Original Article

Survival Probability of Plasmodium Falciparum against Chloroquine and its Combination with Sulphadoxine-Pyremethamine in Punjab, Pakistan

1. M. Saleem Rana 2. Akhtar Tanveer 3. Asma Abdul Latif 4. Ammara Hassan Tahir

1. Assoc. Prof., Health Services Academy, Islamabad 2. Prof. of Parasitology, Punjab University, Lahore
3. Asstt. Prof. of Zoology, Women College University, Lahore 4. Astt. Prof. of Medicine, AIMC, Lahore

ABSTRACT

Purpose of study: Survival probability of P.falciparum was determined against the chloroquine and its combination with sulphadoxine-pyremethamine.

Type of study: Prospective nonrandomized descriptive study.

Place and duration of study: Study was conducted in five districts "Muzaffargarh, D.G.Khan, Jhang, Sheikhupura and Multan" of Punjab, Pakistan. During the non-transmission season of the year 1999 to 2000 and 2008, among the rural populations 5952 persons were screened for malarial parasites.

Methodology: During the malaria non transmission season (November, December & January), 5952 persons were screened for malaria and 1409 positive cases were detected. 404 subjects out of total positive cases were selected to be tested against chloroquine and 50 with combination of chloroquine and sulphadoxine-pyremethamine by in vivo technique. Follow up was carried out for 28 days (on day 1, 2, 3, 7, 14, 21 and 28).

Result: Over all 35.4 % resistance-I was detected against chloroquine monotherapy and 4% with combination therapy (chloroquine and sulphadoxine-pyremethamine). Resistance-III was not found. Two variables were found important predictors of drug resistance; a young child and a high parasitaemia count (>6000/µl) at day 0.

Conclusion: It is concluded that malaria is still significant problem and resistance against monotherapy is increasing, hence adoption of combination therapy as first line treatment for uncomplicated falciparum malaria in Punjab Pakistan is recommended.

Key Words: Plasmodium falciparum, resistance, chloroquine, Pakistan

INTRODUCTION

The efficacy of readily affordable antimalarial drugs is declining rapidly in different parts of the world (1, 2,). Drug resistance (%) was found significantly less 5.3 (4/77) in combination of artesunate and sulphadoxine-pyrimethamine, than monotherapy of chloroquine 71.8 (51/71) or sulphadoxine-pyrimethamine 44.1 (3). Later continuous studies were conducted in Pakistan as per results given in Figure-1. Keeping in view the fluctuated trend in the malaria drug resistance in Punjab the present study was planed and conducted.

MATERIALS AND METHODS

This study was carried out on 404 subjects of uncomplicated *falciparum* malaria with monotherapy of chloroquine and on 50 subjects of uncomplicated *falciparum* malaria with combination therapy of chloroquine and sulphadoxine-pyrimethamine. The study subjects were selected from the both sexes, all age groups except < 6 months babies, with the history of fever ≥38°C, from any occupation. The area of the study was selected on the basis of malaria endemicity in the province of Punjab, Pakistan by record review. To select the subjects for studies important characteristics (4) were parasite density minimum threshold of 1000/µl (asexual parasites per micro-liter) and maximum threshold of 80,000 / µl of blood, positive for *P.falciparum* monoinfection, any subject had not received any antimalarial drugs during previous four weeks. For detection of antimalarial drugs urine test of the patients was conducted and positive subject were excluded. The studies were carried out during the winter season (After 15th November to before 15th January) the non- transmission season in Pakistan to minimize the chances of new infection (5). Test drugs were administrated by weight of the subject as per treatment policy. Follow-up blood slides were obtained on day 1,3, 7,14, 21 & 28 for monitoring the course of asexual parasitaemia. Written or oral consent, as appropriate was obtained from all subjects from whom blood samples were taken. Thick and thin film was prepared by obtaining finger prick blood, stained for 30

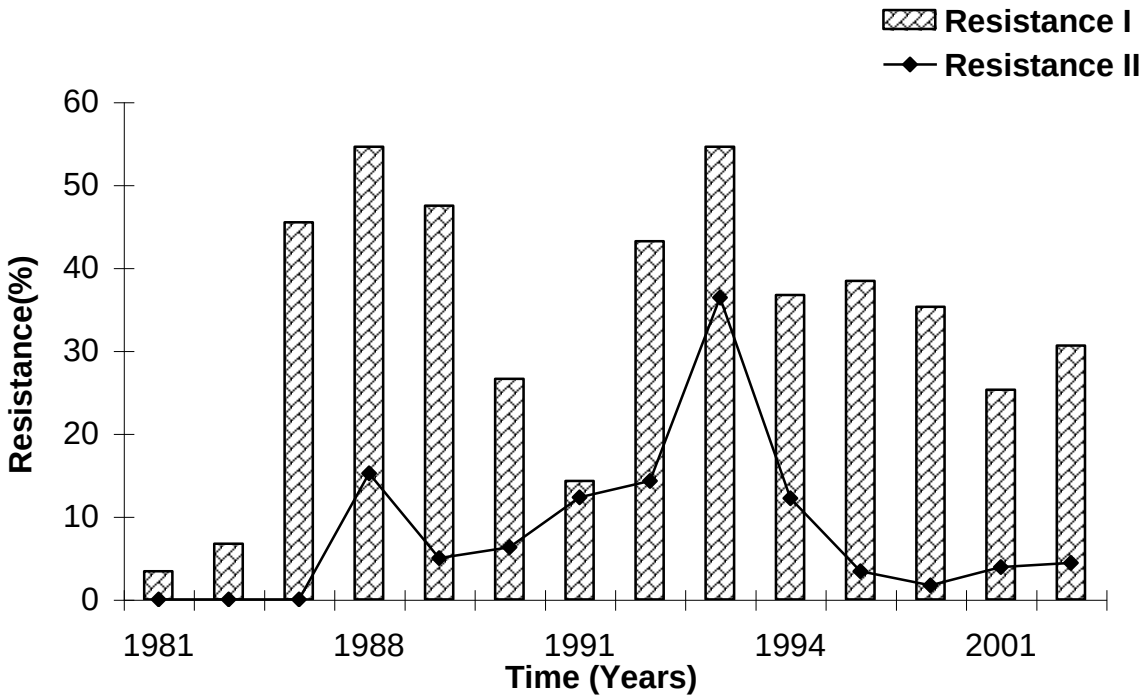

Figure-1: Showing the resistance against chloroquine from 1981 to 2004(Curtsy of Directorate General Health Punjab, Pakistan, 2008).

minutes with Giemsa 1 %(v/v) in the water of pH 7.2 and examine under oil immersion. Subjects for study were not enrolled with the history of pregnancy and lactation or sever malaria cases (cerebral, renal malaria). One or more of the general danger signs or any other sign of severe and complicated malaria, presence of any severe disease, presence of severe malnutrition, febrile diseases other than malaria. Survival probability of *P.falciparum* against chloroquine and its combination with sulphadoxine-pyrimethamine was determined and analyzed by using the Kaplan-Meier method. Early and late failures were distinguish by dividing the follow-up in 28-days. Differences in proportions were analyzed by using chi-square (6).

RESULTS

Chloroquine efficacy was assessed on 404 subjects of uncomplicated *f*alciparum malaria and found 35.4 %(143/404) resistance ($p < 0.001$). As high parasite density is predictor of fatality, similarly high density predicts the resistance development against P.falciparum in various areas.

For all subjects, observations were recorded for 28 days or till treatment failure or loss of follow-up, if either occurred in the mid of study, this information were recorded as drug resistance. Parasite density/µl of resistant subjects and sensitive subjects was also found important factor toward causing the resistance. In resistant subjects 53.84% (77/143) had parasite density >6000 parasite/µl, 28.677% (41/143) had 3000 to 6000 and 17.48% (25/143) had density <3000 parasite/µl, statistically difference was found highly significant (Figure-2). Survival of subjects was estimated by using the Kaplan-Meier method (Figure-3) and found 100 % on day one. Survival (%) of subjects reached at zero with parasite density 28000 /µl on day one having RII level of resistance. Subject having parasite density 30000/µl with RI resistance found zero percent survival.

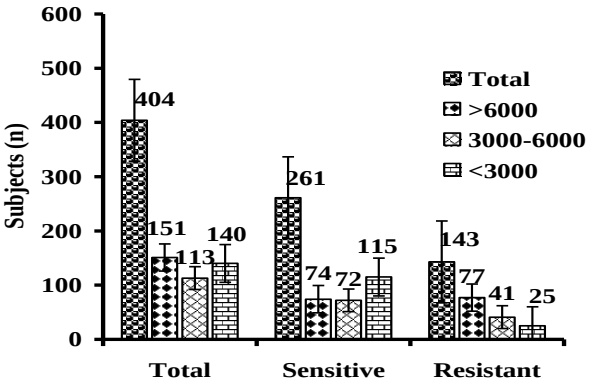

Figure-2: Showing the total, sensitive and resistant subjects of chloroquine against *Plasmodium falciparum* in different groups of subjects having different parasite density /µl by *in vivo* technique in five districts of Punjab, Pakistan from 2003 to 2005.

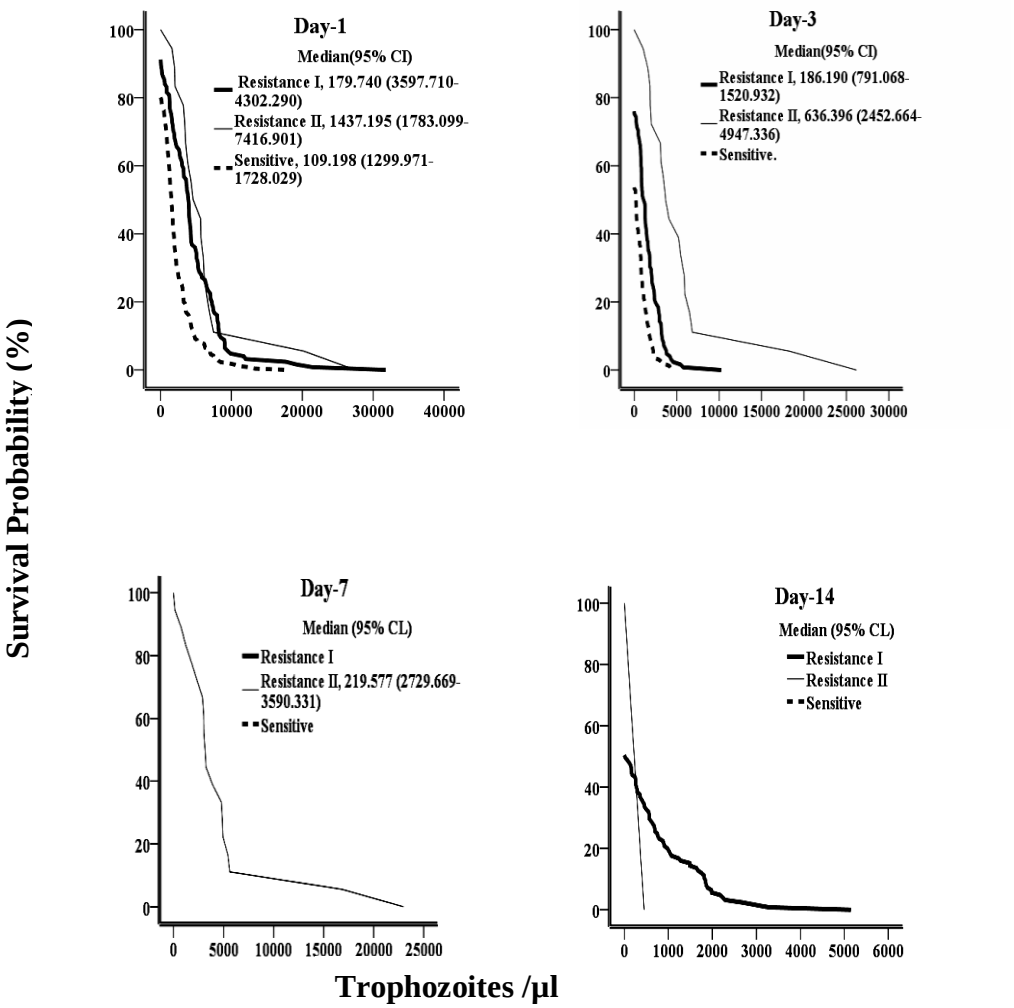

Figure-3: Showing the survival of subjects on every follow-up day (1, 3, 7 and 14) with different parasite density/µl by Kaplan Meier for all subjects studied by *in vivo* technique in five districts of Punjab, Pakistan from 2003 to 2005.

Subject having parasite density 15000 /µl had survival (%) 10 with resistant strains. On day three survival (%) was found 100 with zero parasite density/µl, which decreased with the increase of parasite density/µl and reached zero percent survival with parasite density 3000/µl for sensitive and subjects having parasite density 4000/µl resistance strains (Figure-4).

On day three survival (%) was found 100 with zero parasite density/µl, which decreased with the increase of parasite density/µl and reached zero percent survival with parasite density 28000/µl for RII and subjects having parasite density 10000/µl with RI resistance found zero percent survival. Same pattern was found on all follow-up days.

DISCUSSION

In this trail mono and combination therapeutic effects were tested against P.falciparum on chloroquine and combination of chloroquine/sulphadoxine-pyrimethamine in Punjab, Pakistan. Chloroquine was known as the most effective and safe antimalarial (7) but the development of resistance to chloroquine against P.falciparum has become a serious problem for malaria treatment (8).

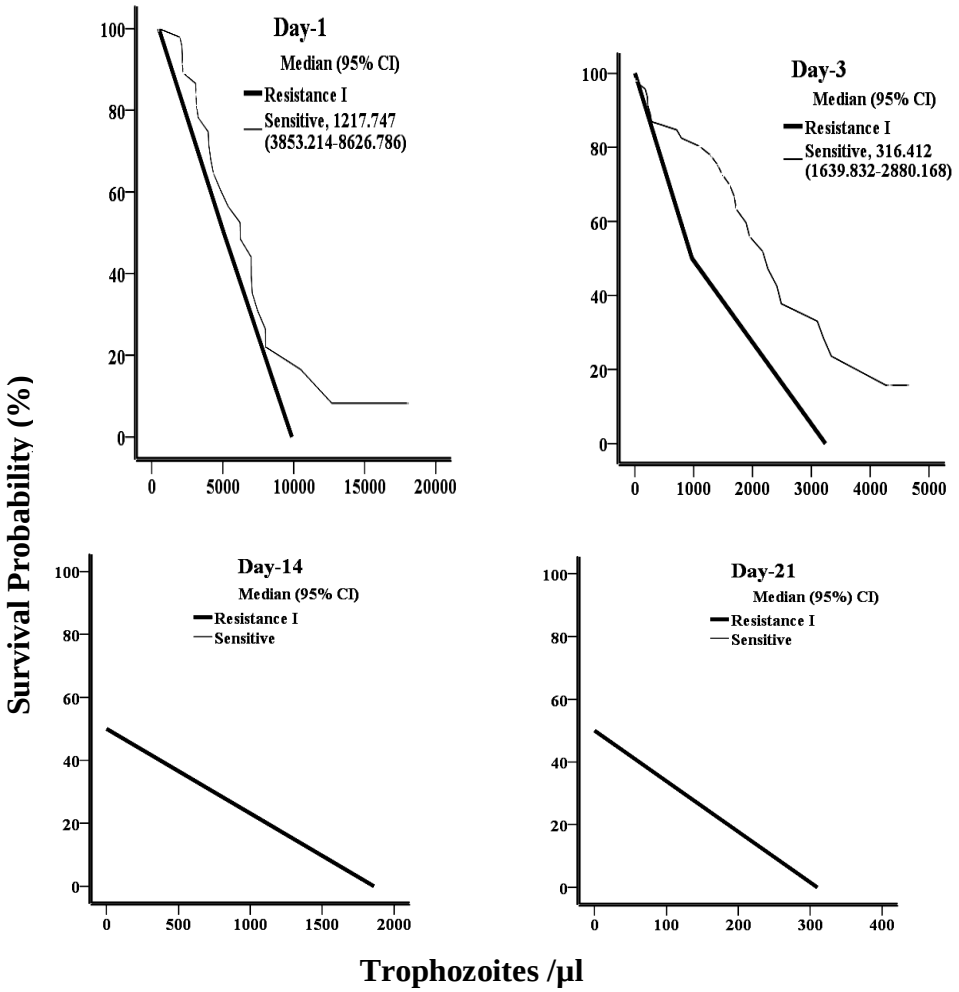

Figure-4: Showing the survival of parasite on every follow-up day by Kaplan Meier for all subjects studied by *in vivo* technique in one district of Punjab, Pakistan on combination therapy against *Plasmodium falciparum*. Study subjects were analyzed on day1, 3, 14 and 21. P=<.000[95% CI=. 000-.000].

In present study SPR noted was 27.4% which is 31.6%, 18% and 40.3% less than reported SPR by 15, 18 and 19 as they reported 59%, 45.4 and 67.7% respectively. This showed that in Punjab, Pakistan still SPR is less than other malarious countries in the world.

The resistance reported in the present study was 35.4% in the same rang (30.6 to 39) as reported by (9). Reported resistance (%) by (10) was less than present study, the reason of low resistance (%) would be the very initial stage of resistance emergence in Punjab, Pakistan in 1985. Globally resistance (%) to chloroquine against P.falciparum has been also reported by many authors (11) noted resistance (%) 47.9, 28, 23.1,16.9, 42.5, 29.4 and 12.8 more respectively than present study. This showed that the development of resistance exists almost in the all malarious countries of the world.

In present and other studies conducted by different researcher in Punjab, Pakistan resistance of grade III was not noted, except one case from district Rahim Yar Khan by (12) in a traveler came from Saudi Arabia. District wise analysis of present study data showed 41.6, 50, 41.6, 44.5, 25, 27.8 and 36.2% resistance in Sheikhupura, Muzaffargarh, Multan, Jhang, Faisalabad, Lahore and D.G.Khan respectively. The reason may be the topographical difference of areas. In 1984 resistance (%) was recorded 20 in district Multan (13), which have been increased 21.6 as per report of present study. The apparent reason of increase in district Multan is high falciparum malaria (31.6%) as compared to other study districts. 54.5 resistances (%) to chloroquine were reported in Faisalabad by (14), now it was detected 25%. In Faisalabad resistance (%) decreased, might be due to low FPR (16.8%). Resistance (%) in district Muzaffargarh was noted 44.4 in 1987, 46.6 in 1996, 31.2 in 1997 and 50 in present study. District Muzaffargarh is one of the districts where resistance (range 31.2-50) has been reported since 1987. In

Sheikhupura resistance was found 33.3% (15) and 41.6 during present study which showed increased trend.

Parasite density/µl was found very significant factor as it is directly proportional to the resistance (%). 53.8% resistance was noted among the subjects having ≥6000 parasite density/ µl. Similarly (16) had reported that high parasitaemia count was one of the important and independent predictors of resistance emergence and spread. This finding offered us unique opportunity to look at predictors of resistance. Our definition of time to develop resistance was based on the parasite's ability to grow despite the presence of chloroquine in the blood and the role of patient's immunity. A similar findings were noted by the (16, 17). In this study, we have examined the efficacy of the combination of chloroquine and sulphadoxine-pyrimethamine for treating uncomplicated falciparum malaria in Punjab Pakistan, compared to either with chloroquine or sulphadoxine-pyrimethamine alone. The resistance (%) of chloroquine monotherapy over 28 days was 35.4% in this study thus chloroquine is no longer useful as first-line treatment for malaria in Punjab Pakistan (18), suggested change of drug if resistance development increases more than 10% in order to prevent deaths. Change of drug is suggested on 25% resistance (19). The combination of chloroquine/ sulphadoxine-pyrimethamine is an efficacious treatment for uncomplicated malaria in Punjab Pakistan as resistance (%) was detected 4 (2 of 50) in the present study. The efficacy of chloroquine/sulphadoxine-pyrimethamine in Gambia had reported 13.9% in 2006 (20). The use of basoquine and sulphadoxine-pyrimethamine in combination is highly effective (21).

These preliminary results demonstrate the need for carefully designed studies to measure the contribution of resistant parasites to inadequate treatment of uncomplicated malaria in Punjab as combination treatments become more widely deployed. The drug resistance of same combination (Sulphadoxine-pyrimethamine and chloroquine) against uncomplicated P.falciparum also checked and reported 98.2%, 92.7% and 97% effectiveness respectively (22). This indicated that combination of sulphadoxine-pyrimethamine with chloroquine is effective remedy for treatment of resistant P.falciparum cases. The ACT ("artimisinine based combination therapy" artimisinine with sulphadoxine-pyrimethamine or artimisinine with 4-aminoquinoline) is a potential drug for the treatment of falciparum malaria.

Monotherapy of chloroquine, basoquine and sulphadoxine-pyrimethamine for treatment of falciparum malaria should be stopped. Artemisinin based combination therapy (ACT) should be adapted, as the combination of a short acting drug like artemisinin with a long acting drug like sulphadoxine-pyrimethamine has the advantage that any parasites remaining after the artemisinin derivative has taken effect are eliminated by a drug with a different mode of action. Rapid test for diagnosis of malaria is recommended for early detection and prompt treatment to achieve the millennium development goal (MDGs). 28-day follow-up test technique is recommended for in vivo resistance studies because it is not possible to detect RI by adopting 7-day or 14-day test technique. It is necessary to first map up the distribution and frequency of resistance through out the country. The use of molecular approaches to enhance the understanding of effective interventions will be useful.

Acknowledgment: Directorate General Health Services Punjab and Directorate of Malaria Control Pakistan is gratefully acknowledged for their assistance.

REFERENCES

1. Babiker HA, Satti G, Ferguson H, Bayoumi R, Walliker D. Drug resistant *Plasmodium falciparum* in an area of seasonal transmission. Acta Trop 2005; 94(3): 260-8.
2. Bjorkman A, Bhattara IA. Public health impact of drug resistant *Plasmodium falciparum* malaria. Acta Trop 2005; 94(3): 163-9.
3. Ferreira ID, Rosario VE, Cravo PV. Real-time quantitative PCR with SYBR Green 1 detection for estimating copy numbers of nine drug resistance candidate genes in *Plasmodium falciparum*. Malar J 2006; 18(5): 11-19.
4. Rowland M, Durrani N, Hewitt S, Sondorp E. Resistance of *falciparum* malaria to chloroquine and sulphadoxine-pyrimethamine in Afghan refugee settlements in western Pakistan: surreys by the general health services using a simplified *in vivo* test. Trop Med Inter Hlth 1997; 2(11): 1049-56.
5. Warhurst DC, Duraisingh MT. Rational use of drugs against *Plasmodium falciparum.* Trans R Soc Trop Med Hyg 2001; 95(4): 345-6.
6. Fontanet AL, Walker AM. Predictors of treatment failure in multiple drug resistant *falciparum* malaria: results from a 42 days follow-up of 224 patients in eastern Thailand. J Trop Med Hyg 1993; 49(4): 465-72.
7. Syafruddin D, Krisin K, Asih P, Sekartuti S, Dewi RM, Coutrier F, et al. Seasonal prevalence of malaria in West Sumba district, Indonesia. Malar J 2009; 8(1):8-10.
8. Asinzai MI, Kakarsulemankhel JK. Incidence of human malaria infection in northern hilly region of Balochistan, adjoining with NWFP, Pakistan: district Zhob. Pak J Biol Sci 2008; 11(12): 1620-24.

9. 13.Rana MS, Naqvi MA, Hussain R, Chaudhry NA. Chloroquine efficacy against *Plasmodium falciparum* in Punjab, *Pakistan.* Pakistan J Hlth 1997; 34(3): 19-22.
10. Khaliq AA, Sarwar M, Fox E, Stickland GT. Chloroquine resistant *Plasmodium falciparum* in Punjab. Pakistan. J Med Res 1985; 24(2): 89-93.
11. Atteke C, Ndong JM, Aubouy A. *In vitro* susceptibility to a new antimalarial organ metallic analogue, ferroquine, of *Plasmodium falciparum* isolates from the haut-Ogooue region of Gabon. J Antimicrob Chemother 2003; 51(5): 1021-24.
12. Yousaf M, Nadeem MA. Multi-drug resistant *falciparum* malaria in a Pakistani. Pakistan J Hlth 1996; 33(1-2): 27-28.
13. Annual Report Directorate of Malaria Control Islamabad Pakistan. 1985; 4-5.
14. Shah IH, Yasin M, Shah AM. Chloroquine resistant *falciparum* malaria in Faisalabad district Punjab. Nation Inst Mal Res Train 1988; 1(1): 82-95.
15. Rana MS, Naqvi MA, Hussain R, Chaudhry NA. Chloroquine efficacy against *Plasmodium falciparum* in Punjab, *Pakistan.* Pakistan J Hlth 1997; 34(4): 19-22.
16. Fontanet AL, Walker AM. Predictors of treatment failure in multiple drug resistant *falciparum* malaria: results from a 42 days follow-up of 224 patients in eastern Thailand. J Trop Med Hyg 1993; 49(4): 465-72.
17. Thwing JI, Odero CO, Odhiambo FO, Otieno KO, Kariuki S, Ord R, et al. *In-vivo* efficacy of amodiaquine-artesunate in children with uncomplicated *Plasmodium falciparum* malaria in Kenya. Trop Med Int Health 2009; 14(3):294-300.
18. Bjorkman A, Bhattara IA. Public health impact of drug resistant *Plasmodium falciparum* malaria. Acta Trop 2005; 94(3): 163-9.
19. World Health Organization, Basic malaria microscopy. WHO. Geneva. 1991; 56-69.
20. Samuel D, Rosalynn O, Hallett R, Jawara M, Walraven G, Mesa E, et al. Randomized trial of chloroquine, sulphadoxine-pyrimethamine in Gambian children with malaria: Impact against multidrug-resistant *Plasmodium falciparum.* PLoS Clin Trials 2006; 1(3):14-19.
21. Sarah GS, Moses RK, Grant D, Gasasira A, Grace N, Rosenthal PJ. Amodiaquine, sulphadoxine-pyrimethamine and combination therapy for treatment of uncomplicated *falciparum* malaria in Kampala, Uganda: a randomized trial. Lancet 2001; 358: 367-4.
22. Gasasira AF, Dorsey G, Nzarubara B, Staedke SG, Nasssali A. Comparative efficacy of aminoquinoline-antifolate combinations for the treatment of uncomplicated *falciparum* malaria in Kampala, Uganda. Am J Trop Med Hyg 2003; 68(2): 127-32.

Address for Corresponding Author:
Muhammad Saleem Rana,
Associate Professor,
Health Services Academy,
Islamabad.