International Journal of General Medicine and Pharmacy (IJGMP)
ISSN 2319-3999
Vol. 2, Issue 4, Sep 2013, 1-10

International Academy of Science, Engineering and Technology
Connecting Researchers; Nurturing Innovations

DRUG RESISTANT MALARIA: BEYOND ARTEMISININ A CHALLENGE TO MEDICAL SCIENCE

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ABSTRACT

Malaria is caused by four species of *Plasmodium* the fifth *P.knowlesi* is prevalent in Malaysia and Southeast Asia. Malaria due to *Plasmodium falciparum* has developed resistant to all first line antimalarial drugs .Chloroquine has been replaced by Sulfadoxine-pyrimethamine (SP) as the first- line treatment of uncomplicated malaria. Resistance tochloroquine SP combination is already reported in Africa, making this combination unsuitable for use in Africa. Chloroquine and SP are replaced by artemisinin combination therapies (ACTs) which are more effective. The emergence of resistance to artemisinin derivatives has increased recently with reports of treatment failures with artesunate-mefloquine and arthemether-lumefantrine in Thai Cambodian malaria control programs. The current generation of ACTs will not maintain the efficacy indefinitely. Malaria control programs and researchers must join efforts to apply in coordinated proactive monitoring programs to detect the emergence and prevent the spread of resistance to ACTs.

KEYWORDS: Malaria, Sulfadoxine, Pyrimethamine, Artesunate Combination Therapies

INTRODUCTION

Malaria parasites *Plasmodium* first observed in a blood sample by Alphonse Laveran in 1880[1]. Other three species were discovered by other scientists, *Plasmodiumvivax* (Grassi andFelette, 1890), *Plasmodium falciparum* (Welch, 897), and *Plasmodium ovale* (Stephens, 1922) A fifth species of malaria *P.knowlesi* was first time reported in humans (Robert Knowles *et al*,1932). It is estimated that at least 250 to 500 million febrile illnesses and up to a million deaths annually [2]. With the introduction of chloroquine and dichlorodiphenyltrichloroethane(DDT) at the end of World War 11 brought new power to malaria control efforts.[3]. With the massive use of chloroquine in the !980s selected for chloroquine- resistant *Plasmodium falciparum* strains that entered and spread in Africa.

The impact of chloroquine resistance was especially evident in young children [4,5]. Chloroquine – resistant *P.falciparum* malaria is wide spread in sub-Saharan Africa, Asia, and Latin America. It has also been reported in areas of the Middle East, including Iran,Yemen,Oman and Saudi Arabia [6,7], but not from Mexico, other regions in Central America west of Panama Canal, Haiti, or the Dominican Republic. High-grade resistance of *P.vivax* malaria to chloroquine has been reported in Oceania and parts of Southeast Asia [8,9]. *P.vivax* malaria not responding to choloroquine treatment have also been reported from Brazil, Guyana, Colomboia, Peru, India, and Myanmar[10-15].

Amodoquine-resistant *P.falciparum*, in Asia and Africa .Mefloquine-resistant *P.falciparum* malaria in Thailand, in Thailand, Cambodia, Myanmar and Vietnam[16-18]. The emergence of resistance to artemisinin derivatives have increased recently with reports of treatment failures with artesunate-mefloquine and artemether-lumefantrine in Thai Cambodian malaria control programs [19]. Emergence of resistance to artemisinin-a hallmark benefit of artemisinin in the treatment of severe malaria, may become less dependable after artemisinin dosing in Southeast Asia [20].

This paper reviews the resistance to antimalarial drugs with emphasis on resistance to artemisinin which has become less reliable.

RESISTANCE TO ANTIMALARIAL DRUGS

Mechanism of Action

Chloroquine

Intraerythrocytic parasites consume the hemoglobin of their host cells, breaking down it with in a large digestive food vacuole and releasinghemoglobinmolecules (heme) that are poisonous if not detoxified. Malaria parasites normally allow these heme molecules to polymerize into inert crystals called hemozoin that can be visualized by light microscopy as intraerythrocytic pigment in thin blood smears. Chloroquine acts by forming toxic complexes with heme molecules and interfering with their crystallization[21].

This mechanism of action explains why chloroquine is effective against developing intraerythrocytictrophozoites but ineffective against other parasite stages i.e. maturegametocytes, liverschizonts that do not actively consume hemoglobin. Chloroquine- resistant *P.faciparum* reduce the amount of drug that accumulates in their digestive vacuoles [22].

The mechanism involves mutations in a conserved transport molecule of the digestive vacuole membrane termed PfCRT (*P.falciparum*chloroquine resistance transporter)[23]. The mutation include a key change from lysine to threonine in the 76^{th} amino acid (K76T) plus additional mutations that depend on their geographic origin [24,25]. Drug selection for mutant PfCRT is evident in association of the K76T marker with increased plasma chloroquine levels and with treatment failures in children receiving drug [26]. Several lines of evidence now indicate that chloroquine resistance involves a specific interaction between chloroquine and the modified form of PfCRT that promotes drug efflux from digestive vacuole [27,28].

While PfCRT is the central determinant of chloroquine resistance, other host and parasite factors also influence treatment outcomes, Forexample, clearance of phenotypically chloroquine resistant parasites can occur after chloroquine treatment and become increasingly prevalent in children as they grow older, presumably owing to the immunity that develops from repeated episodes of malaria [29]. Parasite transport modules in addition to PfCRT have also been proposed to modulate or contribute to the ability of chloroquine-resistant parasites to cope with the drug [30].

Sulfadoxone-Pyrimethamine

Dihydropteronate synthase (DHPS) and dihydrofolatereductase (DHFR) are sequentially involved in the folate pathway of nucleic acid synthesis. Pyrithamine inhibits parasite DHFR and the production of tetrahydrofolate, an essential cofactor for one-carbon metabolism required for the synthesis of nucleic acid and certain amino acids. The substitution of asparagine for serin in position 108 in DHFR is critical for the initial development of pyrithamine resistance., with additional mutation(Ile51, Arg59, Leu 164) increasing the degree of pyrithamine resistance [31]. Part of the sulfadoxine's action is thought to be inhibition of parasites DHPS and point mutations in DHPS reduce its affinity for sulfadoxine [31]. Analysis of the mutant *dhfr* and *dhps*alleles in field studies supports conclusions that clinically significant resistance to pyrithamine arises from multiple mutations in *dhfr* and *dhps* and *dhps* mutations are likely selected after mutations in*dhfr* are already present [32].

Atovaquone-Proguanil-Malarone

Atovaquone binds cytochrome b and inhibits parasites mitochondrial electron transport, leading to collapse of the

mitochondrial membrane potential [33, 34]. This effect is potential by progunanil. The substitution of serine for tyrosine at condon 268 of the cytochrome b gene is associated with resistance to atovaquone and AP combination [35, 23]. Cytoguanil the active metabolite of proguanil, inhibits DHFR. Point mutations in *dhfr* resistance to cytoguanil [36].

Doxycycline

Doxycycline inhibits protein synthesis elongation binding of aminocyl-tRNa to ribosome 30S subunit. Resistance to human malaria parasites have not been described. Doxycycline is successfully used as malaria prophylaxis in IrianJaya [37].

Mefloquine, Qunidine and Quinine

These three antimalarial drugs, mefloquine, quinidine, and quinine are thought to form complex toxic to the parasite by binding to heme. Mefloquine resistance may be associated in part with increases in expression and mutations in the P-glycoprotein homolog-1 gene pfmdr1 [38]. Decreased quinine sensitivity is associated with resistance to other structurally related drugs such as melfoquine and halofantrine, suggesting that drug resistance mechanism may share various genetic determinants [39]. Some studies have implicated *pfmdr1* mutations in mefloquine, quinine, and halofantrine resistance and *pfcrt* mutations in quinine and quinidine responses [40]. The different level of quinine susceptibility among parasites and the relatively slow rate at which quinine resistance has spread throughout the world indicate that quinine resistance is a complex phenotype and is probably affected by other genes in addition to *pfmdr1*. The results of a linkage analysisand surveys of parasites from Southeast Asia, Africa, and South America support a model in which multiple genes can combine in different ways to produce similar phenotypes of reduce quinine response [40].

Artemisinin Derivatives

At present high level of resistance to the artemisinin derivatives has not been found with clinical samples, successful selection of rodent malaria parasites strains with reduced susceptibility, and reports of *P.falciparum* strains with prolonged clearance times in vivo [41,20], raise concerns that strains of human malaria parasites with significant clinical resistance may evolve and spread. No molecular mechanism to account for artemisinin resistance has been established. An S769N mutation in an ATPase enzyme (PfATPase 6) was proposed as a possible determinant of artemisinin resistance [42],. One study associated elevated IC50s with its mutation in strains of *P.falciparum* from French Guiana, but resistance has not been associated with this mutation in field isolates elsewhere nor has mutation been found in rodent malaria parasites selected for resistance[43,41,20].

CLINICAL MANIFESTATION AND DORMANCY OF MALARIA

The malaria parasite incubation period after and infective mosquito bite includes the time required for parasites to progress through liver schizogony and produce symptoms by their propagation in the blood stream. For primary attacks, this period is typically about 8 to 25 days but may be much longer depending on immune status of the infected person, the strain as well as the species of *Plasmodium*, the dose of sporozoites, and the possible effects of partially effective chemoprophylaxis. Relapses from latent hyponozoite may develop months or years after mosquito bites. Late-onset or recrudescent of *P.falciparum* malaria may also occur in individuals who have suppressed parasitemia of drug resistant parasites with chemoprophylatic drugs [44]. Febrile patients presenting within 7 days of entering an endemic area are likely to have malaria, unless there has been earlier exposure to infective mosquito bites.

As a general rule, and because of danger of acute *P.falciparum* infection, all travelers who have visited a malaria-endemic area in the 3 months prior to onset of fever or other suggestive symptoms should be considered to have malaria

until proven otherwise. Even in patients beyond this time frame, it is wise to consider *P.falciparum* malaria, for example, in the recent report of a symptomatic presentation in an 18 years-old patient with sickle cell disease 4 years after visiting an endemic area [45]. There is firm experimental foundation showing that malaria (*Plasmodium)* may persist for long periods in vivo in a viable state but not multiplying state. Latent attacks from reactivation of *P.vivax, P.ovale*hypnozoites usually occur within 3 years and are rare more than 5 years after exposure. Recrudescence *P.malaria* symptoms in individuals with subclinical parasitemia has been reported decades after initial infection [45].

THERAPY OF MULTIDRUG RESISTANT MALARIA

Malaria due to *P.falciparum* can be fatal if not diagnosed and treated promptly and appropriately. This is especially true of nonimmune travelers returning from visits to malaria-endemic areas. Malaria is a disease of protean manifestation [46]. Artemisinin and its derivatives (artesunate, artthemether, dihydroartemisinin) are now commonly used in Africa and Southeast Asia for the treatment of uncomplicated malaria, that caused by multidrug-resistant *P.falciparum* [47]. Parasites recrudescence weeks after therapy with artimisinin does occur, often the elimination of these drugs and recovery of parasitemia without selection of mutant parasites that are truly drug-resistant.

Theaddition of partner drug (e.g. chloroquinine, sulfadoxine-primethamine, or mefloquine) to 3- day course of artemisinin derivative was shown in ameta-analysis to substantially reduce treatment failure and recrudescence [48,49] Artemisinin derivatives (artemisinin, arthemether, duhydroartemisinin) are derived from *Artemisiaannua*,(qinghao) a plant used in China for millennia as therapy for fevers [50]. Artemisinin derivatives are consistently effective against multidrug-resistant parasites and rapid clearance of parasites and clinical improvement usually within 24 to 36 hours. They are well tolerated and safe in adults, children, and pregnant women [51]. Although neurotoxicity can occur with supraphysiologic doses in animals, it has not been documented in humans [52].

P.falciparum, resistant to most standard antimalarial drugs, poses a major problem for the treatment of malaria. Several countries in sub-Saharan Africa have replaced chloroquine with sulfadoxine-pyrimethamine (SP) as the first- line drug for the treatment of uncomplicated *P.falciparum* malaria [53]. In other areas of the world where SP replaced chloroquine, such as South-East Asia, resistance to SP developed within few years of its introduction. In East Africa resistance to SP is present, resulting in a decrease in the effectiveness of this drug [54].

In a study in Gambia, children with uncomplicated *P.faciparum* malaria treated with 3 day of artesunate plus SP had faster resolution of fever, parasite clearance, and gametocyte carriage compared with SP alone[55]. Researchers in Kenya in a randomized, double-blind, placebo-controlled trial, the efficacy, safety and tolerability of artesunate plus SP compared with SP alone in the treatment of uncomplicated *P.faciparum* malaria confirmed that parasite clearance and gametocyte carriage were reduced significantly in both combination groups compared with SP alone. Three day sartesunate were required to reduce significantly the risk of treatment failure by day 28. However, the high background rate of parasitological failure with SP may make this combination unsuitable for widespread use in Kenya [56].

Nosten*et al (*1994) studied 652 adults and children with acute uncomplicated *falciparum* malaria on the Thai-Burmese border and found that a single- dose artesunate (4 mg/kg) plus mefloquine (25 mg of base/kg) gave more rapid symptomatic and parasitological responses than high-dose mefloquine alone but did not improve cure rate[57].

Other researchers in Thailand reported that introduction of artesunate-mefloquine combination in selected areas along Thai-Myanmar borders in 1995 is believed to be one of the multiple factors responsible for stabilizing the multidrug-resistance problems in Thailand [18]. Today the treatment of choice is artemisinin-based combination therapies (ACTs).

Resistance to artemisinin- the core component of the combination- has now been identified in Cambodia, MyanmarThailand and Viet Nam [58].

PREVENTION OF DRUGRESISTANT MALARIA

The concept that resistance could be delayed or prevented by combining drugs with different targets was first developed in the treatment of tuberculosis, and has been adopted widely for the treatment of HIV, leprosy, and cancer. Artemesinin combinations have been proposed as an option for the treatment of drug-resistant malaria [59,60]. The landscape of antimalarial therapy is changing. With new multilateral support for artemisinin combination therapies (ACTs), highly efficacious alternative are becoming available to replace less effective drugs [chloroquine and sulfadoxine-pyrimethamine (SP) that are still used widely despite their impaired efficacy.

Combination therapies presents new challenges for monitoring resistance and efficacy, as well as new prospects for deterring drug resistance [61]. Methods for measuring parasite growth *in vitro* in the presence of increasing drug concentrations were developed for culture-adapted malaria parasites in controlled laboratory testing[62]. Despite the limitations, *in vitro* assays are increasingly important in the era of ACTs because of the inability to rely on molecular methods for monitoring resistance and absence to date of clinically significant resistance to the artemisinin. The early stages of parasite resistance to individual drugs used in combination therapy regimens may not be clinically apparent because of the action of the partner drug(s).

Clinical studies to monitor efficacy may thus be relatively insensitive for heralding the impending failure of drug combinations. While candidate molecular markers for resistance to artemisinin are being studied [63]. Investigators the cases of chloroquine and antifolates, successfully identified the key resistance genes, which, even if they were not the sole genetic contributors to resistance, are clearly its primary determinants. Candidate gene approaches based on non-malaria homologs or on suspected mechanisms of drug action have been used to study genetic determinants of resistance to drugs included in ACTs.

In an example of the homolog candidate gene approach in vitro and clinical evidence suggests that increased *pfmdr*1 copy number is associated with resistance to mefloquine, and artemisinin, as well as other antimalarial drugs [64,65]. ACTs, with their rapid action and excellent efficacy, are being embraced by the policy makers throughout the Africa (and in other malaria endemic areas).However, the current generation of ACTs will not maintain their efficacy indefinitely. Researchers and malaria control workers of all stripes must join efforts to apply *in vitro*, molecular, genomic, pharmacokinetic, and clinical methods in coordinated proactive monitoring programs to detect emergence and deter the spread of resistance to ACTs [61].

CONCLUSIONS

P.falciparumhas developed resistance to all the first line used antimalarial drugs. Artesunate SP combinations are also not the drugs of choice anymore. Resistance toartemisinin combinations is also reported in Southeast Asia. Artemisinin combinations therapies will not maintain their effectiveness indefinitely. Efforts must be made to detect and prevent the spread of resistance to ACTs

ACKNOWLEDGEMENTS

We wish to thank Vice Chancellor and Dean School of Medicine University Malaysia Sabah, Kota Kinabalu, Sabah Malaysia for the permission to publish this article.

REFERENCES

1. Laveran A. Deaxiem note relative a un nouveau trouvedanle sang maladies atteints la fieverpalustre. *Bull Acad Med*.1880;**44:**1346-1327.
2. World Health Organization. *World Malaria Report*. *Geneva: World Health Organization*,2008.
3. Carter R, Mendis KN .Evolutionary and historical aspects of the burden of malaria .*ClinMicrobiolRev*.2002;**15:**564-594.
4. World Health Organization. *World Health Organization Tech Rep Ser*.1990**;805:**1-141.
5. Zucker JR, Rebush Tr, 2nd,Obonyo C,*et al.* The mortality consequences of the continued use of chloroquine in Africa experience in Siaya, Western Kenya.*Am J Trop Med Hyg*.2003;**68**:386-390.
6. al Arishi HM, Awad Ahmed R, al Rishi I.A .Chloroqunine resistant *Plasmodium falciparum* among children seen in a regional hospital Tabuk, Saudi Arabia. *Trans R Soc Trop Med Hyg*.2001;**95:**4:39-440.
7. Al-Maktari MT, Bassiouny HK .Malaria status in Al-Hodeidah Governorate, Republic of Yemen. Part 11:Human factors causing the persistence of chloroquine resistant *P.falciparum* local strain.*J EgyptSoc Parasitolol*.2003;33:829-839.
8. Sumawinata IW, Bernadeta, LekasanB,*et al.* Very high risk of therapeutic failure with chloroquine for uncomplicated *Plasmodium falciparum* and *P.vivax* malaria in Indonesian Papua. *Am J Trop MedHyg*.2003;68:416-420.
9. Murphy GS, Basri H, Purnomo, *et al*. *Vivax* malaria resistant to treatment and prophylaxis with chlorioquine.*Lance*t.1993;**341**:96-108.
10. SotoJ, ToledoJ, Gutierrez R, *et al. Palsmodiumvivax* clinically resistant to chloroquine in Colombia. *Am J Trop Med Hyg*.2001;**65**:90-95.
11. Garavelli PI, Corti R. Chloroquine resistance in *Palsmodiumvivax*: the first case in Brazil. *Trans RSoc Med Hyg*.1992**;86:**128.
12. Ruebusi TK 2nd,Zegarra J,Cair J, *et al.*Chloroquine –resistant Plasmodium vivax malaria in Peru.*Am J Trop Med Hyg*.2003;69:548-552.
13. Marlar T, Myat Phone K, Aye Yu S, *et al*. Development of resistance to chloroquine by *Plasmodium vivax* in Myanmar. *Trans R Soc Trop Med Hyg*.1995; **89:307**-308.
14. Garg M, Gopinathan N, Todhe P, *at al*. *Vivax m*alaria resistant to chloroquine: case report from Bombay.*Trans R Soc Trop Med Hyg*.1995;**89:**656-657.
15. Barret JP, Behrens RH. Prophylaxis Failure Against Vivax Malaria in Guyana, South America.*JTravel Med*. 1996;**3**:60-61.
16. Mandi G, Mockenhaupt FR, Coubilaly B, *et al*. Efficacy of amodiaquine in the treatment on uncomplicated falciparum malaria in young children of rural north Western Burkina Fasso. *Malar* **J**.2008**; 7**:58.
17. Marfurt J, Mueller I, Sie A, *et al*. Low efficacy of amodiaquine or chloroquine plus sufadoxine-pyrimethamine against *Plasmodium falciparum* and *P.vivax* malaria in Papua New-Guniea.*Am J TropMed Hyg*.2007;**77:**947-954.

18. Wongsrichanalai C, Sirichasinthop J, Karwachi JJ, *et al*. Drug resistant malaria on the Thai-Myanmar borders. *Southeast Asian J Trop Med Public Health*. 2001;**32**:41-49.

19. Resistance to artemisinin derivatives along the Thai- Cambodian border. *WklyEpidemiolRec*.2007;**82**:360.

20. White NJ, Qinghaosu (artemisinin): the price of success.*Science*.2008;**320**:330-334.

21. Chou AC, Chevli R, Fitch CD. Ferriprotophyrin IX fulfills the criteria for identification as chloroquine receptor of malaria parasites. *Biochemistry.* 1980;**19**:1543-1549.

22. Verdier R, Le Bras J, Clavier F, *et al.* Chloroquine uptake by *Plasmodium falciparum* infected human erythrocytes during in vitro culture and its relationship to chloroquineresistance. *Antimicrob Agents Chemother*.1985;**27**:561-564

23. Schwobel B, Alifrangis M, Salanti A, *et al.* Different mutation patterns of atovaquone resistance in *Plasmodium falciparum* in vitro and in vivo rapid detection of codon 268 polymorphisms in the cytochrome b as potential in vivo resistance marker. *Malar J*.2003;2:5.

24. Fidock DA, Nomura T, Telley AK, *et al.* Mutations in the *P.falciparum* digestive vacuole trans membrane protein PfCRT and evidence for their role in chloroquineresistance. *Mol Cell*.2000;**6**:861-871.

25. Chen N, Kyle DE, Pasay C, *et a*l. pfrt Allelic types with two novel amino acid mutations in chloroquine resistant *Plasmodium falciparum* isolates from Philippines. *Antimicrob AgentsChemother*.2003;47:3500-3505.

26. May J, Meyer CG .Association of *Plasmodium falciparum* chloroquine resistance transporter variant 176 with age related plasma chloroquinelevels. *Am J Trop MedHyg*.2003;**68:**143-146.

27. Sandez CR, Stein W, Lanzer Trans stimulation provides evidence for a drug efflux carrier as the mechanism of chloroquine resistance in *Plasmodium falciparum.Biochemistry.*2003:**42:**9383-9394.

28. Bray PG, Martin RE, Tilley I, *et al*, Defining the role of PfCRT in *Plasmodium falciparum*chloroquineresistance.*Mol Microbiol*.2005;**56:**323-333

29. Dimde AA, Doumbo OR, Traore G, *et al*.Clearance of drug resistant parasites as a model for protective immunity in *Plasmodium falciparum malaria.Am J Trop Med Hyg*.2003;**69**:558-563.

30. Mu J, Ferdig Mt, Feng X, *et al*. Multiple transporters associated with malaria parasite responses to chloroquine and quinine.*MolMicrobio*. 2003; **49:977**-989.

31. Peterson DS, Waliker D, WellemsTE.Evidence that a point mutation in dihydrofolate-thymidylate synthase confers resistance to pyrimethamine in *falciparum* malaria. *ProcNatlAcadSci* USA.1988;**85**:9114-91168.

32. Plow CV, Cortease JE, Djmde A, *at al.* Mutations in Plasmodium falciparum dihydrofolatereductase and dihydropteroate synthase and epidemiologic patterns of pyrimethaminesulfodoxine use and resistance. *J Infect Dis*.1977; **176:1590**-1596.

33. Sirvastava IK, Morrissey JM, DarrouzetE,*etal*.Resistance mutations reveal the atovaquinine-binding of cytochrome b in malaria parasites. *MolMicrobiol*. 1999;**33**:704-711

34. Sirvastava JK, Rottenberg H, Vaidys AB. Atovaquone a broad spectrum antiparasitic drug, collapses mitochondrial membrane potential in a malarial parasite. *J BioChem*.1997; **272:**3961-3966.

35. Sirvastava JK, Vaidya AB. A mechanism for the synergistic antimalarial action of atovaquine and proguanil. *Anitimicrob Agents Chemother*.1999; **43:1334**-1329.

36. Fidock DA, Nomura T, Wellems TE. Cycloguanil and its parent compound proguanil demonstrate distinct activities against *Plasmodium falciparum* malaria parasites transformed with human dihydrofolatereductase.*Mol Phamacol*.1998; **54:1140**-1147.

37. Taylor WR, Richie TI, Fryauff DJ, *et al*. Malaria prophylaxis using azithromycin: a double blind placebo controlled trial in Irian Jaya, Indonesia. *Clin Infect Dis*.1999;**28**:74-81.

38. Picard AI,Wongsrichanalai C, ParfieldA,*etal*.Resistance to antimalarials in Southeast Asia and genetic polymorphisim in pfmdr 1.*Antimicrob Agent Chemother*.2003;**47**:2418-2423

39. Warsame M,Wernsdorfer WH, Payne D, *et al*. Susceptibility of *Plasmodium falciparum* in vitro to chloroquine, mefloquine and sulfadoxine/pyrimethamine in Somalia: relationships between responses to the different drugs.*Trans R Soc Trop Med Hyg*. 1991;**85**:565-569.

40. FerdigMt,Cooper Ra, Mu J,*et al*.Dissecting the loci of low- level quinine resistance in malaria parasites .*Mol Microbiol*.2004;**52**:985-997.

41. Alfonso A,Hum P, Cheesman S, *et al*. Malaria parasites can develop stable resistance to artemisinin but lack mutation in outside candidate genes atp6 (encoding the sarcoplasmic and endoplasmic reticulum Ca2+ ATPase), tctp,mdr I, and cg10.*Antimicrob AgentsChemother*.2006;**50**:480-489.

42. Eckstein Ludwig, U Webb RJ, Van Goetham ID, *et al* .Artemisinin target the SERCA of Plasmodium falciparum.*Nature*.2003;424:957-911

43. Jambou R, Legrand E, Niang M, *et al*. Resistance of Plasmodium falciparum field isolates in vitro arthemether and point mutations of the SERCA type PfATPase6.*Lancet*.2005;366:1960-1963.

44. Schwartz E, Parise M, KozarskyP,*et al*. Delayed onset of malaria-implications for chemoprophylaxis in travelers*Eng J Med*.2003;**349:**1510-1516.

45. Chadee DD, Tilluckharry CC, Maharaj P, *et al* Reactivation of Plasmodium malariae infection ina Trinidadian man after neurosurgery.*NEng J Med*. 2000;342:1924.

46. Osler W .The study of fevers in the South.*JAMA*.1896; **26:999**-1004

47. NostenF,White NJ, Artemisinin-based combination treatment of falciparum malaria. *Am J Trop Med Hyg*.2007; **77:181**-192.

48. White NJ.The assessment of antimalarial drug efficacy. *Trends Parasitol*.2002; **18:458**-644.

49. Adjuk M, Babiker A, Garner P,*etal*.artesunate combinations for treatment of malaria meta-analysis.*Lancet*.2004;**363:**9-17.

50. van AgtmaelMa,EggelteTA,vanBoxtel CJ. Artemisinin drugs in the treatment of malaria from medicinal herb to registered medication.*TrendsPhamacol Sci*.1999; **20:199**-205.

51. Mcgready R, Brockman A, Cho T, *et al*. Randomised comparison of mefloquine-aretesunate in the treatment of multidrug- resistant malaria in pregnancy.*Trans R Soc TropMed Hyg*.2000;**94**:689-693.

52. Hien TT,TurnerGD,Mai NT, *et al.*Neuropathological assessment of artemether-treated severe malaria.*Lancet*. 2003**; 362:295**-296.

53. Watkins WM,MosoboM.Treatment*of Plasmodium falciparum* malaria with pyrimethamine and sulfadoxine: a selective pressure for resistance is a function of long elimination half-life.*Trans R Soc Trop Med Hyg*.1993;**87**:75-70

54. Anabwani GM, EsamaiFO ,Menya DA.A randomized controlled trial to assess the relative efficacy of chloroquine, amodiaquine,halfantrine and Fansidar in the treatment of uncomplicated malaria in children. *East African Med J*.1996**;73**:155-158.

55. Von Seidlein L, Milligan P, Pinder M, *et al*. Efficacy of artesunate plus pyrimethamine-sulfadoxine for uncomplicated malaria in Gambian children: a double blind,randomized control trial.*Lancet*.2000;**355:**352-357.

56. Charles 0,Obonyo, Francis Ochieng, Walter RJ,*et al.*Artesunate plus sulfadoxine- pyrimethamine for uncomplicated malaria in Kenyan children: a randomized, double- blind, placebo controlled trial. *Trans R Soc Trop Med Hyg*.2003**;97**:585-591.

57. Nosten F, LuxemburgerC,terKuileFO,*etal*.Treatment of multidrug-resistant *Plasmodiumfalciparum* malaria with 3-daya artesunate-melfoquinecombination.*J InfectDis*.1995;**170(**4),971-977

58. World Health Organization.WH0 launches emergency response to antimalarial drug resistance, World Malaria Report.2012.

59. Grosset J, Bacteriologic basis of short-course chemotherapy for tuberculosis. *Clinics inChest Medicins.*1980; **1:231**-234.

60. White NJ.Olliaro PL. Strategies for the prevention of antimalarial drug resistance: rationale for combination therapy for malaria. *Parasitology Today*.1996; **12:399**-401.

61. Miriam K, LauferAboulayeA,Djimde,ChristopherVP.Monitoring and deterring drug-resistant malaria in the era of combination therapy. *Am J Trop Med Hyg*.2007;**77**(6),160-169.

62. RickermannKH,McnamaraJV,FrischerH,*et al* Effects of chloroquine, quinine and cyloguanil upon the maturation of asexual erythrocytic forms of two strains of *Plasmodiumfalciparum* in vitro.*Am J Trop Med Hyg*.1968;1**7**:661-671.

63. Jambou R, Legrand E, NiangM,*et al.* Resistance of *Plasmodioum falciparum* field isolates to in vitro artemetheranf point mutations of the SERCA-type PfARPase 6.*Lancet*.2005;**366:**1960-1963.

64. Price RN ,UhlemannAC,VanVM,*etal*.Molecular and phamachiological determinants of the therapeutic response of artemether-lumefantrine in multidrug-resistant *Plasmodiumfalciparum*malaria.*Clin Infect Dis*.2006;**42**:1570-1577.

65. Price RN,UhlemannAC,BrockmanA,*et a*l. Mefloquine resistance in *Plasmodiumfalciparum* and increased pfmdr 1 gene copy number.*Lancet.*2004;**364**:438-447.