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Malaria is still a life threatening disease review

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Abstract
Malaria is one of the most severe life threatening disease worldwide. It is a leading cause of death and disease in many developing countries, where young children and pregnant women are the groups most affected. In fact, new antibiotic and possible vaccine are needed but the importance of effective anephelos mosquitoes control and education of the community is compulsory. Malaria kills 3000 children everyday world wide. In Pakistan, half a million malaria cases occur annuall are more affected in Khyber pakhtunkhwa provinces. Malaria is cause of estimated 50000 deaths each year worldwide mostly in infants, children and pregnant women, he added. In countries where Malaria is common, women are 4 times more likely to suffer malaria attacks during pregnancy resulting in low weight babies and stillbirths. Moreover, nearly 60% of miscarriages in hyper endemic areas are also due to malaria. Morocco a Muslim country remarkably reduced malaria cases to 00% Vector control measures; strong political will and Community participation are main way to reduce malaria transmission at the community level. It is the only intervention that can reduce malaria transmission from very high levels to close to zero. Malaria in Pakistan was mainly caused because of a lack of awareness and improper sewerage system. For this, a proper method of prevention and treatment should be designed which not only provide benefit to the patient and facilitate the physician but also provide an important socioeconomic benefit to everyone.

Keywords: Malaria, life threatening, mosquitoes

Introduction
The word malaria is a combination of two Italian words “Mala” means bad and “Aria” means the air [1]. It was the belief of that time (in 1753) that the bad air transmits the disease, inhalation of poisonous gases emanating from a marshy place was supposed to be the chief factor [2]. About Seven Million years ago, Malaria was identified as one of the most serious and deadliest among blood infections, particularly found in Chimpanzees, but in few hundred thousand years it was observed that malaria not only attacks on chimpanzees but also targets human beings swell [3]. Malaria is documented in a Chinese medical document Nei Ching (*Canon of the drugs*) about 2700 BC. Nei Chingwas abbreviated by Chinese *royal leader “Huang Ti” in which several clinical signs and symptoms were named as Malaria*. In Fourth century BCE Malaria killed a large number of populations in Greece, hence it was hot issue of that time [4]. Ancient Greeks starts studies on Malaria. Empedocles of Agrigentum in 550 BC, Homer in 850BC while Hippocrates in 400 BC became conscious of the Malarial Signs and symptoms like Poor Health of the patients living in Marshy places, Enlargement of the Spleen and fevers etc. [5] Malaria was then called “fever and ague” in United States, because that time early American people were unaware about the parasites that transmits malaria [6]. USA has no historical roll in the tragedy of Malaria. It was estimated that in USA Malaria incidence was very high in 1875. But after 1914 more than 600,000 occurs per year. During Vietnam War and the World War II, Malaria was a serious issue in USA military campaigns. US military lost more time due to malaria than to ammunition and bullets [6].

In 1880 a French military doctor Charles Louis Alphonse Laveran examined the blood of a human being infected with Malaria and found Parasites in Red Blood Cells. For his discovery he was rewarded in 1907 with Nobel Prize [4]. An Italian neurophysiologist Camillo Golgi (discoverer of the Golgi Bodies in the cell) suggested that the parasites (merozoites) that reside in the RBCs, upon maturity produce a large number of new parasites which are releases in the bloodstream. He also observed that there are about 2 types of the malaria infection, one with the fever of every other day (tertian periodicity) and a new with the fever of every 3rd day

(quartan periodicity) In 1890 Raimondo Filetti and Giovanni Batista Grassi introduced the terms *Plasmodium malariae* and *Plasmodium vivax* for two of the malarial parasites which attacks on human [4] Laveran believed that there was only one specie that infects human being and named it as Oscillariamalariae but later on in 1897 William H. Welch identified malignant tertian malarial parasite and named it as *Plasmodium falciparum*. In 1922 *Plasmodium knowlesi and Plasmodium ovale were identified by* Biraj Mohan Das Gupta and Robert Knowles in long-tailed macaques (Monkeys). In 1965 the first case of human infection with *Plasmodium knowlesi was reported. Later on in an experiment* Giuseppe Bastianelli and Amico Bignami collected mosquitos of the specie *Anopheles claviger and they were fed by Plasmodium. As a result, an absolute* sporogonic round of *Plasmodium malariae, P. vivax* and *P. falciparum was observed.* In 1899, infected mosquitoes that were fed by a patient in Rome, they were sent to London where they fed on 2 volunteers, as a result of which both of them were infected by malaria [4]

Malarial Parasite

A Single cell protozoon parasite which belongs to genus Plasmodium is the main cause of malarial infection. More than one hundred species of Plasmodium exists which cause malaria in humans, birds, reptiles and in chimpanzees' as well. There are about four species of plasmodium that infects human beings i.e. *Plasmodium vivax, Plasmodium falciparum*, *Plasmodium malariae* and *Plasmodium ovale* [7]. Moleculer studies revealed that *Plasmodium vivax* is derived from an antientparqaa site of macaque from Africa [8]. All the Malarial parasites have different morphologies and appearances, and can be differentiated from one another. They also cause different clinical symptoms [9]. In high endemic areas there are many chances of multiple infections. At the same time two or more than two species of a parasite may attack a single host [9]. Among all plasmodium species, Infection with the *Plasmodium falciparum* is deadliest because of its severity of infection and serious complications [10, 11]. It causes life threatening fever that leads to high level mortality in a population [9]. Any stage of the Red Blood Cells can be easily invaded by *Plasmodium Falciparum* which results in high parasitemia that makes it a virulent Parasite. Cerebral malaria, black water fever, non-cardiopulmonary edema, lower Glucose level, Anemia and acute renal failure are the most serious clinical manifestations that are linked with the infection of *P. falciparum* [10, 11]. The multiplication of the parasite is so high that they become twenty fold just in two days [12]. Plasmodium vivax is the 2nd most common parasite that is distributed worldwide and comparatively less severe than P. Falciparum P. vivax is mostly found in tropical areas especially *in entire Asia* [13]. P. vivax affects about 75 million people worldwide. Most of the cases are in S. America and Asia [9]. *Relapse cases of the Plasmodium vivax can occur up to 3 years* [13]. Infection of the Plasmodium ovale is very rare, but sometimes found in W. Africa. Plasmodium malariae *is eliminated from temprate regions, a person who is infected with the* Plasmodium malariae may be *asymptomatic (show no symptoms), and the* Plasmodium malariae can stay in the blood for *decades without any malarial signs and symptoms* [13]. In Pakistan only two species of Plasmodium are dominant i.e. Plasmodium falciparum and Plasmodium vivax [14].

Vector of the Malarial Parasite

In 1897 a British officer Ronald Ross studied on birds and mosquitoes, and suggested that malaria can be transferred from infected bird to healthy bird through Anopheles mosquito. This study revealed the secrets of the life cycle of the parasite that a part of the life of Plasmodium is spent in mosquitoes, later on it was termed as Sporogonic Cycle. Ronald Ross was then rewarded by Nobel Prize in 1902for his discovery [4]. Major vectors for the transmission of Plasmodium are *Anopheles funestus, A. bwambae, A. nili, A. merus, A. arabiensis and A. gambiae*. About six other species in *A. gambiae* also act as vectors of malarial infection in South and Eastern Africa [15, 16]. Genus Anopheles of mosquitoes is an important vector of plasmodium transmission on islands and coastal areas of Southeast Asia. Molecular studies showed a large number of polymorphism in *A. funestus*, *An. Gambiae* and *A. arabiensis* [18].

Vectors of Malarial parasites in Pakistan

Studies were conducted on the Plasmodium vectors of Pakistan which resulted that there are about 24 species of anopheles mosquitoes including *A. sergenti, A. pulcherrimus, A. subpictus, A. dthali, A. culicifacies, An. pallidus, A. turkhudi, A. annularis, A. fluviatilis, A. stephensi, A. superpictus, A. multicolor, A. willmori, A. lindesayi, A. moghulensis, A. theobald, A. maculates, A. claviger, A. gigas, A. barianensis, A. splendidus, A. barbirostris, A. nigerrimus. A. peditaeniatus, A. culicifacies and A.* stephensi are the species that gain resistance to many of the insecticides including Dieldrin, DDT, carbamates and organophosphates (malathion, fenitrothion) [19]. Primary vector species in Pakistan are *A. culicifacies* and *A. stephensi* [20-23]. In Khyber Pakhtunkhwa province of Pakistan, a large number of *A. stephensi and A. culicifacies* were reported as vectors of plasmodium [24]. But the number of *A. stephensi* is much more then *Anopheles culicifacies. Anopheles culicifacies* is dominant in Punjab province of Pakistan. It is more active before September while it disappears after the month of September [20-24]. *An. stephensi* is a sub-tropical species which is distributed in the entire the middle east and South Asia. In Arid and Semi-arid zone of Rajasthan and Gujarat *A. subpictus* Grassi were found to be more common species [25]. *An. stephensi* is an exclusive vector of malaria in Pakistan, India and in Afghanistan. It has an amazing characteristic of proficient breeding in underground water reservoirs mostly in urban areas [26].

Epidemiology

Malaria is a tropical disease which worldwide distributed mostly in tropical areas, in entire Sub-Saharan Africa and in South Africa, South America Southeast Asia, India, Central America and Pacific Islands. Pakistan is located in the middle of the malaria belt around the globe encompassing tropical and subtropical areas [27]. Malaria cause approximately 8, 63,000 deaths in 2008 and About 243 million malaria cases are reported every year [28]. *About 1.2 billion populations of the Pacific countries and South East Asia are at higher risk of malarial transmission, representing about one third of total world population* [29, 14]. There are about 109 of malaria-endemic countries, where about 3.3 billion people are at risk for malaria. 50% of global population at risk for malaria, whereas 90% deaths in Africa are caused by Malaria in which 5.85% are children [30]. *Plasmodium vivax* is chiefly a widespread cause of malaria in Asia and Central and South America and in some parts of [31]. Probable figure of yearly malaria episodes in Pakistan is 1.5 million [28]. *Plasmodium vivax is internationally distributed and is the chief species in many countries* [29, 14] 2.6 billion Population is at risk of vivax infection throughout the world [31]. *Infection by Plasmodium*

vivax is generally regarded as benign malarias with low mortality level [29, 14]. On the other hand, the rate of infection of the *P. falciparum* is comparatively narrower than that of *Plasmodium vivax but it is the serious cause of* mortalities in Pakistan. In 2005, *falciparum* malaria was one of the 33 percent of reported definite malaria cases, this rate reduced to 24 percent in 2008 [28].

In 2004 the number of malaria cases were 103,416, in 2006 they were 124910, in 2007 they were 128570 while in 2008 the number of malaria cases were 104454 (WHO, 2005). Pakistan is a tropical country with the improper irrigation and dumping of garbage system and other wastes. Majority of the population of Pakistan live in rural areas where there is unhygienic environment, all these factors leads to malariogenic potential [29]. Because of a countrywide malaria eradication fight in 1961, malaria was almost eradicated in Pakistan for the period of 1960s with 9500 positive cases in 1967 but due to Financial and administrative crises and political disturbances Malaria again arouse in 1970s reaching epidemic level in 1972-73 [30]. It is estimates that the rate of malarial infections is enlarged by 40 percent in the middle of 1970 and 1997 in sub-Saharan Africa. [31] While the number of deaths due to malaria worldwide has decreased from 985,000 in 2000 to 781,000 in [32]. To deal with this infection, public health personnel selected cautiously many avoidance methods appropriate to a exacting area or environment [6] Latest studies reveled that there were 225 million cases of malarial infection and an estimated 781,000 deaths reported in 2009 [33].

About 300-500 million people infected with the malaria infection while 1.1 - 2.7 million deaths occur worldwide annually. Children under age group <5 years are more susceptible for the mortality rate caused by malarial infection. In 2004, 20 percent deaths of the children were reported [33]. In Eastern Mediterranean Region (EMR), there were 5.7 million confirmed malaria cases, of which 17% cases were registered in Pakistan [34]. In 2010, Due to flood estimated cases reported from Pakistan [35]. In 2003 Malaria control program stated serious outbreaks in many districts. This may clarify the elevated rates of authenticated cases in 2002 and 2003 in KP. By 2005 guidance had been imparted in 16 districts of KP. Improved investigative techniques (diagnostic tools) could have condensed false identification, and afterward report for the decrease in figure of confirmed malaria cases after 2003, and the outbreaks could have been controlled [36].

Malaria in Afghan Refugees

Malaria became a major health problem during emergency in Afghanistan [37]. During soviet war in 1979, more than three million Afghani peoples migrated to Pakistan. Pakistan government settled them all in more than two hundred camps located at the western border. Prevalence of malarial parasites among Afghan refugees is much higher as compare to native population of the Pakistan. Afghanis settled in the Khyber Pakhtunkhwa are at higher risk of malarial infection which can be transfer to the native population. It is fact that Afghan refugees are very badly treated in Pakistan, afghan camps are mostly waterlogged and having no proper drainage system hence they provide a best site for mosquitoes breeding [38]. After migration poor Afghan people were unable to prevent themselves from mosquitos as they were unable to but mosquito nets and repellents [39]. Hence they were more susceptible to malarial infection [38]. About 150000 malaria cases were registered in different afghan refugee camps in 1990 United Nations High Commission for Refugees (UNHCR) [40]. During migration of Afghanis people they transfer many of the malaria infection from Afghanistan to Pakistan [41].

Transmission of the parasite

Ronald Ross discovered in 1897 that the avian malaria parasite *Plasmodium relictum transmitted by* culicine mosquitoes. He suggested that human malaria may also be transmitted my mosquitoes. After his study and research then he proved that human malaria is indeed transmitted by anopheline mosquitoes. It was proved by many of the Italian scientist. Ross was a well-known military medical doctor he was operational in India till 1894 he didn't believed that a parasite is the cause of malarial infection that resides in blood, but Manson proved him with the malarial positive slides that this infection is actually a blood infection that is transmitted by blood meal of female *Anopheles* mosquito [5]

Life Cycle

Asexual Stage

Asexual stage begins when a female *Anopheles* mosquito (infected with malaria) bites a healthy human being and infuses the sporozoites in the bloodstream of that healthy person. These sporozoites move in the hepatic circulation and attack the liver cells [5].

Incubation Period

Incubation Period of the *Plasmodium vivax & P. ovale* is 14 days, *P. falciparum* 12 days while *P. malariae* has 30 days [4].

Exoerythrocytic Schizogony

In liver cells sporozoites undergoes multiple rounds of asexual division as a result of which many uninucleated merozoites are produced. These merozoites released in the general circulation and attack on Red Blood Cells [5]

Erythrocytic Schizogony

Merozoites invade RBCs and each merozoite undergoes second phase of asexual multiplication as a result of which 8-16 new merozoites are produced in each RBC. Each new merozoite again invades a new RBC, this cycle continues and clinical manifestations (fever, Headache, Anemia) are appeared [5]

Gametogony

Some of the young merozoites are transformed into gametocytes (male and female) that flow in the peripheral bloodstream until they are gulp by a female anopheles' mosquito during blood meal [5]

Sexual Stage

In the gut of the mosquito ex-flagellation of the male gametocytes takes Place. Then both the male and female gametocytes become fuse together and converted in to a motile zygote. Zygote is then transformed into ookinete [5]. The ookinete then penetrate the wall of the gut and converted in to a conspicuous oocyst, which is then converted in to sporocyst. Sporocyst results in the development of sporozoites which migrate towards the salivary glands of female *anopheles* mosquito and are ready to be infuse in to the new host during blood meal of the mosquito [5] The life cycle of the plasmodium within the mosquito is about 8-35 days, after [4] Sometime the malarial parasites become dormant in the host body, show no signs and symptoms (Hypnozoite stage)

parasite wakes up suddenly and cause attacks of malarial fever [42]. Human malaria is caused by any species of the plasmodium [43]. But In spite of human the parasitic *Plasmodium* species also infect rodents, birds, monkeys, chimpanzees and reptiles [44]. Number of White Blood Cells becomes low in the malaria infection [45].

Symptoms

Clinical symptoms and manifestations appear in the asexual stage of the parasite, mostly in erythrocytic cycle when the parasite reproduces in Red Blood Cells. Parasite count in the blood is directly proportional to the clinical presentations [46]. Sometime the parasite remains inactive in the human body, intemperate areas one out of five cases of *Plasmodium vivax* began relapse in a year after mosquito bite, which is called the hypnozoite stage of the parasite [47]. Neutrophils are activated in the infection of *Plasmodium falciparum* which results in endothelial damage and organ failure [48]. Other clinical presentations in the Malaria infection includes Temperature, Headache, juindus, enlargement of spleen and liver, thrombocytopenia (platelets deficiency), diarrhea, apnea, respiratory distress [49]. Hemolysis or cytokine disturbances and decreased rate of RBC production and maturation which leads to anemic condition or dyserythropoiesis (defective development of erythrocytes) [50]. Enlargement of spleen may lead to death of an individual [51]. Plasmodium infection is a major cause of pediatric anemia in malaria patients. During erythrocytic cycle when RBCs are under attack, Hemoglobin concentration becomes lower due to RBC destruction and also due to the removal of parasitised and non-parasitised RBCs [52]. When the Haemoglobin level become less or equal then 11.0 gm/dl then this condition may be termed as mild anemia [52]. This criterion was used 1995 to investigate the anemia burden [53]. When the Hb level becomes 7-8 gm/dl, that condition is called moderate anemia while if it is less than 5 gm/dl then the anemia is called severe anemia [52].

Eradication Efforts

In 1955 WHO stars working on the eradication of malaria worldwide. Dichlorodiphenyltrichloroethane (DDT) and other synthetic anti-malarial were used to eradicate vectors as well as malarial parasites throughout the world. Efforts were started on anti-mosquito insecticides spraying, anti-malarial drug management, and observation. Four major step were taken to eliminate malaria i.e. preparation for the attack on malaria, attack on vectors as well as on parasites, consolidation, and maintenance. Successes were gain in the countries with moderate climates and recurrent malaria transmission [4]. Many countries including Sri Lanka and India had razor-sharp decrease in the figure of malarial cases, then followed by sudden boost to extensive levels after hard work ceased. Other nations had insignificant improvement (such as Haiti, Indonesia, Afghanistan, and Nicaragua). Some countries were completely excluded from the campaigns of malaria eradication (most of sub-Saharan Africa) which are now at higher risk of malarial infection [4]. Drug and insecticidal resistance of parasites and vectors, lake of community participation, massive population migrations / travel and wars were the major factors that made resistances in the eradication efforts of malaria [4]. In Pakistan Malaria eradication program was started in 1950, but later on it was termed as Malaria Control Program [35]. Malaria control program was decentralized in 1975 and bonded with primary healthcare infrastructure. In Khyber Pakhtunkhwa malaria remains a public health problem. KP was identified as a higher risk of malarial infection [53].

Diagnosis

Diagnosis of malaria includes identification of *Plasmodium* or its antigens within the blood of the infected person. Malaria may be diagnosed by various techniques available commercially [54].

Microscopy

A gold standard technique and established method for the diagnosis of malarias is the Microscopy. But without technical expertise it may not produce a good result [55]. It is based on finger pricked blood which is poured on a slide, thin and thick blood films are prepared. Giemsa stain is used for staining then with the help of immersion oil slides are examined under x100 lens of the compound microscope [56].

Advantages of Microscopy

Microscopy is an informative and sensitive diagnostic test which can be used by skilled technical expertise and can detect 5 – 10 parasites per µl of blood [57]. All the species and different stages of the Plasmodium can be differentiated in microscopy. Due to recent drug treatment any of the morphological change in the parasites on blood film can be detected by expert microscopists. Parasite can also be quantifies easily that how many parasites are there in a single RBC, Such studies are required to elaborate hyperparasitaemia. It is a cheap method of diagnosis. Costs estimated about 30 to 40 Pakistani Rupees per slide examination. It provides an everlasting evidence (the blood films) of the analytic discovery [57].

Disadvantages of Microscopy

Microscopy is labor and time consuming, normally it requires one hour from blood collection to finals result. Without technical expertise, and good reagents microscopy is impossible. In these situations, microscopic finding hazards become an untrustworthy instrument that uses up scarce resources for uncertain outcomes. There are regularly delays in the microscopy outcomes to the clinician, so that decisions on medication are often taken without the assistance of the lab results [57].

Rapid Diagnostic Test

RDT tests are based on the finding of antigens of the malarial parasites in RBCs by using an immunochromatographic technique. RDT also called Immunochromatographic Technique (ICT). It is based on antigen-antibody interaction. For the field diagnosis it is very good and sensitive diagnostic technique. Malaria diagnosis is improved with the help of world wide implementation of RDTs [28, 58]. ICT tests that detect *P. falciparum* are based on histidine rich protein 2 (HRP-2), that is specific to *P. falciparum* While those that detect *P. vivax* are based on Plasmodium aldolase and Plasmodium lactate dehydrogenase (LDH) detection using monoclonal antibodies which start reaction with LDH of all species including *P. falciparum*. Trophozoites and gametocytes of the *P. falciparum* produced Histidine-rich protein II (HRP-II) which is a water-soluble protein. HRP-2 may persist in the blood for days or weeks after treatment, whereas LDH is only detected if live parasites are present. Parasite lactate dehydrogenase (pLDH) is an enzyme which is produced by gametocytes of plasmodium. Currently Test kits are available to detect pLDH from all four Plasmodium species. They can distinguish *P. falciparum* from the non-falciparum species, but cannot differentiate between *P. vivax, P. ovale* and *P. malariae*. Some of the RDT kits detect all four malarial parasites mention in their brand name or their

marketing material only two species i.e. "PF/PV". This lead to confusion about their diagnostic capabilities [59]. ICT tests are obtainable in many designs like plastic cards, cassettes or dipsticks. The quality of the ICT format depends upon producer as well as on conditions in which it is being stored. This test is 90 percent more sensitive than microscopy. For the field diagnosis RDT/ ICT can tolerate temp and humidity depending upon the manufacturer [58].

Mode of action

RDT format consists of a nitrocellulose strip, on which dye labeled specific antibodies are coated on a thin line for malarial antigen. And either antibody specific for the labeled antibody, or antigen, is bound at the control line. A drop of blood is poured on the nitrocellulose strip then a buffer is also added which flush the blood towards the antibodies. RBCs are lysed and the parasite antigen becomes naked and mixed with the labeled antibody. If there is specific antigen, that will react with the antibody on the strip and become visible in the test line. While other labeled antibodies will be tapped on the control line [32].

RDT have many disadvantages as well, kits available in markets, targeting HRP-II can detect *Plasmodium falciparum* only and gives positive results for up to 14 days after chemotherapy and parasite clearance, which is very confusing. Such diagnostic kits are not fit for identifying cases of malaria other than *Plasmodium falciparum* from. RDTs are expensive than microscopic examination, with unstable costs per test from 80 to 150 PKR. RDT strips are qualitative, not quantitative. This is not a detailed investigation; therapeutic efficacy of antimalarial drugs is suffered. We are not able to count parasites as we do in microscopy. Those RDT which identify both *P. falciparum* and non-falciparum species are unable to distinguish between *P. ovale, P. vivax,* and *P. malariae,* or mix infections [59].

Polymerase Chain Reaction (PCR)

PCR is an expensive but sensitive and more specific molecular diagnostic method than all other techniques. It is a lengthy diagnostic test that needs expensive reagents and tools along with sterilized conditions. It cannot be performed in field [60, 61]. This molecular test depends on the quality of the Plasmodium DNA, more is the purified DNA, accurate will be the result, as there are a number of factors that inhibit the reaction like presence of haemoglobin compromise the sensitivity of the reaction [61, 62] For Malaria detection using the Phusion™ enzyme that amplifies DNA by nested PCR from dried blood spots on filter papers [63]. Different oligonucleotide primers are commercially designed to amplify the DNA of the parasite.

Plasmodium vivax Circumsporozoite (Pvcs) Protein Primers

Name	Sequence	Reference
VCS-OF	ATGTAGATCTGTCCAAGGCCATAAA	[9]
VCS-OR	TAATTGAATAATGCTAGGACTAACAATATG	
VCS-NF	GCAGAACCAAAAAATCCACGTGAAAATAAG	
VCS-NR	CCAACGGTAGCTCTAACTTTATCTAGGTAT	

Briefly, the external oligonucleotide primers VCS-OF (5′-ATGTAGATCTGTCCAAGGCCATAAA-3′) and VCS-OR (5′-TAATTGAATAATGCTAGGACTAACAATATG-3′), targets the conserved domains at non repetitive 5′ and 3′ domains, are used to amplify a fragment of 1100 base pairs, from 1 µl of purified parasite DNA, with 25 cycles and 58 °C annealing temperature. The internal primers VCS-NF (5′-GCAGAACCAAAAAATCCACGTGAAAATAAG-3′) and VCS-NR (5′-CCAACGGTAGCTCTAACTTTATCTAGGTAT-3′) amplified a 680-bp fragment, starting with 1 µl of the primary PCR product, with 30 cycles and 62 °C annealing temperature [64]. PCR genotyping protocols are based on polymorphic a locus that is located in two *P. vivax* genetic markers, Pvcs [9].

Other Primer Sequences

Name	Sequence	Reference
Pvmsp1 Sequence		[9]
VM1-O1R	CCACTCCATGAAACTGAAGTGTTTA	
VM1-N1F	CGATATTGGAAAATTGGAGACCTTCATCAC	
VM1-N1R	CTTTTGCGCCTCCTCCAGCTGGCTCGTGT	
Pvmsp1 F2 Sequence		
VM1-O2F	GATGGAAAGCAACCGAAGAAGGGAAT	
VM1-O2R	AGCTTGTACTTTCCATAGTGGTCCAG	
VM1-N2F	AAAATCGAGAGCATGATCGCCACTGAGAAG	
Pvmsp1 F3 Sequence		
VM1-3F	CAAGCCTACCAAGAATTGATCCCCAA	
VM1-3R	ATTACTTTGTCGTAGTCCTCGGCGTAGTCC	

Other Diagnostic Techniques

There are few other malaria diagnostic methods but they are not used widely as RDT and Microscopy. They include antibody recognition by serology and microscopy using fluorochromes, such as acridine orange, on blood smears [65] or on centrifuged blood samples [66].

Treatment

For the development of malarial drugs sexual stages malarial parasites were cultured but they gain resistance to drug, as culturing of liver stages, were extra hard to accomplish, made it probable to build up and experimentally test the drugs in opposition to this stage, this provided significant information about the immune reaction in the liver. Finally, the culture of sporogonic stages has enabled researchers to discover that in the mosquito vector what happens to the parasite [5]. Drugs that is required for malarial treatment includes sulfadoxine/pyrimethamine, mefloquine, atovaquone-proguanil, quinine or quinidine, clindamycin, doxycycline, chloroquine, and primaquine [67]. The artemisinin are the most

effective medicines that have ever been invented for [32]. A lot of work has been done on malarial vaccines with limited success [68]. The circum sporozoite protein (CSP) is an antigen that is present on the outside of sporozoites, has been used broadly as an objective for the development of vaccines [69]. Intermittent preventive treatment (IPT) that is taken time to time regardless of malarial infection is recommended by the World Health Organization especially for pregnant women. IPT immune the body for the specific parasite encounters [43].

Resistively of Malarial Parasites to drugs

More is the drug resistance spreads; the number of yearly deaths could double [32]. Malaria is the one of the most common parasitic blood infections that has developed resistance to many anti-malarial drugs [70]. Many patients in Pakistan suffer from chloroquine resistant malarial infection [71]. *P. falciparum* which is the deadliest strain of plasmodium is resistant to multiple drugs. As a result, the cost of treating malaria in these regions is high [33]. In a study 100 children were examined to determine the effect of persistent malaria parasitemia ontheair packed cell volume, as a result of which 49 patients were found chloroquine resistant, 32 where fansidar resistant [72].

Prevention

With the discovery mosquitoes in the malaria transmission, provided researches with a new weapon against malaria. It is experimentally proved that by the reducing the contact of the healthy person with the infected persons declines the risk of malarial infection [73]. Other methods to prevent the malarial infection include the distraction of mosquito breeding places, use of anti-mosquitoes oils and use of mosquito nets, appropriate clothing, mosquito repellents, particularly in the evening and after dark (long-sleeved shirts, trousers, socks, etc.) [32]. Biological control includes larvivorous fish that eat upon the lavas of the mosquitoes [74]. Some entomopathogenic bacteria, such as Bacillus thuringiensis serovar. *Israelensis* (Bti) and *Bacillus sphaericus* (Bsph) are used to control the vectors [75-78]. Chemoprophylaxis of Malaria is recommended for those people who travel to malaria-endemic areas and travel medical specialists must be contacted before travel. As malaria is a blood infection, blood donors must be properly examined for the history of malarial parasites [4]

Conclusion

From the obtained related study it may be concluded that malaria is a life threatening disease. Prevention and treatment should be designed which not only provide benefit to the patient and facilitate the physician but also provide an important socioeconomic benefit to everyone.

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