Madonna University Journal of Medicine and Health Sciences. Volume 2 issue 2(2022), Pp. 22-40

http://madonnauniversity.edu.ng/journals/index.php/medicine

MALARIA VACCINE: PROSPECTS AND CHALLENGES

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ABSTRACT

Malaria is a life-threatening disease caused by the plasmodium genus, malaria can cause a range of symptoms and life –threatening complications so an early diagnosis is crucial. In many parts of the world, the parasites have developed resistance to a number of antimalarial drugs. Interventions control the spread malaria has been embarked on over the years, such interventions include the use of herbs, and insecticide treated nets, covering or oiling the surface of open water source, treatment with artemisinin-based combination therapies. The most recent intervention is the malaria vaccine just approved by the World Health Organization known as RTS, S(ASO1). This vaccine confers immunity to children between 2-18 months. Before the approval of the malaria vaccine various clinical trials had been embarked on to demonstrate the variable safety and immunogenicity of the vaccine. In this review, we summarised the latest research progress in combating the spread of

Hassan, A.O., Oso, O.V., Obeagu, E.I. and Adeyemo, A.T. (2022). Malaria Vaccine: Prospects and Challenges.Madonna University Journal of Medicine and Health Sciences. 2(2): 22-40

malaria, life cycle of malaria, pathogenesis of malaria, the prospects and challenges of the newly released malaria vaccine.

Keywords*: malaria, vaccine, prospects, challenges*

INTRODUCTION

The history of malaria stretches back from its prehistoric origin as a zoonotic disease in the primates of Africa through to the 21st century. Its prevention and treatment have been targeted in science and medicine for hundreds of years. Our understanding of the malaria parasite began in 1800's with the discovery of the malaria parasite in the blood of malaria patients by Alphnose Laveran. The sexual stage in the blood was discovered by William MacCallum in birds infected with a related Haematozoan in 1897(Cox, 2010).

For thousands of years' traditional herbal remedies have been used to treat malaria (willcox and Bodeker, 2013) however, the first effective treatment for malaria came from the back of the cinchona tree which contains quinine. Also, in the quest for a solution to treating malaria, control measures such as widespread use of insecticide; DDT (Dichlorodiphenyltricholoroethane) swamp drainages, covering or oiling the surface of open water sources, indoor residual spraying and use of insecticide treated nets was initiated but these weren't enough to curb the spread of the malaria parasite.

Prophylactic quinine was prescribed in malaria endemic areas and new therapeutic drugs, including, chloroquine, and artemisinins were used to resist the scourge. Today artemisinin is present in every remedy applied in the treatment of malaria. Going forward, the application of

artemisinin only reduced the mortality rate of malaria in Africa by half, thereby bringing up the need to search for the perfect cure, and this led to the discovery of the first malaria vaccine.

The search for the perfect vaccine took over 30 years due to the complex life cycle of the malaria parasite; malaria has been a tricky target to vaccine markers due this reason (Roxby, 2021).

The first approved malaria vaccine was announced by the World Health Organizations on 6th of October 2021 "RTS, S" with the brand name MOSQURIX. The "RTS, S" acts against the plasmodium species known as plasmodium *falciparum (*Roxby, 2021*).*

CONCEPTUAL CLARIFICATION

MALARIA

Malaria is a mosquito-borne disease caused by a parasite. People with malaria often experience fever, chills, and flu-like illness. Left untreated, they may develop severe complications and die. In 2020 an estimated 241 million cases of malaria occurred worldwide and 627,000 people died, mostly children in sub-Saharan Africa. About 2,000 cases of malaria are diagnosed in the United States each year. Malaria is an acute febrile illness caused by *Plasmodium* parasites, which are spread to people through the bites of infected female *Anopheles* mosquitoes. There are 5 parasite species that cause malaria in humans, and 2 of these species. *P. falciparum* and *P. vivax* – pose the greatest threat. *P. falciparum* is the deadliest malaria parasite and the most prevalent on the African continent is the. *P. vivax.* The Plasmodium life cycle is very complex and takes place in two phases; sexual and asexual, the vector mosquitoes and the vertebrate hosts. In the vector mosquito host, the sexual phase of the parasite's life cycle occurs, while the asexual phase of the life cycle occurs in humans which are the intermediate host for malaria (Obeagu *et al.,* 2019; Obeagu *et al.,* 2017; Obeagu and Obeagu, 2019; Obeagu *et al.,* 2021; Ogbonna *et al.,* 2021).

Human Malaria is transmitted only by female mosquitoes of the genus *Anopheles*. The parasite in the form of sporozoites after a bite by an infected female mosquito enters the human blood and after half an hour of blood circulation, enters the hepatocytes. The first phase of *Plasmodium* asexual development occurs in the hepatocytes, and then in the erythrocytes (Talapko *et al.,* 2019).

The first symptoms which are fever, headache and chills usually appear 10–15 days after the infective mosquito bite which may be mild and makes it difficult to recognize as malaria, if left untreated, *P. falciparum* malaria can progress to severe illness and death within a period of 24 hours.

According to a 2020 report by the World Health Organization, it was reported that nearly half of the world's population was at risk of malaria. The report also stated that some population groups are at considerably higher risk of contracting malaria and developing severe disease: infants, children under 5 years of age, pregnant women and patients who are immunocompromised moving to areas with intense malaria transmission such as migrant workers, mobile populations and travellers. (WHO, 2021).

Malaria is transmitted all over Nigeria; 76% of the population live in high transmission areas while 24% of the population live in low transmission areas. Transmission period can last all year round in the south and is about 3 months or less in the northern part of the country. According to the 2020 world malaria report, Nigeria had the highest number of global malaria cases (27% of global malaria cases) and accounted for the highest number of death (WHO, 2020).

VACCINE

Vaccination is the administration of a vaccine to help the immune system develop protection from a disease. Vaccines contain a microorganism or virus in a weakened, live or killed state, or proteins or toxins from the organism. In stimulating the body's adaptive immunity, they help prevent sickness from an infectious disease. Vaccination is the most effective method of preventing infectious diseases.

The first vaccine was introduced by British physician Edward Jenner, who in 1796 used the cowpox virus (vaccinia) to confer protection against smallpox, a related virus in humans. Prior to

that use, however, the principle of vaccination was applied by Asian physicians who gave children dried crusts from the lesions of people suffering from smallpox to protect against the disease. While some developed immunity, others developed the disease. Jenner's contribution was to use a substance similar to, but safer than, smallpox to confer immunity. He thus exploited the relatively rare situation in which immunity to one virus confers protection against another viral disease. In 1881 French microbiologist Louis Pasteur demonstrated immunization against anthrax by injecting sheep with a preparation containing forms of the bacillus that causes the disease. Four years later he developed a protective suspension against rabies (Emily, 2021).

The activation occurs through priming the immune system with an immunogen. Most vaccines are administered before a patient has contracted a disease to help increase future protection. However, some vaccines are administered after the patient already has contracted a disease. Vaccines given after exposure to some diseases (e.g. smallpox) are reported to offer some protection from disease or may reduce the severity of disease.

MALARIA LIFE CYCLE

Malaria is caused by single-cell microorganism of the plasmodium group. It is spread exclusively through bite of infected female Anopheles mosquitoes. The mosquito introduces the parasites from the mosquito saliva into a person's blood. The parasite travels to the liver, where they mature and reproduce.

Five species of *Plasmodium* can infect and be spread by humans. Most deaths are caused by *P. falciparum*, whereas *P. vivax*, *P. ovale*, and *P. malariae* generally cause a milder form of malaria. The species *P. knowlesi* rarely causes disease in humans (Caraballo, 2014).

Parasites are typically introduced by the bite of an infected *Anopheles* mosquito. These introduced parasites, called "sporozoites", follow the bloodstream to the liver where they invade hepatocyte. They grow and divide in the liver for 2–10 days (White, 2011) each infected

hepatocyte eventually harbouring up to 40,000 parasites. The infected hepatocytes breakdown, releasing this invasive form of *Plasmodium* cells, called "merozoites" into the blood stream. In the blood, the merozoites rapidly invade individual red blood cells, replicating over 24–72 hours to form 16–32 new merozoites. The infected red blood cell lyses, and the new merozoites infect new red blood cells, resulting in a cycle that continuously amplifies the number of parasites in an infected person. Over rounds of this infection cycle, a small portion of parasites do not replicate, but instead develop into early sexual stage parasites called male and female "gametocytes". These gametocytes develop in the bone marrow for 11 days, then return to the blood circulation to await uptake by the bite of another mosquito. Once inside a mosquito, the gametocytes undergo sexual reproduction, and eventually form daughter sporozoites that migrate to the mosquito's salivary glands to be injected into a new host when the mosquito bites (Crown *et al*., 2016).

Within the red blood cells, the parasites multiply further, again asexually, periodically breaking out of their host cells to invade fresh red blood cells. Several such amplification cycles occur. Thus, classical descriptions of waves of fever arise from simultaneous waves of merozoites escaping and infecting red blood cells (Bledsoe, 2005)

Some *P. vivax* sporozoites do not immediately develop into exoerythrocytic-phase merozoites, but instead, produce hypnozoites that remain dormant for periods ranging from several months (7–10 months is typical) to several years *(*White, 2011). After a period of dormancy, they reactivate and produce merozoites. Hypnozoites are responsible for long incubation and late relapses in *P. vivax* infections, although their existence in *P. ovale* is uncertain many of the symptoms associated with severe malaria are caused by the tendency of *P. falciparum* to bind to blood vessel walls, resulting in damage to the affected vessels and surrounding tissue.

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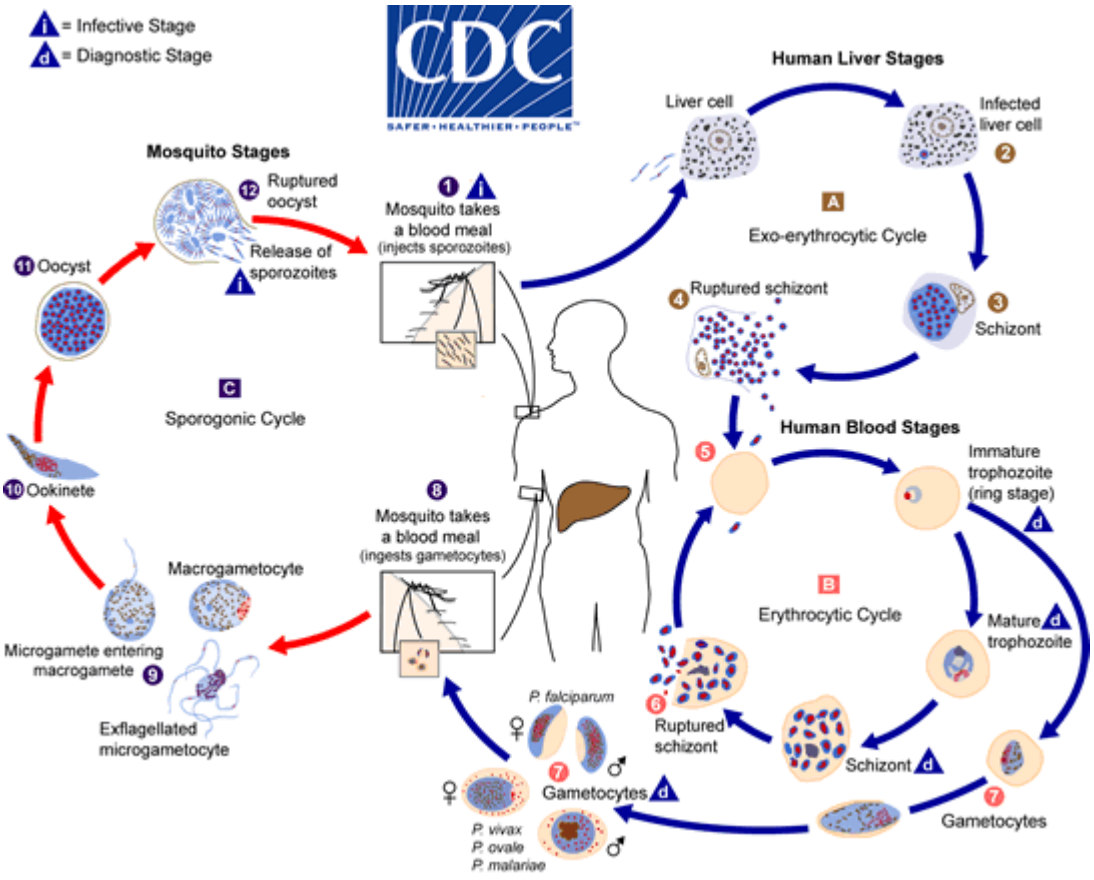

(CDC,2018)

SIGNS AND SYMPTOMS

In uncomplicated malaria, symptoms progress as follows, through cold, hot, and sweating stages:

- a sensation of cold with shivering
- fever, headaches, and vomiting
- seizures sometimes occur in younger people with the disease

- sweats, followed by a return to normal temperature, with tiredness

In areas where malaria is common, many people recognize the symptoms as malaria and treat themselves without visiting a doctor (Peter and Jill, 2018)

In severe malaria, clinical or laboratory evidence shows signs of vital organ dysfunction.

Symptoms of severe malaria include:

- fever and chills
- impaired consciousness
- prostration, or adopting a prone position
- multiple convulsions
- deep breathing and respiratory distress
- abnormal bleeding and signs of anemia
- clinical jaundice and evidence of vital organ dysfunction

Severe malaria can be fatal without treatment.

LABORATORY DIAGNOSIS

Malaria must be recognized promptly in order to treat the patient in time and to prevent further spread of infection in the community via local mosquitoes.

Malaria should be considered a potential medical emergency and should be treated accordingly. Delay in diagnosis and treatment is a leading cause of death in malaria patients in Nigeria.

Malaria can be suspected based on the patient's travel history, symptoms, and the physical findings at examination. However, for a definitive diagnosis to be made, laboratory tests must demonstrate the malaria parasites or their components

Diagnosis is based on the patient's symptoms and on physical findings at examination.

The first symptoms of malaria (most often fever, chills, sweats, headaches, muscle pains, nausea and vomiting) are often not specific and are also found in other diseases (such as the "flu" and common viral infections). Likewise, the physical findings are often not specific (elevated temperature, perspiration, tiredness).

In severe malaria (primarily caused by *Plasmodium falciparum*), clinical findings (confusion, coma, neurologic focal signs, severe anemia, respiratory difficulties) are more striking and may increase the index of suspicion for malaria.

Methods used in laboratory diagnosis of malaria include;

- Microscopic Diagnosis.
- Antigen Detection Method (RDT).
- Molecular Detection Method (PCR).
- Serology Detection Method (ELISA).

Clinical findings should always be confirmed by a laboratory test for malaria. Malaria Parasite can be identified by examination under the microscope, a drop of the patient's blood, spread out as a "blood smear" on a microscope slide. Prior to examination, the specimen is stained (most often with the Giemsa stain) to give the parasites a distinctive appearance. This technique remains the

gold standard for laboratory confirmation of malaria. However, it depends on the quality of the reagents, of the microscope, and on the experience of the laboratorian (CDC, 2018).

The microscopic detection and identification of *Plasmodium* species in Giemsa-stained thick blood films (for screening the presenting malaria parasite), and thin blood films (for species' confirmation) remains the gold standard for laboratory diagnosis (Bharti *et al.*, 2015*).* Malaria is diagnosed microscopically by staining thick and thin blood films on a glass slide, to examine malaria parasites. Briefly, the patient's cubital fossal is cleaned with 70% ethyl alcohol, allowed to dry and then the site is pricked with a sterile needle and poured into a sample bottle. To prepare a thick blood film, a drop of blood is placed on a clean grease free slide and is stirred in a circular motion with the corner of the slide, taking care not make the preparation too thick, and allowed to dry without fixative. After drying, the spot is stained with diluted Giemsa (1: 20, vol/vol) for 20 min, and washed by placing the film in buffered water for 3 min. The slide is allowed to air-dry in a vertical position and examined using a light microscope. As they are unfixed, the red cells lyse when a water-based stain is applied. A thin blood film is prepared by immediately placing the smooth edge of a spreader slide in a drop of blood, adjusting the angle between slide and spreader to 45° and then smearing the blood with a swift and steady sweep along the surface. The film is then allowed to air-dry and is fixed with absolute methanol. After drying, the sample is stained with diluted Giemsa (1: 20, vol/vol) for 20 min and washed by briefly dipping the slide in and out of a jar of buffered water (excessive washing will decolorize the film). The slide is then allowed to air-dry in a vertical position and examined under a light microscope (Chotivanich *et al*.,2011). The wide acceptance of this technique by laboratories all around the world can be attributed to its simplicity, low cost, its ability to identify the presence of parasites, the infecting species, and assess parasite density-all parameters useful for the management of malaria.

TREATMENT

Malaria is treated with antimalarial drugs. The type of medications that are used to treat malaria depends on the severity of the disease and the likelihood of resistance. The drugs available to treat malaria include:

- Chloroquine
- Quinine
- Hydroxychloroquine (Plaquenil)
- Atovaquone (Mepron)
- Proguanil (sold as a generic)
- Mefloquine
- Clindamycin (Cleocin)
- Doxycycline

Artemisinin-based combination therapies (ACTs). ACT is a combination of two or more drugs that work against the malaria parasite in different ways. This is usually the preferred treatment for chloroquine-resistant malaria. Examples include artemether-lumefantrine (Coartem) and artesunate-mefloquine. (Mayo Foundation for Medical Education and Research (MFMER) (2021).

MALARIA VACCINE

A Malaria vaccine is a vaccine that prevents malaria. The only approved vaccine as of 2021, is the RTS, S known by the brand name MOSQUIRIX it requires four injections (European Medicines Agency, 2019).

World Health Organization (WHO) announced the use the RTS, S vaccine on the 6th of October 2021. The world's first mass vaccination program against malaria, is set to prevent millions of children from catching malaria and thousands dying from this debilitating disease. The WHO

has recommended widespread use of the RTS, S/AS01 (Mosquirix) vaccine in young children who are most at risk of malaria in Africa.

RTS, S was developed by PATH MALARIA VACCINE INITIATIVE (MVI) And GLAXOSMITHKLINE (GSK) with support from the Bill and Melinda Gates Foundation has been in development since mid-1980s (Daniel and Michael, 2021)

The RTS, S is the most recently developed recombinant vaccine. It consists of the *P. falciparum* circumsporozoite protein (CSP) from the pre-erythrocytic stage. The CSP antigen causes the production of antibodies capable of preventing the invasion of hepatocytes and additionally e licits a cellular response enabling the destruction of infected hepatocytes. The CSP vaccine presented problems in the trial stage due to its poor immunogenicity. RTS, S attempted to avoid these by fusing the protein with a surface antigen from hepatitis B, creating a more potent and immunogenic vaccine. When tested in trials an emulsion of oil in water and the added adjuvants of monophosphoryl A and QS21 (SBAS2), the vaccine gave protective immunity to 7 out of 8 volunteers when challenged with *P. Falciparum.*

RTS,S/AS01 (commercial name Mosquirix), was engineered using genes from the outer protein of *P. falciparum* malaria parasite and a portion of a hepatitis B virus plus a chemical adjuvant to boost the immune response. Infection is prevented by inducing high antibody titres that block the parasite from infecting the liver. In November 2012, a Phase III trial of RTS, S found that it provided modest protection against both clinical and severe malaria in young infants. *(*Agnandji *et al.,* 2014).

The vaccine Is a protein type vaccine that is administered by the intramuscular route. This protein is coupled with an "adjuvant (ASO1)", a molecule designed to stimulate a strong immune response. The vaccine works mainly by stimulating the body to make antibodies against the parasite, neutralising it, and preventing it from entering liver cells. These are the first cells the

parasite invades when it enters the body. The vaccine also works by helping to mount an inflammatory response, when a different part of the immune system responds.

THE PROSPECTS OF MALARIA VACCINE

The major prospect of the malaria vaccine is the complete eradication of malaria. The vaccine could prevent millions of children from catching malaria and save thousands from dying.

CHALLENEGES OF MALARIA VACCINE

The CDC noted that several challenges had to be overcome to reach this point, including the lack of a traditional market, few developers, and the technical complexity of developing any vaccine against a parasite. Malaria parasites have a complex life cycle, and there is poor understanding of the complex immune response to malaria infection. Malaria parasites are also genetically complex, producing thousands of potential antigens (Welkhoff, 2021).

It is only 65% effective against severe malaria, does not work very well in older children or adults, requires a specific adjuvant that limits its supply, and loses most of its protection for young children over the first 18 months.

The RTS, S acts only against one species of the plasmodium parasite known as the plasmodium falciparum (Roxby, 2021).

Protective immunity also decreases rapidly over time. This means regular booster doses will be required. Alternative immunisation schedules are also being evaluated.

CONCLUSION

This current version of Mosquirix is not expected to be the last. Preliminary results for a new modified vaccine, called R21, are encouraging. Other malaria vaccines in development include whole parasite vaccines. These use the whole malaria parasite that has been killed or altered so it cannot cause a malaria infection but can still stimulate an immune response. Passive vaccines are also being investigated. These involve injecting long-lasting antibodies to prevent malaria infection.

It is evident that although many promising malaria vaccines are under various stages of development, the RTS, S vaccine is the only approved malaria vaccine currently available for immunisation. Further advances are still required for malaria vaccine development, based on empirical approaches and basic research, to identify new target antigens and provide improved understanding of how different adjuvants will affect the balance and durability of effector, memory and regulatory responses.

REFERENCES

Agnandji, S.T., Lell, B., Fernandes, J.F., Abossolo, B.P., Methogo, B.G., Kabwende, A.L., Adegnika, A.A., Mordmüller, B., Issifou, S., Kremsner, P.G., Sacarlal, J., Aide, P., Lanaspa, M., Aponte, J.J., Machevo, S., Acacio, S., Bulo, H., Sigauque, B., Macete, E., Alonso, P., Abdulla, S., Salim, N., Minja, R., Mpina, M., Ahmed, S., Ali, A.M., Mtoro, A.T., Hamad, A.S., Mutani, P., Tanner, M., Tinto, H., D'Alessandro, U., Sorgho, H., Valea, I., Bihoun, B., Guiraud, I., Kaboré, B., Sombié, O., Guiguemdé, R.T., Ouédraogo, J.B., Hamel, M.J., Kariuki, S., Oneko, M., Odero, C., Otieno, K., Awino, N., McMorrow, M., Hassan, A.O., Oso, O.V., Obeagu, E.I. and Adeyemo, A.T. (2022). Malaria Vaccine: Prospects and Challenges.Madonna University Journal of Medicine and Health Sciences. 2(2): 22-40

Muturi-Kioi, V., Laserson, K.F., Slutsker, L., Otieno, W., Otieno, L., Otsyula, N., Gondi, S., Otieno, A., Owira, V., Oguk, E., Odongo, G., Woods, J.B., Ogutu, B., Njuguna, P., Chilengi, R., Akoo, P., Kerubo, C., Maingi, C., Lang, T., Olotu, A., Bejon, P., Marsh, K., Mwambingu, G., Owusu-Agyei, S., Asante, K.P., Osei-Kwakye, K., Boahen, O., Dosoo, D., Asante, I., Adjei, G., Kwara, E., Chandramohan, D., Greenwood, B., Lusingu, J., Gesase, S., Malabeja, A., Abdul, O., Mahende, C., Liheluka, E., Malle, L., Lemnge, M., Theander, T.G., Drakeley, C., Ansong, D., Agbenyega, T., Adjei, S., Boateng, H.O., Rettig, T., Bawa, J., Sylverken, J., Sambian, D., Sarfo, A., Agyekum, A., Martinson, F., Hoffman, I., Mvalo, T., Kamthunzi, P., Nkomo, R., Tembo, T., Tegha, G., Tsidya, M., Kilembe, J., Chawinga, C., Ballou, W.R., Cohen, J., Guerra, Y., Jongert, E., Lapierre, D., Leach, A., Lievens, M., Ofori-Anyinam, O., Olivier, A., Vekemans, J., Carter, T., Kaslow, D., Leboulleux, D., Loucq, C., Radford, A., Savarese, B., Schellenberg, D., Sillman, M. and Vansadia, P. (2012). A phase 3 trial of RTS, S/AS01 malaria vaccine in African infants. *New England Journal of Medicine*, **367**(24):2284-95.

Amaratunga, C., Sreng, S., Suon, S., Phelps, E.S., Stepniewska, K., Lim, P., Zhou, C., Mao, S., Anderson, J.M., Lindegardh, N., Jiang, H., Song, J., Su, X.Z., White, N.J., Dondorp, A.M., Anderson, T.J., Fay, M.P., Mu, J., Duong, S. and Fairhurst, R.M. (2012). Artemisinin-resistant Plasmodium falciparum in Pursat province, western Cambodia: a parasite clearance rate study. *Lancet Infectious Disease*, **12**(11):851-8.

Bharti, A.R., Patra, K.P., Chuquiyauri, R., Kosek, M., Gilman, R.H., Llanos-Cuentas, A. and Vinetz, J.M. (2015). Malaria Diagnosis of malaria infection-a short review of methods. *Australia Journal of Medical Science*, **11**:15.

Bledsoe, G. H. (2005). Malaria primer for clinicians in the United States. *Southern Medical Journal-Birmingham Alabama*, **98**(12):1197.

Caraballo, H. and Kelvin, K. (2014). "Emergency department management of mosquito-borne illness: malaria, dengue, and West Nile virus". *Emergency Medicine Practice*, **16**(5):1-23 .

Centre for Disease Control and Prevention (CDC). (2018). Laboratory Diagnosis of malaria.

Chotivanich, K., Silamut, K. and Day, N.P.J. (2011). Laboratory diagnosis of malaria infection-a short review of methods. *Australia Journal of Medical Science*, **27**:11-15.

Cowman, A. F., Healer, J., Marapana, D. and Marsh, K. (2016). Malaria: biology and disease. *Cell*, **167**(3):610-624.

Cox, F. E. (2010). History of the discovery of the malaria parasites and their vectors. *Parasites & vectors*, **3**(1):1-9.

Crompton, P. D., Pierce, S. K. and Miller, L. H. (2010). Advances and challenges in malaria vaccine development. *The Journal of clinical investigation*, **120**(12):4168-4178.

Dondorp, A. M., Nosten, F., Yi, P., Das, D., Phyo, A. P., Tarning, J. and White, N. J. (2009). Artemisinin resistance in Plasmodium falciparum malaria. *New England journal of medicine*, **361**(5):455-467.

European Medicines Agency. (2019). Mosquirix: Opionion on medicine for use outside EU. https://www.ema.europa.eu/en/opinion-medicine-use-outside-EU/human/mosquirix

Good, M. and Daniel, S. (2021*).* New malaria vaccine :key question answered. *World Economic Forum, https://www.weforum.org/agenda/2021/10/world-s-first-mass-malaria-vaccine-rollout-could-prevent-thousands-of-children-dying/*

Hill, A. V. (2011). Vaccines against malaria. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **366**(1579):2806-2814.

Mackinnon, M.J. and Marsh, K. (2010). *The selection landscape of malaria parasites*. *Science*, **832**:866–71.

Malaria Vaccine Initiative Path. (2013). Accelerating Malaria vaccine development, https://www.malariavaccine.org/

Mayo Foundation for Medical Education and Research (MFMER). (2021). Malaria,https://www.mayoclinic.org/diseases-conditions/malaria/symptoms-causes/syc-20351184

Nicholas, J.W. (2011). "Determinants of relapse periodicity in Plasmodium vivax malaria". *Malaria Journal*, **10**:297

Obeagu, E.I., Ochei, K.C. and Mbah P.C. (2019). Haemolysis associated with malaria infection: Athreat to human existence. World Journal of Pharmaceutical and Medical Research 5 (6): 47- 49.

Obeagu, E.I., Didia, B.C.,,Obeagu, G.U. andAzuonwu, O (2017). Evaluation of changes inhaematological profile of cerebral malaria patients in Enugu State, Southeast, Nigeria. Ann. Clin. Lab. Res. 5 (4): 202.

Obeagu, E.I. and Obeagu, G.U. (2019). Pathological changes associated with Malaria:Haematological Perspective. Int. J. Adv. Res. Biol. Sci. 6(9): 81-82. DOI: http://dx.doi.org/10.22192/ijarbs.2019.06.09.010

Obeagu, E. I., Busari, A. I., Obeagu, G. U., Okafor, C. J., Okpala, P. U., Obi-Ezeani, C. N., Ochiabuto, O. M. T. B., Ofodile, A. C., Ezeoru, V. C., & Ogbonna, L. N. (2021). Gender Based Variations of Haematological Parameters of Patients with Asymptomatic Malaria in Akure, Ondo State, Nigeria. *Journal of Pharmaceutical Research International*, *33*(8), 75-80. https://doi.org/10.9734/jpri/2021/v33i831216

Ogbonna, L. N., Ufelle, S. A., Obeagu, E. I., Okpala, P. U., Esimai, B. N., Agu, C. C., Ibekwe, A. M., & Offie, D. C. (2021). Fibrinogen and C-Reactive Protein Significance in Children Infected by Plasmodium falciparum Species in Enugu, Enugu State, Nigeria. *Journal of Pharmaceutical Research International*, *33*(15), 1-8. https://doi.org/10.9734/jpri/2021/v33i1531280

(Obeagu *et al.*, 2019; Obeagu *et al.*, 2017; Obeagu and Obeagu, 2019; Obeagu *et al.*, 2021; Ogbonna *et al.*, 2021)

Hassan, A.O., Oso, O.V., Obeagu, E.I. and Adeyemo, A.T. (2022). Malaria Vaccine: Prospects and Challenges.Madonna University Journal of Medicine and Health Sciences. 2(2): 22-40

Peter, L. and Jill, S. (2018). *What To Know About Malaria*. https://brainly.in/question/35074053

Roxby, P. (2021). Malaria vaccine hailed as potential breakthrough. https://www.bbc.com/news/health-56858158

Sallusto, F., Lanzavecchia, A., Araki, K. and Ahmed, R. (2010). *From vaccines to memory and back. Immunity*, **33:**451–63.

Schijns, V.E. and Lavelle, E.C. (2011). *Trends in vaccine adjuvants. Expert Review of Vaccines*, **10**:539–50.

Talapko, J., Škrlec, I., Alebić, T., Jukić, M. and Včev, A. (2019). Malaria: The Past and the Present. *Microorganisms*, **7**(6):179.

Tuteja, R. (2007). Malaria - an overview. *The FEBS journal*, **274**(18):4670-4679.

Vaughan, A. M. and Kappe, S. H. (2012). Malaria vaccine development: persistent challenges. *Current opinion in immunology*, **24**(3):324-331.

Welkhoff, P. (2012). What will it take outsmart malaria? https://www.gatesfoundation.org/ideas/articles/malaria-rtss-vaccine

World Health Organization (WHO). (2012). World Malaria Report.

World Health Organization (WHO). (2020). World Health Organisation. Retrieved from World Health Organisation.