum malaria. The sickle cells are more prevalently found on Africa ancestry populations .Where as, people who have hemoglobin-related disorders and other blood cell dyscrasias, such as Hemoglobin C, the thalassemias and G6PD deficiency [10], are more prevalent to get malaria. In the sub saharan Africa regions such as Nigeria included 99.9% malaria cases such as P.falciparum. The children under 5 years old accounted for 66.7% of deaths while 11% of maternal deaths contributed to malaria.

3. Current Diagnosis Techniques

3.1. Malaria Rapid Diagnostic Testing (RDT)/ Histidine-Rich Protein 2 (HRP2) mRDT /Paracheck-Pf RDT

An immune-chromatographic technique called a rapid diagnostic test (RDT) is used to identify parasite-specific antigens in finger-prick blood samples. RDT uses dye-labeled antibodies that form visible bands on nitrocellulose strips to help in the diagnosis of malaria by demonstrating the presence of malaria parasites in human blood. These strips are often housed in plastic containers called

cassettes [11]. Fast detection times, low costs, simple findings interpretation, and ease of use make RDT an attractive option to clinical or microscopy-based diagnosis [12].

Histidine-rich protein 2 (PfHRP2), lactate dehydrogenase (LDH), and aldolase are among the antigens that are the focus of commercially available RDTs [11]. The most popular test at the moment is HRP2 mRDT, but it has the following limitations: PfHRP2 persists in the blood for 1–5 weeks after effective treatment, making it impossible to distinguish between recent infections that have been successfully treated and new infections; variable frequencies of HRP2 deletions in *P. falciparum* parasites exist in countries in the Amazon region, rendering HRP2-based tests inappropriate in this region; and low sensitivity for *P. malariae* and *P. ovale* detection., etc [11].

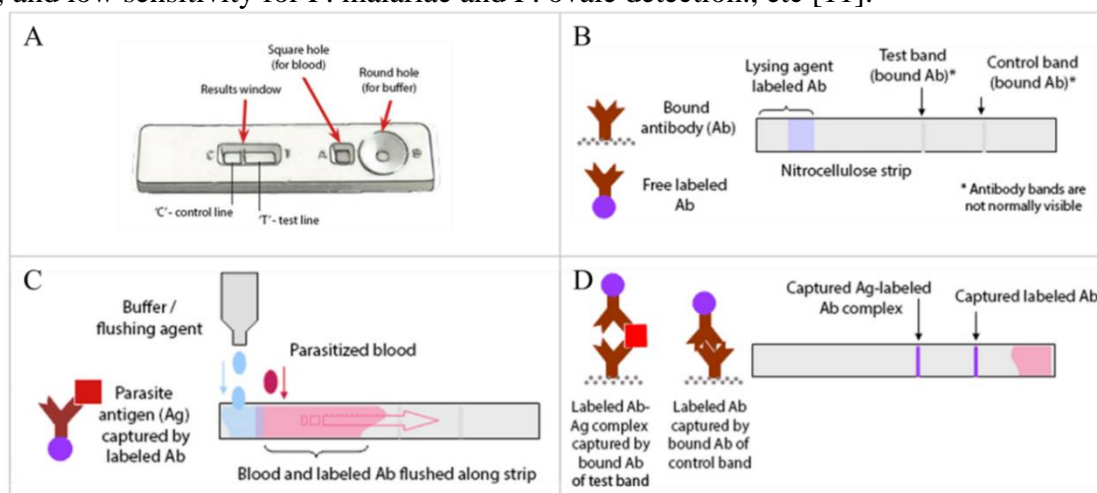


Figure 1: RDT cassette (A), (B), and (C) (D) RDT format for common malaria's mode of action [11]. In populations of patients who are HIV-positive, the Paracheck-Pf RDT (a histidine-rich protein-II based malaria rapid diagnostic test) is accurate and reliable in identifying malaria parasite densities 200/L. With rising parasite density, the Paracheck-Pf RDT's sensitivity and specificity rose [11].

3.2. Microscopy

The gold standard for detecting malaria is microscopy, an established and very easy technique that offers a highly sensitive malaria-specific diagnosis and can quantify malaria parasites and identify infecting species [13]. Through a chemical procedure known as staining, parasites can be successfully located and recognized visually under a microscope. Giemsa is a well-liked and reasonably priced stain frequently used in the dyeing process. Giemsa staining reveals parasites, white blood cells, platelets, and other artifacts while only slightly staining red blood cells. Thin and thick malaria smears can be taken in any laboratory that can perform basic hematology testing [13], and trained microscopists can find asexual parasites at a density of less than 10/L of blood [13]. In addition to identifying patients with active malaria, microscopy can also provide details on the density of the parasites, which can be used to track treatment effectiveness. The subjective identification and counting of parasites by microscopists, the dearth of skilled microscopists and dependable equipment in many malaria-endemic countries like Nigeria, and the difficulty of an unpredictable power supply are some drawbacks of microscopy, though [9]. Therefore, these situations may result in overdiagnosis or underdiagnosis, overuse or underuse of antimalarial medications, increasing parasite resistance to malaria, and drug-induced clinical disorders [9].

3.3. Nested PCR (nPCR)

One of the problems with malaria control and eradication is the sensitivity of the numerous diagnostic tools used to detect the disease. Even while microscopy remains the gold standard for identifying malaria, more precise and trustworthy diagnostic techniques, such as polymerase chain reactions (PCR), are used in research settings to find submicroscopic infections and track treatment [14]. In order to employ them for the nested PCR, already-existing primers that target the 18S rRNA

of Plasmodium species were changed in cycling conditions (nPCR). Isolated gDNA and the primary amplicon, respectively, were employed for the PCR amplification in the primary and nested PCRs [15]. The highly sensitive nested PCR is commonly used for microscopy accuracy assessment, epidemiological research, diagnosis confirmation, and pharmaceutical efficacy evaluation. The high cost, complexity, material requirements, and strenuous exercise requirements of nPCR techniques may prevent many resource-poor populations from finding them to be a suitable diagnostic tool [14]. This is despite the fact that they can detect parasitaemia at low microliter levels with remarkable accuracy.

3.4. Quantitative PCR (qPCR)

Using tagged probes in quantitative real-time PCR (qPCR) is necessary for greater specificity. According to several writers, the use of such molecular approaches has aided in the discovery of "hidden non-falciparum species." In determining the current malaria infection rate, these tools have proven to be quite useful. However, in locations with limited resources, such tools are not readily available, and in endemic populations, the need for a skilled technician, quality control, and equipment maintenance may be "far-reaching." It is concerning to see that qPCR has a high proportion of false positive results [15].

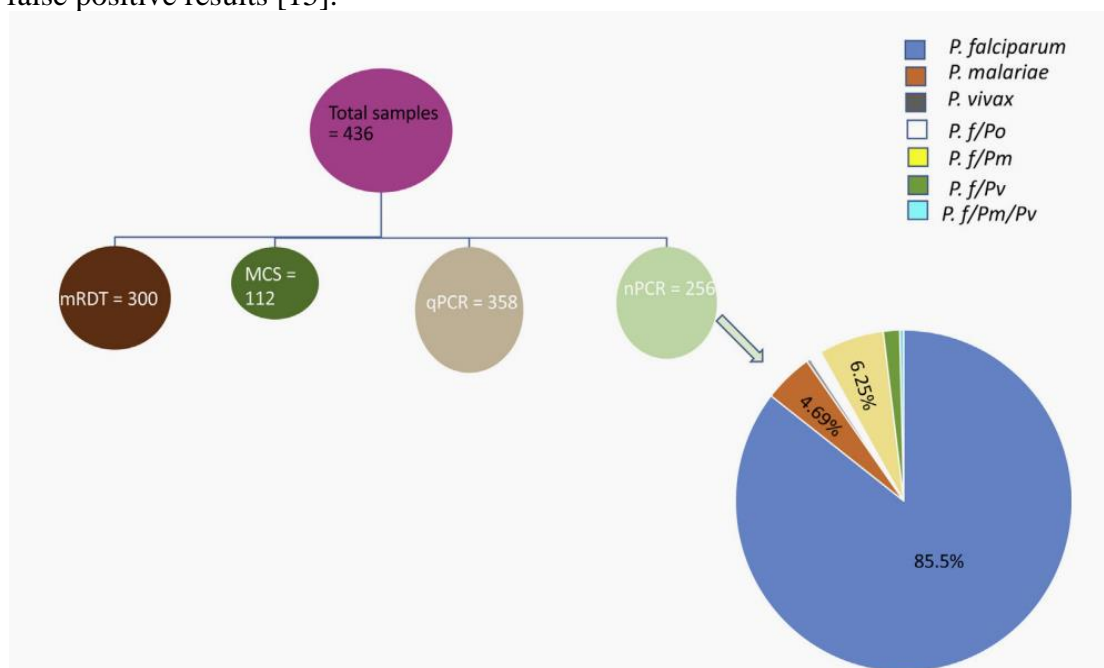


Figure 2 shows a visual comparison of the effectiveness of the various malaria diagnostic techniques as measured by prevalence (quantitative PCR, nested PCR, mRDT, and microscopy). Advances Diagnosis Techniques [12]

3.5. Loop-mediated Isothermal Amplification (LAMP)

Using tagged probes in quantitative real-time PCR (qPCR) is necessary for greater specificity. According to several writers, the use of such molecular approaches has aided in the discovery of "hidden non-falciparum species." In determining the current malaria infection rate, these tools have proven to be quite useful. However, in locations with limited resources, such tools are not readily available, and in endemic populations, the need for a skilled technician, quality control, and equipment maintenance may be "far-reaching." It is concerning to see that qPCR has a high proportion of false positive results [15]. Because RDT has a limited sensitivity to low parasite densities, LAMP can be employed in reactive active case detection (RACD) to find sub-patent infections that were overlooked. Although LAMP is currently more expensive than RDT, it is nevertheless likely to be employed for quality control and the identification of malaria hotspots. On the other hand, one significant drawback of the LAMP test is its inability to calculate parasite density [17].

3.6. CRISPR-based Diagnostic-using the nucleic acid detection platform SHERLOCK (specific high-sensitivity enzymatic reporter unlocking)

Using the nucleic acid detection platform SHERLOCK, a CRISPR-based diagnostic for ultrasensitive detection and differentiation of *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae* is available (specific high-sensitivity enzymatic reporter unlocking). As shown in figure 5 below, the diagnostic procedure involved a 10-min S-PREP followed by SHERLOCK for *Plasmodium* species-specific identification using fluorescent or lateral flow strip readout. The main benefits include a streamlined sample preparation process that does not require nucleic acid extraction, isothermal determination conditions (40°C) independent of the thermal circulator, freeze-drying integrated determination, and readings that can be used in the field, including the use of handheld fluorimeters or transverse flow strips. The field environment[18] still lacks samples from asymptomatic individuals with complete blood and performance testing.

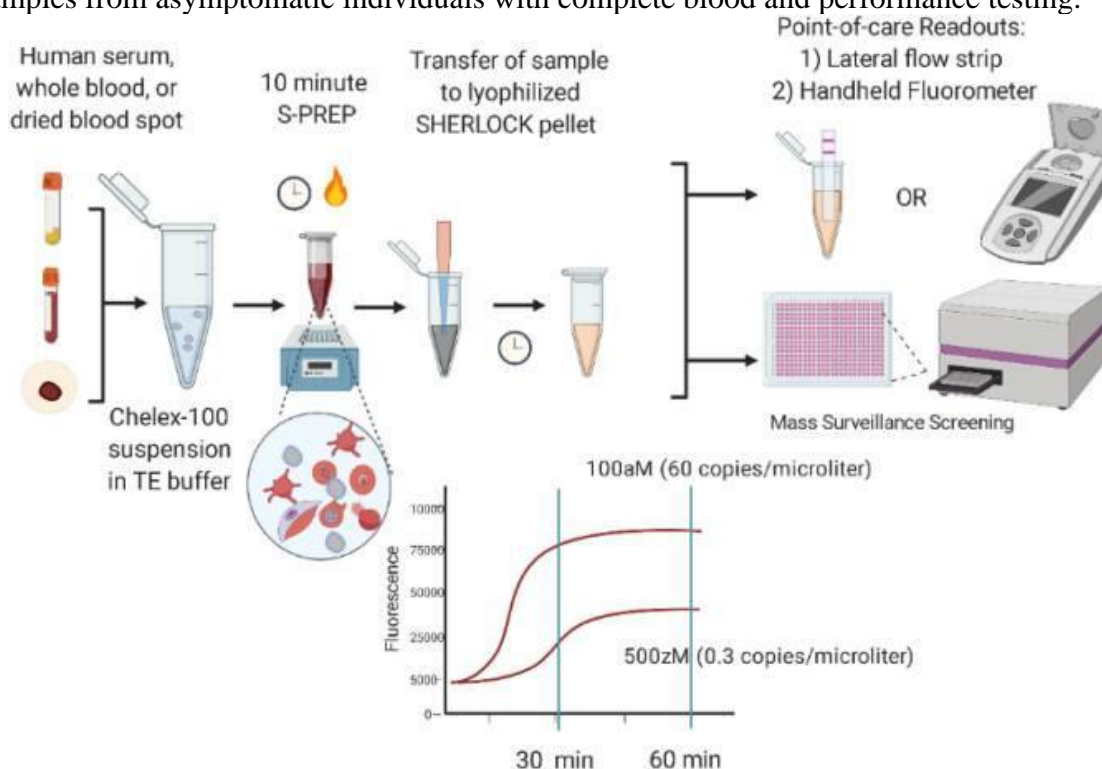


Figure 3 shows the SHERLOCK diagnostic workflow. 1) Human serum, whole blood, or DBS samples go through a 10-min S-PREP protocol in which the sample is suspended in 20% (wt/vol) Chelex-100 in TE buffer with 50 mM DTT and incubated at 95 °C for 10 min; and 2) the suspended sample is transferred to lyophilized SHERLOCK pellet followed by incubation at 40 °C for 60 min before endpoint analysis via fluorescence or lateral flow strip[13].

4. Treatment

Malaria, with no doubt, is one of the world's most devastating tropical mosquito-borne diseases. [19]There has been an increasing number of people who are infected by malaria or have succumbed to the disease, especially young children and pregnant women. People with no or low immunity against the disease are prone to its attacks and it remains a major threat in tropical countries. [20]However, despite the decreased drug sensitivity against the disease, new methods and drugs have emerged in a bid to control this parasitic infection. The treatment continues to change and more discoveries have been made on how to treat the disease.

When it comes to this parasitic disease, the treatment methods differ depending on the age of the patient, severity of the infection, cost, and availability of drugs as well as the patient's immunity level. The drugs administered therefore, need regular review and should be administered as recommended.

For example: Chloroquine phosphate. *P. oval*, *P. vivax* and *P. malaria* are classified as the least dangerous type of malaria and can be treated with chloroquine medication.[21] These parasites are sensitive to this drug and once administered in the body, the treatment helps fight off the infection. However, there have been some reported cases of the drug causing itchy skin for black people. On rare occasions, the patient may experience side effects such as nausea. The drug is taken for three days, 25mg per kilogram of body weight for 24 hours and 5 mg per kilogram at 48 hours. This treatment totally removes the parasite from the body.

The other category is *P. falciparum* which is more severe than the other three. The treatment varies because the parasite's sensitivity to antimalarial drugs differs. Depending on the area of the infection, the parasite can be treated using chloroquine. However, this drug may fail and when this happens, the next drug is amodiaquine. However, in Africa, parts of South America and South Asia where the parasite is fully resistant to chloroquine, they treat it by using sulfoxide and pyrimethamine. However, there is a form of *P. falciparum* parasite that is resistant to multiple drugs, in such a case, mefloquine, quinine or halofantrine is administered instead. Most are taken for a maximum of three days and research shows that they have been successful in getting rid of the parasite. These drugs have minimal side effects to the patient but the effects are severe in young children. Some of these drugs are better administered orally though some like quinine are extremely bitter.

Although these drugs have proved useful over the years, with the fact of these drugs can no longer fight the infection. This is the reason why there is constant research on the treatment of malaria. For example, in Nigeria, they have recommended a therapy called ACT (Artemisinin-based Combination Therapy). ACT treatment consists of an artemisinin derivative coadministered with a longer-acting partner drug. Moreover, three Nigerian medicinal plants have played a huge role. First one is named *Markhamia tomentosa*. The other is called *Polyalthia longifolia* plant. The third plant is the *Trichilia heudelotii* plant. They have been effective in treating severe malaria without complications or side effects.

5. Summary

In conclusion, malaria is threatening the WHO African Region people's lives mainly, especially Nigeria and leading to disproportionate deaths. The mechanism of malaria consists of parasite invasion, parasite biology and host defense. Infected female Anopheline mosquito is the first host and spreads blood parasite plasmodium to human and other vertebrates by biting them. When they successfully invade the human body and evade the scan of the immune system, the sporozoites cells are able to invade the liver and reproduce. They will infect red blood cells and make them vulnerable. Malaria Rapid Diagnostic Testing (RDT) is the most common method for malaria testing since it takes little detection time and low cost. However, it is unable to distinguish new infections from recently effectively treated infections. Still, it's a recommended way for Nigeria to eliminate malaria cases. The common treatments are starting to lose their strength against malaria so Nigeria should choose mefloquine, quinine or halofantrine. The major issue of Nigeria in the fight against malaria is enough supply of these medicines and other resources that could help their national programs against malaria.

References

- [1] R.E. Phillips, G. Pasvol, Anaemia of *Plasmodium falciparum* malaria 1992, *Bailliere's clinical haematology* 5(2) 315-330.
- [2] J.M. Cohen, D.L. Smith, C. Cotter, et al., Malaria resurgence: a systematic review and assessment of its causes 2012, *Malaria journal* 11(1) 1-17.
- [3] L. Aravind, L.M. Iyer, T.E. Wellems, et al., *Plasmodium* biology: genomic gleanings 2003, *Cell* 115(7) 771-785.

- [4] R.W. Steketee, M. Choi, A. Linn, et al., World Malaria Day 2021: Commemorating 15 Years of Contribution by the United States President's Malaria Initiative 2021, *The American journal of tropical medicine and hygiene* 104(6) 1955.
- [5] S.K. Mishra, C.R. Newton, Diagnosis and management of the neurological complications of falciparum malaria 2009, *Nature Reviews Neurology* 5(4) 189-198.
- [6] K. Baer, C. Klotz, S.H.I. Kappe, et al., Release of hepatic Plasmodium yoelii merozoites into the pulmonary microvasculature 2007, *PLoS pathogens* 3(11) e171.
- [7] H. Caraballo, K. King, Emergency department management of mosquito-borne illness: malaria, dengue, and West Nile virus 2014, *Emergency medicine practice* 16(5) 1-23; quiz 23.
- [8] I.A. Clark, L. Schofield, Pathogenesis of malaria 2000, *Parasitology Today* 16(10) 451-454.
- [9] M.V. Sharakhova, A. Xia, Z. Tu, et al., A physical map for an Asian malaria mosquito, *Anopheles stephensi* 2010, *The American journal of tropical medicine and hygiene* 83(5) 1023.
- [10] H. Fujioka, M. Aikawa, The malaria parasite and its lifecycle 1999, *Malaria: molecular and clinical aspects*. First edition. Amsterdam: Harwood Academic Publishers 21-36.
- [11] J.M. Cohen, F. Okumu, B. Moonen, The fight against malaria: Diminishing gains and growing challenges 2022, *Science Translational Medicine* 14(651) eabn3256.
- [12] M.A. Oboh, E.C. Oriero, T. Ndiaye, et al., Comparative analysis of four malaria diagnostic tools and implications for malaria treatment in southwestern Nigeria 2021, *International Journal of Infectious Diseases* 108 377-381.
- [13] M.T. Visser, R. Zonneveld, T.J. Peto, et al., Are national treatment guidelines for falciparum malaria in line with WHO recommendations and is antimalarial resistance taken into consideration?—A review of guidelines in non-endemic countries 2022, *Tropical Medicine & International Health* 27(2) 129-136.
- [14] W. Zhu, X. Ling, W. Shang, et al., High value of rapid diagnostic tests to diagnose malaria within children: A systematic review and meta-analysis 2020, *Journal of global health* 10(1).
- [15] O. Shittu, O.A. Opeyemi, O. Ajibaye, et al., Evaluation of the clinical proficiency of RDTs, microscopy and nested PCR in the diagnosis of symptomatic malaria in Ilorin, North-Central, Nigeria 2018, *Open Access Macedonian Journal of Medical Sciences* 6(6) 1032.
- [16] Y. Mori, H. Kanda, T. Notomi, Loop-mediated isothermal amplification (LAMP): recent progress in research and development 2013, *Journal of infection and chemotherapy* 19(3) 404-411.
- [17] J.G. Mwangi, Evaluation of the Colorimetric Malachite Green Loop-Mediated Isothermal Amplification (MG-LAMP) Assay for the Detection of Plasmodium Species at Two Different Health Facilities in a Malaria Endemic Area of Western Kenya, JKUAT-COHES, 2021.
- [18] R.A. Lee, H.D. Puig, P.Q. Nguyen, et al., Ultrasensitive CRISPR-based diagnostic for field-applicable detection of Plasmodium species in symptomatic and asymptomatic malaria 2020, *Proceedings of the National Academy of Sciences* 117(41) 25722-25731.
- [19] B. Hanboonkunupakarn, N.J. White, Advances and roadblocks in the treatment of malaria 2022, *British Journal of Clinical Pharmacology* 88(2) 374-382.
- [20] B.S. Uzochukwu, O.P. Ezeoke, U. Emma-Ukaegbu, et al., Malaria treatment services in Nigeria: A review 2010, *Nigerian Medical Journal* 51(3) 114.
- [21] N.J. White, The treatment of malaria 1996, *New England journal of medicine* 335(11) 800-806.