REVIEW ARTICLE

Vaccines as a Preventive Modality for Malaria: A Review

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Abstract

Malaria is one among the several parasitic infections that continue to claim lives despite the tremendous progress in the techniques for diagnosis, prevention, and treatment. Although the figures of malaria-ridden patients are decreasing in all the countries due to the collective efforts from all sectors of the society, the tropical countries are still facing severe setbacks in the implementation of the health measures due to the emergence of resistant strains and non-availability of appropriate medications at the required time. An efficient strategy would be to develop vaccines to prevent malaria from occurring than treating it. This review reflects the significant progress made in the field of vaccines and the challenges associated with it. A comprehensive summary is provided for the various diagnostic tests available for detecting malaria and the aspects of treatment related to infections during pregnancy.

Key words: Diagnosis, malaria, parasite, pregnancy, vaccine

INTRODUCTION

A plague of the poor - malaria is a deadly disease caused by parasites.[1,2] In the year 2016, the number of new cases of malaria was reported to touch 216 million, as a result of which the total number of deaths caused by malaria was 4,45,000 worldwide.[3] The highest number of cases and deaths due to malaria occurred in the tropical areas majorly the African region followed by the South-East Asia region and the least affected was the Eastern Mediterranean region. The age group most affected by malaria is children under five. The total number of deaths of the under-five due to malaria was accounted to be 3,03,000 globally, of which 2,92,000 deaths took place in the African region alone.[4]

Globally, the incidence of malaria occurrence has fallen by 30%, and the deaths caused due to malaria have decreased by 40% between the years 2000 and 2013 resulting in 4.3 million lives being saved.[5] The mortality rate has reduced by 58% in the Western Pacific region, by 46% in the South East region, by 37% in the American territories, and finally by 6% in the East Mediterranean region since the year 2010. The mortality rate among the under-five has declined by 35% since 2010. Despite this decrease in the percentage of malaria occurrence and deaths caused by malaria, it remains a threat to the population accounting for the death of one child (under the age of 5 years) every 2 min.[4]

The World Health Assembly has adopted a "Global Technical Strategy for Malaria 2016–2030" in the year 2015, which gives guidance to all the countries in the efforts they are putting in for malaria eradication. This is posed to result in a global reduction of malaria incidences and deaths due to malaria by 90% by the year 2030.[6]

A DISEASE: MALARIA

Causative organisms

Malaria is a deadly disease caused by the parasitic protozoans of the *Plasmodium genus*, commonly transmitted by an infected female *Anopheles* mosquito. When the mosquito bites a human or an animal, the malarial parasites are transferred into the body through the saliva of the mosquito, thus, reaching the liver where the maturation and reproduction

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of the parasites take place. Then, the cells burst in the red blood cells and release more parasites thus infecting the other healthy cells in the human body which eventually results in malaria.[1,2]

There are five species of *Plasmodium* which can cause malaria - *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae,* and *Plasmodium knowlesi*.[7] The dominant species responsible for causing malaria in humans is *P. falciparum*. The incidences of malaria caused by *P. knowlesi* in humans are sporadic.[8]

Symptoms

When a female mosquito bites a human and after the cells burst, within a span of 2–3 days, symptoms of malaria start to appear. The various symptoms likely to occur are fever, chills, sweating, nausea, muscle pain, increased heart rate, shivering, fatigue, and headache. Symptoms such as yellow skin, coma, seizures, or death can be observed in severe cases of malaria infection. The disease may re-occur after several months if it is not treated adequately at the first contact.[9]

Diagnosis

The current malaria policy believes in the early diagnosis and proper treatment of malaria.[2] The two main criteria for the determination of malaria according to the World Health Organization (WHO) are fever and the presence of the *Plasmodium* parasites.[9] There have been several effective but costly antimalarial drugs introduced to treat malaria caused by the resistant parasite,[1,10] but there has not been enough identification of the parasitaemic cases in which these drugs have shown any benefit. Some countries do avail diagnostic programs which are parasite based. Despite the availability of the parasite-based diagnostic programs, a large number of patients with tropical fever are devoid of it. Field microscopy is one of the techniques for the diagnosis of malaria, but it does often meet with the essential requirements (well-organized infrastructure providing proper quality reagents, good quality microscopes, maintenance, preparation of good blood smears, and proper work environment) of a good quality effective microscopy.

Another technique was introduced for use in the places where microscopy was not possible to perform, for the detection of tropical fever, in the early 1990s named lateral flow immunochromatographic assay with the help of which parasitaemic and non-parasitaemic febrile illness were distinguished by a village health worker for the first time till date.[11] The diagnosis using the microscopy[9] method still has the edge over other techniques for the quantification of the malaria parasites.[11,12]

There are several advantages of the microscopic techniques which include the ability to distinguish between the different species of the malaria parasite, quantification of the parasite with sensitivity up to 0.001% and also observation of the asexual stages of the malaria parasites.[13,14] The limitations of the microscopic technique include lack of expertise in analysis of Giemsa-stained blood smears, individualism, chances of non-reproducibility, and also it is time-consuming (it may take >5 min to analyze one slide).[15]

Flow cytometry is an advanced technique used in a higher clinical setup to diagnose malarial infection. It provides higher sensitivity and is less time consuming than microscopy but due to its high cost and labeling process it is not widely used. Micromagnetic resonance relaxometry (MRR) was introduced to overcome the limitation of flow cytometry. It is a label-free technique used for the quantification of the parasite in the blood. The principle of this technique is the detection of the various spin-spin relaxation times of the signals observed in MRR forms the hemozoin particles which were paramagnetic.[12] Raman Spectroscopy is a label-free imaging technique which gives unique and particular Raman fingerprint spectrum of various biological samples. This technique was used to observe what changes took place in the molecular composition of a mice spleen tissue when malaria-infected it as compared to standard or non-infected spleen tissues.[16] Various other techniques are available for the diagnosis of malaria, including as enzyme-linked immunosorbent assay and polymerase chain reaction (PCR). The main limitation of these techniques is that there is a need for expertise in these fields for the analysis of the results. According to reports, there had been the introduction of paper-based analytical devices which proved sensitive and cost-efficient. It was introduced for the early diagnosis of malaria caused due to *P. falciparum* by the detection of the histidine-rich protein - 2 (HRP2) biomarker in the blood.[17]

The detection of the malarial parasites can also be done by rapid diagnostic tests (RDT). The diagnosis of the disease is assisted by the exposure of the human to the endemic region. The level of transmission of the parasites can be correlated with the clinical expression of the *Plasmodium* species infection.[9] These are reliable tests but provide results only qualitatively and are costly with a short half-life. The most recent technique, light emission diode fluorescence microscopy (LED FM), is reliable and could be used in the daily diagnosis. There had been a study carried out to determine the effectiveness of LED FM in the diagnosis of the disease. Acridine orange has been used as the staining agent to stain the blood smears. It was concluded from the study that this technique could be of much use in the clinical practices.[18]

CURRENT TREATMENT MODALITIES

The various drugs used in the treatment of malaria are Artemether, Arteether, Artesunate, Chloroquine, Artemether/Lumefantrine, Sulfadoxine/Pyrimethamine (SP),

Chlorproguanil/Dapsone, Mefloquine, Atovaquone/ Proguanil, Primaquine, Artemisinin, and Quinine.[19]

MALARIA VACCINES

Why a vaccine?

Vaccines are biological preparations which are used for providing active acquired immunity against the disease in question. These are the most cost-efficient means of controlling, preventing, eliminating, and eradicating any infectious disease.[20] For any vaccine to be an ideal means of treatment against any disease, it should develop immune responses against all the strains of the disease and also should be efficient in providing sterile protection for life with a few doses as possible.[21] Various studies have proven that vaccines are a feasible mode of prevention or treatment of malaria disease.[22] Some of the multiple reasons to support the above statement are:

- Immunization of the rodents, monkeys,[23] and humans with the irradiated sporozoites, partially or fully protect them thus preventing the sporozoites to cause an infection in them.[24,25]
- When malaria repeatedly occurs in a human, there is the development of naturally acquired immunity (NAI) which then helps in the protection against the disease.[26] Hence, if a vaccine could be developed reproducing the NAI, humans could be protected from the disease as the vaccine would increase the immunity of the body.
- Immunization studies have proved that the already existing vaccines in hand have the potential to work against the disease in the animal models as well as in the humans.[27]
- The development of transmission-blocking vaccines has also shown that mosquitoes could be protected from getting infected by *P. falciparum* and *P. vivax*.

Types and development of vaccines

There are various approaches to developing a vaccine for malaria in which the vaccines target different life cycle stages of the malaria vector[28] as depicted in Figure 1.

Pre-erythrocytic vaccines

The sporozoites that invade or enter the hepatic cells during the malaria infection are blocked by these vaccines which induce antibodies against the invasion of these sporozoites. The Phase III, clinical trials of the most explored and advanced malaria vaccine, being developed globally, the candidate which is RTS, S/AS01, was completed in the year 2014.[29] It has taken a very long span of 30 years for the development of the first malaria vaccine, RTS, S/AS01, which got its approval from the European Medicines Agency in the year 2015.[30] It was developed by Glaxo Smith Kline and the Program for Appropriate Technology in Health Malaria Vaccine Initiative. It is commercially known as Mosquirix. RTS, S vaccine constitutes a recombinant protein of the sporozoites of the *P. falciparum* along with the surface antigen hepatitis B virus and a proprietary adjuvant.[31] It acts against *P. falciparum* and offers no protection against *P. vivax* malaria. The Phase III trials of this vaccine were conducted at 11 centers in about seven countries in sub-Saharan Africa (one site in Burkina Faso, Gabon, and Malawi and Mozambique, two sites in Ghana and Tanzania, and three sites in Kenya).[30] This study was carried out for over a period of 4 years.[32] The results of the study demonstrated that the cases of clinical malaria were reduced by 28% in the youngsters and 18% in the infants (a follow up of 38 months was done after the administration of the first dose) when a series of 3 treatments (primary dose) is administered to them. Once a dose was administered, a booster dose of RTS, S was administered after 18 months of primary dosing; the number of clinical malaria cases was reduced by 36% in the youngsters (5–17 months) and by 26% in the infants (6–12 weeks).[29] The trials were refused to be done in the infants as the vaccine did not prove highly efficacious in them by the strategic advisory group of experts and the malaria policy advisory committee.[33] In both, the youngsters and the infants, there were adverse effects such as pain or swelling and fever, frequently observed, when they were administered with RTS, S vaccine. The frequency of occurrence of the adverse effects in the control groups was lesser compared to those delivered with the RTS, S vaccine (31% vs. 21% of RTS, S vaccine doses in the infants, and 31% vs. 13% in the youngsters). There were a few severe adverse events also observed, but overall the drug profile was safe and efficacious.[29]

It has been proven long ago that sterilized protection can be achieved by immunizing the body with the bites of the mosquitoes which are infected with radiation-attenuated *P. falciparum* sporozoites (PfSPZ), the reason behind this being the arrest of the sporozoites getting matured in the early liver stage itself.[25,34,35] This observation brought hope that a vaccine could be developed, but it was yet to be tested practically. Recently, a cryopreserved vaccine - PfSPZ vaccine was proven safe after the subcutaneous and intradermal injection as it fulfilled all the regulatory requirements.[36] When this irradiated vaccine was administered intravenously, it showed promised results by achieving a high level of sterile immunization against the controlled human malaria infection with homologous (3D7 clone) of the *P. falciparum* parasites.[5] A novel approach for vaccination was the administration of a combination of the infected PfSPZ along with Chloroquine (antimalarial drug) enabling the maturation of the *Plasmodium* parasites in the liver stage thus reaching the blood to finally eradicate it pharmacologically was used as an alternate way of immunization. This approach was named as the PfSPZ Challenge Vaccine or PfSPZCVac. This approach also led to an increased level of immunization and protection against the disease.[37–39]

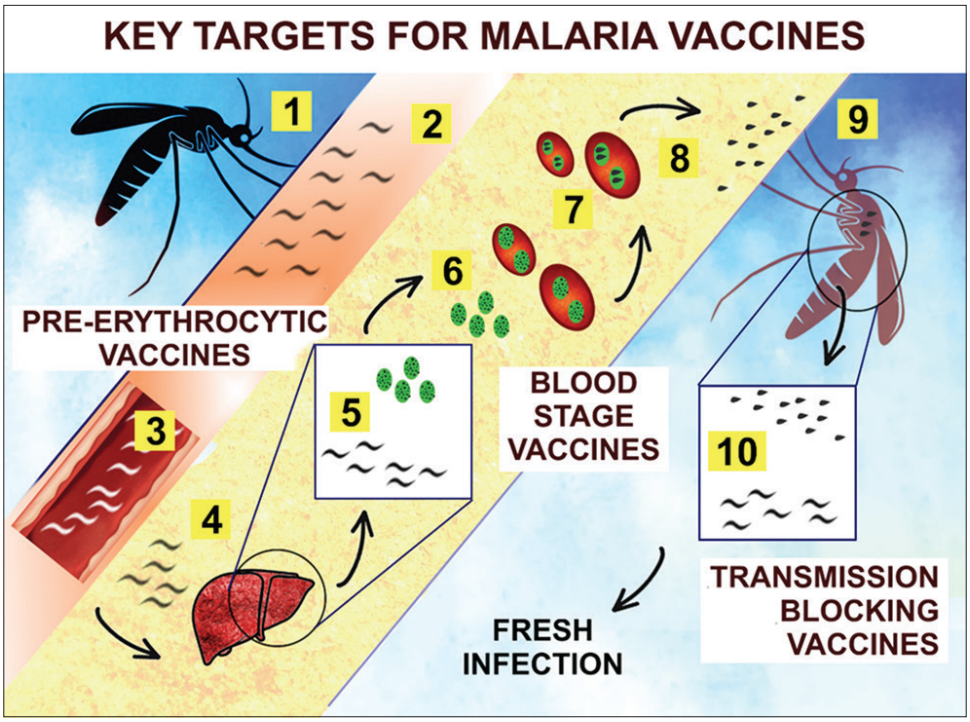

Figure 1: Key main targets for Malaria Vaccines based on the life cycle of malaria parasites (1) bite of a female anopheles mosquito, (2) injection of sporozoites to a healthy human being, (3) movement of the sporozoites in the bloodstream to the (4) liver, (5) development of the sporozoites into merozoites in the liver cells, (6) release of the merozoites into the bloodstream by the vacuoles, (7) infection of the red blood cells (RBCs) and (8) rupture of the RBCs to release of gametocytes which is (9) taken up by a new female anopheles mosquito, and (10) development of the gametocytes into the ookinete, oocyst and then into sporozoites that concentrates in the salivary gland of the mosquito which is injected into human beings beginning a new cycle of the parasitic infection. The symptoms start appearing at the step (7) of the malarial parasitic life cycle

Blood-stage vaccines

The clinical illness associated with the malaria infection is aimed to be minimized by these vaccines.[5] There are various blood-stage vaccine candidates in trials, out of which, most vaccines act on the antigens (highly polymorphic),[40] which are either expressed on the merozoite surface or the proteins which are responsible for the invasion of the parasite in the erythrocytes. The antigen present on the merozoite surface called the merozoite surface protein-1 is the target for these vaccines, and there was an observation made that a bivalent apical membrane protein 1 showed no protective efficacy in the African children.[41,42] It was also observed that there was no efficacy specifically related to strains of the parasite.[43] Later, in the children of Mali, there was a strain-specific efficacy or effectiveness (60% above) observed when the monovalent apical membrane antigen-1[22] vaccine was combined with a potent liposomal adjuvant system (AS0).[44] Despite the pre-erythrocytic vaccines being preferred over the blood stage vaccines as pre-erythrocytic vaccines are successfully emerging, the research in this field is continuous with a promising future.[5]

Transmission-blocking vaccines

These vaccines act on the antigens present in the gamete or different sexual stages of the mosquito which hinders the life cycle of the malaria parasite. The rationale behind the development of this vaccine is to block or interrupt the transmission of malaria in the human population on a large scale. There have been various target antigens tested against malaria caused by *P. falciparum* and *P. vivax* which include Pfs25[45], Pfs230, Pfs47, and Pfs48/45.[46–48] Out of these, Pfs25 was tolerable,[45] but it showed undesirable interactions when combined with the adjuvant montenide ISI 51.[49]

PREGNANCY AND MALARIA

There is a very high risk of occurrence of malaria caused by *P. falciparum* in pregnant women leading to adverse outcomes. The susceptibility of malaria in the pregnant women is very high as during pregnancy, the immunity of the mother decreases.[50] On the global level, there are about 125 million pregnant women who are at risk of malaria, of which 56 million women live in the regions where there is the stable transmission of malaria. These areas pose a threat not only to the mother but also to the newborn baby and thus the reason for problems caused during pregnancy and birth outcomes. There is a reported estimate that annually, there are 75,000–2, 00,000 infant deaths taking place in pregnancy-associated malaria cases.[51] Malaria during pregnancy leads to low birth weight and also an increase in the deaths of the infants. When malaria occurs in a pregnant woman, it can lead

to various conditions such as abortion and pre-term delivery (both due to the release of prostaglandins due to fever caused in malaria),[50] cerebral malaria, intrauterine growth retardation, and fetal, and maternal death due to maternal anemia.[52,53] The placenta in the mother has decidual vessels to which the *P. genus* has a very high liking, so it gets attracted to it highly. Therefore, the occurrence of malaria during pregnancy is very high along with human immunodeficiency virus infection.[50,54] *P. vivax* also causes malaria in pregnancy leading to maternal anemia and low birth weight, but, the severity of the disease is less than that produced by the *P. falciparum* species.[52]

Mechanism of disease progression

The adverse effects observed during malaria in pregnancy are due to the accumulation of the infected erythrocytes (IE) in the placenta of the mother. This accumulation of the IE takes place with the help of VAR2CSA which is a specific variant or variety of the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1). VAR2CSA is expressed on the surface of the IE which is found to be bound to the placental chondroitin sulfate A (CSA) selectively.[51] CSA is a glycosaminoglycan expressed by syncytiotrophoblast which is present on the surface of the villi of the placenta and also on the fibrinoid in the intervillous spaces. The multiplication of the parasites in the placenta can lead to the formation of an inflammatory infiltrate in the spaces between the villi which ultimately leads to severe, maternal anemia, and low birth weight in the newborn babies.[52] Immunity against VAR2CSA is developed naturally with successive pregnancies which help in the protection against the adverse birth outcomes. Thus, development of a vaccine against VAR2CSA could be a beneficial way to decrease the adverse consequences taking place during malaria in pregnancy.[51]

Prevention of malaria in pregnancy

There are various recommendations made by the WHO to prevent malaria during pregnancy including the use of insecticide-treated bed nets (ITNs), efficacious treatment of malaria in the areas where there are more chances of the transmission of malarial *P. falciparum* and administration of intermittent preventive treatment in pregnancy (IPTp) with SP. The administration of IPTp-SP is a therapeutic dose to the mother at the starting of the second trimester. After that subsequent doses are administered such that at least three doses are administered during the tenure of pregnancy. The usage of ITNs and IPTp-SP has resulted in a decrease in malaria associated pregnancy and has also produced an increased birth weight of the newborns.[55]

CHALLENGES TO EFFECTIVE TREATMENT

The public and private institutions have not paid much attention to providing support to the development of various treatments against malaria which is the actual need of the hour. There are a lot of drugs available for the treatment of malaria, but they face many challenges. One of the most significant challenges to the prevention of malaria is the resistance of the mosquito strains to the medicines against it. The other drawbacks of the therapies available currently for malaria prevention are either poorly bioavailable or have an extremely low solubility in both hydrophilic and hydrophobic media.[19] The major challenge in the development of a vaccine for malaria is the complex, multistage, multi-antigen life-cycle of the malaria parasite, and also its genetic variability.[56,57]

INVESTMENTS FOR THE ERADICATION OF MALARIA

The treatment of malaria requires massive investments which adversely affect the economy of the developing countries. According to Gallup and Sachs, the economic burden on the African countries, due to malaria, was estimated to be about US$ 12 billion annually whereas in the years 1965–1990, there was a reduction in the economic growth of the developing countries by 1.3% per person per year due to malaria. According to the WHO, the total investment in malaria prevention and elimination may be estimated up to US$ 101.8 million between the years 2016 and 2030 along with the US$ 673 million investment done in the research and development of the drugs, annually.[6] It is also estimated that, to eliminate malaria by the year 2040, there will be investments up to US$ 90–US$ 120 billion required.[58]

CONCLUSION

Malaria till date is being considered a serious threat to the younger age groups and the pregnant women in addition to the ever-prevailing infection in the adults. Treating the infection seldom results in complete remission of the parasites and lead to renascence making it challenging to treat again. Synergistic to this, the emergence of resistance to the first-line therapy modalities is alarming, and therefore the need of the hour is to develop vaccines against the parasites causing malaria. The vaccines enable the host immunity to work against the parasite and would be a significant player in the eradication of the disease. However, the incompatibility of these vaccines, if any when administered to the young children and during pregnancy needs to be validated and analyzed. Several well-randomized clinical trials are required to be conducted to ascertain the benefits of the vaccines before recommending them to large-scale programs.

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