ejpmr, 2021,8(10), 49-55

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

IMMUNOCHROMATOGRAPHIC TEST FOR MALARIA SCREENING IN SUDANESE BLOOD DONORS – WAD MADANI – GEZIRA STATE

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Article Received on 11/08/2021 **Article Revised on 01/09/2021** **Article Accepted on 22/09/2021**

ABSTRACT

Objectives: To detect the prevalence of malaria parasite among Sudanese blood donors. To detect the antigen of plasmodium Falciparum and plasmodium vivax among Sudanese blood donors. To perform Rapid Diagnostic Test (RDT) for blood donors using SD Bioline Malaria Ag P.f./Pan. **Material and Methods:** Venous blood samples were taken from 100 apparently healthy male donors, aseptically by standard phlebotomy technique by trained phlebotomist from each patient and dispensed in to tri-potassium Ethylenediamine tetra-acetic acid (K3 EDTA) anticoagulant containers about 10-15 minutes after the hemodialysis. **Results:** The study revealed that 3 blood donors (3%) were positive for malaria parasite 2 blood donors with plasmodium Falciparum (2%) and 1 donor with plasmodium vivax (1%). **Conclusion:** The study revealed that a significant number of blood donors with positive rapid diagnostic test.

KEYWORDS: Malaria, Plasmodium falciparum, Plasmodium vivax, Blood transfusion.

INTRODUCTION

The modern transfusion medicine is concerned with proper selection and utilization of blood components. Safe and efficient blood transfusion practice, depends on elimination of clerical errors within the laboratory. Consideration also given to the patients clinical history particularly with respect to pervious transfusion, pregnancy and drugs and a satisfactory pre-transfusion testing to ensure donor-recipient compatibility is essential. About 5% of the general population donates blood. Almost all donations are from volunteers. The first step in the donation process, registration, makes a record of the donor who can be contacted in the future, if necessary. The information requested include, name, sex, date of birth, telephone number, the donor must also sign a consent. Very little whole blood is used, this enables each product to be stored under ideal conditions, prolonging its life and making available the appropriate product for a particular clinical situation to allow proper selection and utilization of blood components. The blood components, red cells, platelets, granulocytes, fresh frozen plasma and cryoprecipitate are made directly from a unit of whole blood.

The major goal of transfusion medicine practice has been to reduce the risk of transfusion transmitted infection to as low level as possible. In order to approach the desired level of zero risk from transfusion of allogeneic blood multiple layers of safety are needed. Methods used in attempting to maximize safety from donated allogeneic units, include donor selection criteria, donor medical history, the confidential unit exclusion (CUE) option, donor deferral registries, laboratory testing of donated units and modification of the blood units after collection either by leucocyte removal or physicochemical procedures for pathogen inactivation.

All blood donors are asked about their medical history to help determine if they can safely donate blood without experiencing any negative health effects.

Malaria is one of the most important parasitic diseases in the world and remains a major challenge to mankind. The disease occurs mostly in tropical and subtropical regions, particularly in sub-Saharan Africa and Southeast Asia.[30] About 90% of all malaria infections in the world occur in Africa South of the Sahara. Majority of infections in the region are caused by Plasmodium falciparum, the most dangerous and the most effective malaria vector of the four human malaria parasites.[31]

The administration of blood to a patient is potentially a lifesaving procedure and the demand for blood has greatly increased over the years.[32] Although this therapy helps to save lives, blood can nonetheless be a vehicle for transmission of infections including parasitic

diseases.[33] Malaria can be efficiently transmitted by transfusion of cellular blood components, and it is undoubtedly responsible for the majority of transfusion transmitted diseases in the world.[34,35]

Induced malaria by blood transfusion was first reported in 1911.[36,37] It is well established that all four human malaria parasites (P. falciparum, P. vivax, P. ovale and P. malariae) may be transfusion-transmitted.[38] The fact that, malarial parasites can survive in red blood cells at refrigerator temperatures (2-6oC) for days or weeks.[39]

When malaria is transmitted through blood transfusion to a non-immunized recipient, it can progress rapidly and may lead to significant morbidity and mortality, specifically when diagnosis is delayed.[40] There are no evidence-based international guidelines for the prevention of transfusion-transmitted malaria (TTM) in sub-Saharan Africa, and there is lack of harmonisation between policies produced by blood safety programmes and policies by malaria programmes.[41] The World Health Organization (WHO) recommends that donated blood should be tested for malaria ‘‘where appropriate and possible’’[41], but there is currently no method for screening blood for low-level parasitaemia that is sensitive, practical and affordable for use by transfusion services in endemic countries.[41] This study aimed to determine the prevalence of malaria parasitaemia in blood transfusion donors in Gezira, Sudan; using rapid diagnostic test (RDT).

Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected female mosquitoes. About 3.2 billion people – almost half of the world’s population – are at risk of malaria. Young children, pregnant women and non-immune travelers from malaria-free areas are particularly vulnerable to the disease when they become infected. Malaria is preventable and curable, and increased efforts are dramatically reducing the malaria burden in many places. Between 2000 and 2015, malaria incidence (the rate of new cases) fell by 37% globally.

In that same period, malaria death rates fell by 60% globally among all age groups, and by 65% among children under 5. Sub-Saharan Africa carries a disproportionately high share of the global malaria burden. In 2015, the region was home to 89% of malaria cases and 91% of malaria deaths. Malaria is caused by Plasmodium parasites. The parasites are spread to people through the bites of infected female Anopheles mosquitoes, called "malaria vectors.” Plasmodium falciparum is the most prevalent malaria parasite on the African continent. It is responsible for most malaria-related deaths globally. P. vivax has a wider distribution than P. falciparum, and predominates in many countries outside of Africa.

Life cycle

In the life cycle of Plasmodium, a female Anopheles mosquito (the definitive host) transmits a motile infective form (called the sporozoite) to a vertebrate host such as a human (the secondary host), thus acting as a transmission vector. A sporozoite travels through the blood vessels to liver cells (hepatocytes), where it reproduces asexually (tissue schizogony), producing thousands of merozoites. These infect new red blood cells and initiate a series of asexual multiplication cycles (blood schizogony) that produce 8 to 24 new infective merozoites, at which point the cells burst and the infective cycle begins anew.[1] Other merozoites develop into immature gametocytes, which are the precursors of male and female gametes. When a fertilised mosquito bites an infected person, gametocytes are taken up with the blood and mature in the mosquito gut. The male and female gametocytes fuse and form an ookinete—a fertilized, motile zygote.

Ookinetes develop into new sporozoites that migrate to the insect's salivary glands, ready to infect a new vertebrate host. The sporozoites are injected into the skin, in the saliva, when the mosquito takes a subsequent blood meal.[1]

Only female mosquitoes feed on blood; male mosquitoes feed on plant nectar, and do not transmit the disease. The females of the Anopheles genus of mosquito prefer to feed at night. They usually start searching for a meal at dusk, and will continue throughout the night until taking a meal.[2] Malaria parasites can also be transmitted by blood transfusions, although this is rare.[3]

PATHOPHYSIOLOGY

Malaria infection develops via two phases: one that involves the liver (exoerythrocytic phase), and one that involves red blood cells, or erythrocytes (erythrocytic phase). When an infected mosquito pierces a person's skin to take a blood meal, sporozoites in the mosquito's saliva enter the bloodstream and migrate to the liver where they infect hepatocytes, multiplying asexually and asymptomatically for a period of 8–30 days.[4] After a potential dormant period in the liver, these organisms differentiate to yield thousands of merozoites, which, following rupture of their host cells, escape into the blood and infect red blood cells to begin the erythrocytic stage of the life cycle.[4] The parasite escapes from the liver undetected by wrapping itself in the cell membrane of the infected host liver cell.[5]

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.[4] 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. After a period of dormancy, they reactivate and produce merozoites. Hypnozoites are responsible for long incubation and late relapses in P. vivax infections,[4] although their existence in P. ovale is uncertain.[6] The parasite is relatively protected from attack by the body's immune system because for most of its human life cycle it resides within the liver and blood cells and is relatively invisible to immune surveillance. However, circulating infected blood cells are destroyed in the spleen. To avoid this fate, the P. falciparum parasite displays adhesive proteins on the surface of the infected blood cells, causing the blood cells to stick to the walls of small blood vessels, thereby sequestering the parasite from passage through the general circulation and the spleen.[7] The blockage of the microvasculature causes symptoms such as in placental malaria.[9] Sequestered red blood cells can breach the blood–brain barrier and cause cerebral malaria.[10]

Symptoms

Malaria is an acute febrile illness. In a non-immune individual, symptoms appear 7 days or more (usually 10–15 days) after the infective mosquito bite. The first symptoms – fever, headache, chills and vomiting – may be mild and difficult to recognize as malaria. If not treated within 24 hours, P. falciparum malaria can progress to severe illness, often leading to death.

Children with severe malaria frequently develop 1 or more of the following symptoms: severe anaemia, respiratory distress in relation to metabolic acidosis, or cerebral malaria. In adults, multi-organ involvement is also frequent. In malaria endemic areas, people may develop partial immunity, allowing asymptomatic infections to occur.[12]

Who is at risk?

In 2015, approximately 3.2 billion people – nearly half of the world's population – were at risk of malaria. Most malaria cases and deaths occur in sub-Saharan Africa. However, Asia, Latin America, and, to a lesser extent the Middle East and parts of Europe, are also at risk. In 2015, 97 countries and territories had ongoing malaria transmission. Some population groups are at considerably higher risk of contracting malaria, and developing severe disease, than others. These include infants, children under 5 years of age, pregnant women and patients with HIV/AIDS, as well as non-immune migrants, mobile populations and travelers. National malaria control programmes need to take special measures to protect these population groups from malaria infection, taking into consideration their specific circumstances.[12]

Disease burden

According to the latest WHO estimates, released in September 2015, there were 214 million cases of malaria in 2015 and 438 000 deaths. Between 2000 and 2015, malaria incidence fell by 37% globally; during the same period, malaria mortality rates decreased by 60%. An estimated 6.2 million malaria deaths have been averted globally since 2000. Sub-Saharan Africa continues to carry a disproportionately high share of the global malaria burden. In 2015, the region was home to 89% of malaria cases and 91% of malaria deaths.

Some 15 countries – mainly in sub-Saharan Africa – account for 80% of malaria cases and 78% deaths globally. Since 2000, the decline in malaria incidence in these 15 countries (32%) has lagged behind that of other countries globally (54%).

In areas with high transmission of malaria, children under 5 are particularly susceptible to infection, illness and death; more than two thirds (70%) of all malaria deaths occur in this age group. Between 2000 and 2015, the under-5 malaria death rate fell by 65% globally, translating into an estimated 5.9 million child lives saved.[15]

Transmission

In most cases, malaria is transmitted through the bites of female Anopheles mosquitoes. There are more than 400 different species of Anopheles mosquito; around 30 are malaria vectors of major importance. All of the important vector species bite between dusk and dawn. The intensity of transmission depends on factors related to the parasite, the vector, the human host, and the environment. Anopheles mosquitoes lay their eggs in water, which hatch into larvae, eventually emerging as adult mosquitoes. The female mosquitoes seek a blood meal to nurture their eggs. Each species of Anopheles mosquito has its own preferred aquatic habitat; for example, some prefer small, shallow collections of fresh water, such as puddles and hoof prints, which are abundant during the rainy season in tropical countries.[15]

Transmission is more intense in places where the mosquito lifespan is longer (so that the parasite has time to complete its development inside the mosquito) and where it prefers to bite humans rather than other animals. The long lifespan and strong human-biting habit of the African vector species is the main reason why nearly 90% of the world's malaria cases are in Africa.

Transmission also depends on climatic conditions that may affect the number and survival of mosquitoes, such as rainfall patterns, temperature and humidity. In many places, transmission is seasonal, with the peak during and just after the rainy season. Malaria epidemics can occur when climate and other conditions suddenly favour transmission in areas where people have little or no immunity to malaria. They can also occur when people with low immunity move into areas with intense malaria transmission, for instance to find work, or as refugees.

Human immunity is another important factor, especially among adults in areas of moderate or intense transmission conditions. Partial immunity is developed over years of exposure, and while it never provides

complete protection, it does reduce the risk that malaria infection will cause severe disease. For this reason, most malaria deaths in Africa occur in young children, whereas in areas with less transmission and low immunity, all age groups are at risk.[15]

Prevention

Vector control is the main way to prevent and reduce malaria transmission. If coverage of vector control interventions within a specific area is high enough, then a measure of protection will be conferred across the community. WHO recommends protection for all people at risk of malaria with effective malaria vector control. Two forms of vector control – insecticide treated mosquito nets and indoor residual spraying – are effective in a wide range of circumstances.[41]

Diagnosis

Malaria is usually confirmed by the microscopic examination of blood films or by antigen-based rapid diagnostic tests (RDT).[8],[9] Microscopy is the most commonly used method to detect the malarial parasite—about 165 million blood films were examined for malaria in 2010.[10] Despite its widespread usage, diagnosis by microscopy suffers from two main drawbacks: many settings (especially rural) are not equipped to perform the test, and the accuracy of the results depends on both the skill of the person examining the blood film and the levels of the parasite in the blood. The sensitivity of blood films ranges from 75–90% in optimum conditions, to as low as 50%. Commercially available RDTs are often more accurate than blood films at predicting the presence of malaria parasites, but they are widely variable in diagnostic sensitivity and specificity depending on manufacturer, and are unable to tell how many parasites are present.[10]

In regions where laboratory tests are readily available, malaria should be suspected, and tested for, in any unwell person who has been in an area where malaria is endemic.

In areas that cannot afford laboratory diagnostic tests, it has become common to use only a history of fever as the indication to treat for malaria—thus the common teaching "fever equals malaria unless proven otherwise". A drawback of this practice is over diagnosis of malaria and mismanagement of non-malarial fever, which wastes limited resources, erodes confidence in the health care system, and contributes to drug resistance.[11] Although polymerase chain reaction-based tests have been developed, they are not widely used in areas where malaria is common as of 2012, due to their complexity.[12]

Treatment

Malaria is treated with antimalarial medications; the ones used depends on the type and severity of the disease. While medications against fever are commonly used, their effects on outcomes are not clear.[13] Uncomplicated malaria may be treated with oral medications. The most effective treatment for P. falciparum infection is the use of artemisinins in combination with other antimalarials (known as artemisinin-combination therapy, or ACT), which decreases resistance to any single drug component.[14] These additional antimalarials include: amodiaquine, lumefantrine, mefloquine or sulfadoxine/pyrimethamine.[15]

Another recommended combination is dihydroartemisinin and piperaquine.[16][17] ACT is about 90% effective when used to treat uncomplicated malaria.[19] To treat malaria during pregnancy, the WHO recommends the use of quinine plus clindamycin early in the pregnancy (1st trimester), and ACT in later stages (2nd and 3rd trimesters).[18] In the 2000s (decade), malaria with partial resistance to artemisins emerged in Southeast Asia.[20][21] Infection with P. vivax, P. ovale or P. malariae is usually treated without the need for hospitalization. Treatment of P. vivax requires both treatment of blood stages (with chloroquine or ACT) and clearance of liver forms with primaquine.[22]

Recommended treatment for severe malaria is the intravenous use of antimalarial drugs. For severe malaria, artesunate is superior to quinine in both children and adults.[24] Treatment of severe malaria involves supportive measures that are best done in a critical care unit. This includes the management of high fevers and the seizures that may result from it. It also includes monitoring for poor breathing effort, low blood sugar, and low blood potassium.[23]

Prognosis

When properly treated, people with malaria can usually expect a complete recovery.[25] However, severe malaria can progress extremely rapidly and cause death within hours or days.[26] In the most severe cases of the disease, fatality rates can reach 20%, even with intensive care and treatment. Over the longer term, developmental impairments have been documented in children who have suffered episodes of severe malaria.[27] Chronic infection without severe disease can occur in an immune-deficiency syndrome associated with a decreased responsiveness to Salmonella bacteria and the Epstein–Barr virus.[28]

During childhood, malaria causes anemia during a period of rapid brain development, and also direct brain damage resulting from cerebral malaria.[27] Some survivors of cerebral malaria have an increased risk of neurological and cognitive deficits, behavioral disorders, and epilepsy.[29] Malaria prophylaxis was shown to improve cognitive function and school performance in clinical trials when compared to placebo groups.[27]

MATERIAL AND METHODS

Study area

The study was carried out in the Central blood bank, Wad Medani teaching hospital. Wad Medani is the

capital of Gezira state, it is considered one of the largest states in Sudan with an area of 35.304 km and population of 4.5 millions. The Central Blood Bank provide blood donation services to 7 governmental hospitals and other special hospitals in Wad Medani. About 1600 to 1700 donors attend the central blood bank monthly. Different types of blood components (whole blood, packed red cells, platelets, fresh frozen plasma) are prepared from whole blood using large refrigerated centrifuges. All donors are selected according to the accepted criteria for donation including age, weight, physical and medical examination and screening for viral infections (hepatitis B, C and HIV) and the test for syphilis.

Study population: All blood donors.

Study design: Descriptive, prospective cross sectional study was conducted in wad medani central blood bank, during the period from 16\10\2017 to 16\11\2017.

Methods

Sample collection: A total of 100 adult male blood donors were screened for malaria parasite. This analysis was conducted at the Wad Medani central blood bank. Venous Blood samples were taken from an antecubital vein by a 5ml syringe. The site of collection was cleaned using 70% alcohol and left to dry. 2.5 ml of blood was taken into a container with 0.05ml (K2 EDTA) as an anticoagulant with a concentration of 1.5- 2.2 mg/ml and then the sample gently mixed. The blood samples were tested immediately using SD Bioline Malaria Antigen P.f./Pan (HRP-II P.f and pLDH P.v). The test was performed for the detection of plasmodium falciparum and vivax according to manufacturer's instructions, in brief, whole blood was added into sample wells and an assay buffer were added into assay buffer wells. The blood -buffer mixture were allowed to run toward the test and control window.

RESULTS AND DISCUSSION

Result

The study included 100 blood transfusion donors. All donors were male; their median age was 25.4 years with minimum age of 20 and maximum age of 38 years. All blood samples were tested for the presence of malaria parasites antigen by RDT. Malaria parasites antigen were detected in 3 blood donors (3%), 2 of them were positive for plasmodium falciparum antigen (2%) and 1 blood donor was positive for plasmodium vivax (1%).

Table: 1 frequency of malaria parasitaemia among donors by Rapid Diagnostic.

Plasmodium falciparum frequency (%)	Vivax plasmodium species frequency (%)	Total frequency (%)
2 (2)	1 (1)	3 (3)

DISCUSSION

Malaria can be transmitted by inoculation of blood from infected donor to patient.[33] In certain geographical locations (endemic areas), transmission of malaria by blood transfusion poses a real threat.[34] However, in non-endemic areas transmission of malaria by blood transfusion is an uncommon complication of blood transfusion, but because of the delay in diagnosis, it may have a relatively high fatality rate particularly in pregnant women, splenectomized and immuno-compromised patients.[35] Worldwide, different strains of malaria species viz Plasmodium falciparum, Plasmodium vivax, Plasomdium malriae and Plasomdium ovale are responsible for transfusion-transmitted disease for the capability of plasmodia to survive well in stored blood and even in frozen blood.[31]

Manson reported that P. falciparum was present in the blood of donors who were exposed to malaria 20 months to three years previously[36] Plasmodium vivax has been transmitted by transfusion from donors infected four years previously and P. malariae from donors infected 17 years earlier[37] Moreover, McClure and Lam (1945) reported that quartan malaria (P. malariae) has been accidentally transmitted in blood previously stored for 5 days[38] and in another case reported by Black (1960), a donor had carried P. malariae parasites for 19 years.

Screening blood donors for malaria (as recommended by the WHO) is currently not included in the protocols of Sudanese Blood Banks. This risk is worsened by the facts that absence of symptoms even for a long period does not necessarily ensure lack of infectivity and malaria parasites survive well in stored blood.

The detection of malarial antigen with RDTs was originally intended as a more rapid and objective alternative to direct microscopy[36] RDTs detect Plasmodium-Specific parasite proteins, such as pan-malarial lactate dehydrogenase (pLDH), and P. falciparum specific histidine-rich protein 2 (HRP2). Most of these assays are in a 'dipstick' format that can be used with minimal training, are field applicable, and provide a result within 10-20 minutes.

CONCLUSION

1. The prevalence of infected asymptomatic blood donors is moderately considerable.
2. The hazard of transmitting malaria through blood transfusion is much greater than the cost of testing donors.
3. Although Sudan is now considered to be an endemic area for malaria, the present effort will eventually lead to eradication of the disease. Screening blood donors for malaria will help in this effort and in the future will become an important factors in continuous control of the disease, and will lead to reduction of transfusion-induced malaria and does not affect the donation flow negatively.

Recommendations

1. Alternative technique (PCR, simple blood film) will be considered.

2. The screening of blood donors for malaria prior to donation is mandatory.

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