IN VITRO RESPONSE OF *p.falciparum* TO CHLOROQUINE, AMODIAQUINE, QUININE AND SULFADOXINE/PYRIMETHAMINE IN THREE COMMUNITIES IN GHANA

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SUMMARY

In Vitro asexual parasite sensitivity to chloroquine, amodiaquine, quinine and sulfadoxine/pyrimethamine (SDZ/PYR) combination was determined for Plasmodium falciparum isolates from children in three schools at Nima (Urban), Madina (Periurban) and Gomoa Fetteh (Rural), during the rainy season. Chloroquine resistance was present in 62.5% of children at Nima, 69.6% (23/36) of children at Madina and 50% (16/32) of those in Gomoa Fetteh. Resistance of P. falciparum to amodiaquine was recorded in 30% (6/20), 35.7% (5/14) and 12.5% (1/8) of children in Nima, Madina and Gomoa Fetteh respectively. In addition parasite resistance to SDX/PYR was found in children in Nima, Madina and in one child at Gomoa Fetteh. Resistance to both chloroquine and amodiaquine was present in 3 children at Nima, 5 at Madina and in a single child at Gomoa Fetteh. Resistance to both chloroquine and SDX/PYR and to Amodiaquine and SDX/PYR was restricted to only Nima (2/15, 1/15) and Madina 3/14, 2/15). There was no resistance to quinine in any of the areas studied. A progressive increase in minimum inhibitory concentrations (IC_{90}) for chloroquine was observed from Nima and Madina to rural Gomoa Fetteh. Similarly IC_{90} for amodiaquine was highest in Madina. The presence of multiple-drug resistant P. falciparum in these children represents a challenge to the control and management of falciparum malaria and this data serves as a baseline for monitoring any changes in parasite sensitivity to antimalarial drugs in the study areas.

Key Words: Chloroquine, Quinine, Sulfadoxine/Pyrimethamine, P. falciparum, In vitro sensitivity.

INTRODUCTION

Multiple drug resistant *Plasmodium falciparum* occurs in most malaria endemic parts of the world and poses a serious threat to the management and control of malaria.

In some parts of South East Asia for instance, chloroquine and sulfadoxine/pyrimethamine (SDX/PYR) combination (Fansidar), hitherto considered as potent antimalarial are no longer useful for malaria treatment and prophylaxis[1,2]. Resistance to mefloquine which is a relatively new drug has also been reported in different parts of the world including West Africa[3,4].

The development and introduction of techniques for the in vitro cultivation of *P. falciparum*[5]

has made an important turn in the study of chemotherapy of malaria. These *in vitro* microtests, although not meant as replacement for the *in vivo* assessment of the response of patients to treatment, however, provide useful means for quantitative monitoring of parasite sensitivity to drugs and are convenient for the early detection of the emergence of drug resistance and the evaluation of new compounds without any significant influence of the host immune status[6]. In addition drug-resistant malaria parasites can reliably be picked up and with relative ease in field studies. Such studies have been used to delineate areas or regions where drug-resistant infections are prevalent[7,8]. In a study in 1985 Hogerzeil and others failed to detect any *in vitro* chloroquine resistance in Ghana[9]. The resistance of *P. falciparum* to chloroquine has now been established *in vivo*[10,11] and subsequently in both in vivo and *in vitro*[12] tests. This resistance has largely been attributed to drug pressure, partly due to subcurative doses and/or self-medication[13,14]. A consequence of emergence of chloroquine resistance has been the increased use of other antimalarial drugs.

The present study was conducted to determine the *in vitro* response of *P. falciparum* from children in three different communities (urban, periurban and rural) in southern Ghana to chloroquine, amodiaquine, SDX/PYR combination and quinine.

In vitro Microtest: The standard WHO microtest (MarkII) was used and the procedure was as outlined in the manual, with some modifications. Briefly, preculture blood films were made for each child selected following screening. Simultaneously 100 microlitres of blood was drawn into a heparinised capillary tube and immediately transferred into 900 microlitres of RPMI 1640 medium supplemented with L-glutamine HEPES buffer, sodium hydrogen carbonate and gentamicin sulphate. Specimen were transported to the laboratory within 3 hours of collection and 50 microlitres each added to wells of microtitre plates predosed with varying concentrations of each antimalarial drug. An undruged well served as a control. All plates were incubated at 37.5^{0}C in a candle jar for 30 hours. The culture supernatant was carefully siphoned off with pastuer pipetes and separate smears made of the contents of each well. Slides were stained as before and examined. Counts of mature schizonts per 200 asexual (mature) parasites were made. With regard to SDX/PYR, only mature schizonts with at least 8 distinct nuclei were counted. Chloroquine concentrations were 1,2,4,8,16,32 and 64 picomoles per well, amodiaquine concentrations were 0.25, 0.50, 1.00, 2.00, 4.00, 8.00 and 16.00 picomoles per well, quinine concentrations were 4,8,16,32,64,128 and 256 picomoles and SDX/PYR concentrations were 10,30, 100,300,1000,3000, and 10000 (dose shown for sulfadoxine but ratio of 80:1 maintained).

RESULTS

Twenty-four successful chloroquine susceptibility microtests were performed for children in Nima, 39 in Madina and 32 in Gomoa Fetteh while the number of successful amodiaquine microtests were 20 in Nima, 14 in Madina and 8 in Gomoa Fetteh. In the case of SDX/PYR, a total of 15 tests were done in Nima, 14 in Madina and 9 in Gomoa Fetteh.

Resistance was considered present when mature schizonts were encountered in 8 pmol/well ($1.6x10^{-6}$ mol/L blood) or more chloroquine, 4 pmol/well ($0.8x10^{-6}$ mol/L blood) amodiaquine of 256 pmol/well ($51.2x10^{-6}$ mol/L blood) of quinine. On the basis of these, chloroquine-resistant *P. falciparum* was present in 62.5% (15/24) of children at Nima, 60.6% (23/36) of those in Madina and 50% (16/32) children at Gomoa Fetteh. Resistance to amodiaquine was found in 30% (6/20) children in Nima, 35.7% (5/14) of those at Madina and in a single child at Gomoa Fetteh. There was no resistance to quinine in all three communities. The effective SDX/PYR concentration (IC_{90}) determined for parasites from the three communities were 184.6 pmol/well SDX and 2.3 pmol/well PYR (Nima), 3769.2 pmol/well SDX and 47.1 pmol/well (Madina) and 3285.7 pmol/well SDX and 41.1 pmol/well PYR (Gomoa Fetteh). As a result *P. falciparum* resistance to SDX/PYR was seen in 5 children in Nima, 3 in Madina and one child in Gomoa Fetteh.

FIG.1: IN VITRO SENSITIVITY PATTERN OF *P. FALCIPARUM* FROM CHILDREN IN NIMA, MADINA AND GOMOA FETTEH, 1988.

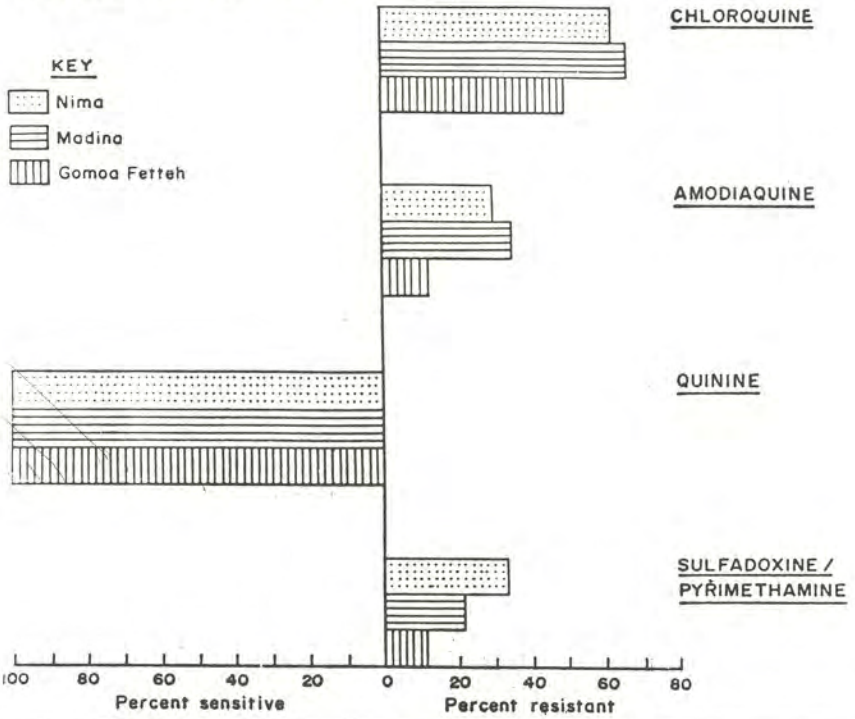


FIG.2: RESPONSE OF *P. FALCIPARUM* TO QUININE IN THREE COMMUNITIES IN SOUTHERN GHANA. 1988

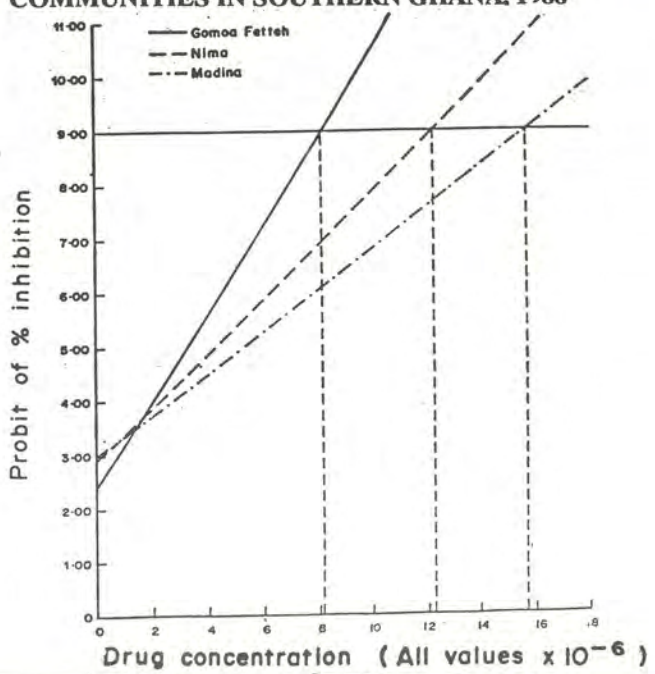


Fig. 1 illustrates the proportion of children with resistance and those with drug sensitive parasites in all the communities.

Graphs of probits of percent inhibition against drug concentrations for chloroquine, amodiaquine SDX and quinine are presented in Figs. 2-5. The IC_{90} values obtained for chloroquine are 10.7×10^{-6} mol/L (53.5 pmol/well) for Gomoa Fetteh, 14.9×10^{-6} mol/L (74.5 pmol/well) for Madina and 16.2×10^{-6} mol/L (81.0 pmol/well) for Nima. Figures for amodiaquine are 1.5×10^{-6} mol/L (5.7 pmol/well) for Gomoa fetteh, 1.02×10^{-6} mol/L (5.1 pmol/well) for Nima and 1.80×10^{-6} mol/L (9.0 pmol/well) for Madina. In the case of quinine, IC_{90} values are 12.2×10^{-6} mol/L (61.2 pmol/well) for Nima, 12.7×10^{-6} mol/L (78.5 pmol/well) for Madina and 8.1×10^{-6} mol/l (40.5 pmol/well) for Gomoa Fetteh. The IC_{90} values for quinine were well below the recommended concentration of quinine (51.2×10^{-6} or 256 pmol/well) required for complete inhibition of schizont maturation while for chloroquine and amodiaquine IC_{90} values were above the recommended values of 1.6×10^{-6} mol/L (8 pmol/well) and 0.8×10^{-6} mol/L (4 pmol/well) respectively.

DISCUSSION

Although chemotherapy remains the most important tool for the management and control of malaria, the emergence and spread of drug resistant strains of *P.falciparum* poses a problem to its effective use. The results of the present study confirm the existence of chloroquine-resistant strains of *Plasmodium falciparum* in both rural and urban communities in Ghana.[10,11,12] Resistance to chloroquine was more prevalent among children in Nima, an urban area followed by Madina a periurban area and finally Gomoa Fetteh, a rural community. Similarly, IC_{90} values which indicate minimum concentration of chloroquine required to achieve a 90% inhibition of schizont maturation was highest in Nima, followed by Madina and Gomoa Fetteh having the least. These values are all higher than the World Health Organization (WHO) recommended critical concentration needed to achieve complete inhibition of schizont maturation. Thus for chloroquine, 78% inhibition was achieved in Gomoa Fetteh, 72.5% in periurban Madina and 62.5% in urban Nima. It is evident that there is a loss of sensitivity to chloroquine by *P. falciparum* in the communities studied. In a similar study in East Africa, in vitro resistance to chloroquine was demonstrated in 72% of the children in an urban community, [4] a value higher than ours.

Extensive use of any antimalarial as a single entity inevitably leads to the emergence of strains of highly resistant parasites sooner or later. This situation has arisen regarding chloroquine in Ghana, where self-medication with chloroquine is common and subcurative doses are employed.[8,13,14] Once *P. falciparum* has become resistant to the 4-aminoquinolines, notably chloroquine, the resistant parasites acquire great facility for the development of resistance to compounds chemically related or even to compounds which are quite different from it. The resistance of parasites to amodiaquine is therefore hardly surprising. In Rwanda for instance, amodiaquine has been found to have lost its effectiveness in treating falciparum malaria following development of chloroquine resistance[15] Resistance of *P. falciparum* to SDX/PYR and maloprim (Dapsone/Pyrime thamine combinations) has been reported in South East Asia and parts of East Africa[16] No report has however come from the West African subregion. We observed in vitro resistance to SDX/PYR in 5 children in Nima, 3 in Madina and a single child in Gomoa Fetteh.

Although resistance to quinine has been reported in some parts of the world[17], in Africa only a reduction in sensitivity has been recorded. Our results indicate no resistance to quinine in the three areas studied. This is very important as quinine remains the drug of choice in the treatment of severe and complicated malaria[18].

In conclusion the presence of chloroquine resistant *P. falciparum* in children studied presents a challenge to the control and management of falciparum malaria. The data serves as a baseline

FIG.3: IN VITRO RESPONSE OF P. FALCIPARUM TO CHLOROQUINE IN THREE COMMUNITIES IN SOUTHERN GHANA - 1988

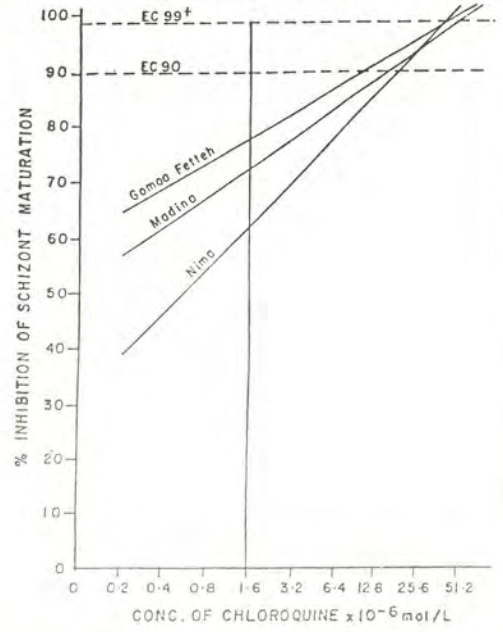


FIG.4:RESPONSE OF P. FALCIPARUM TO AMADIAQUINE IN CHILDREN IN THREE COMMUNITIES IN SOUTHERN GHANA - 1988

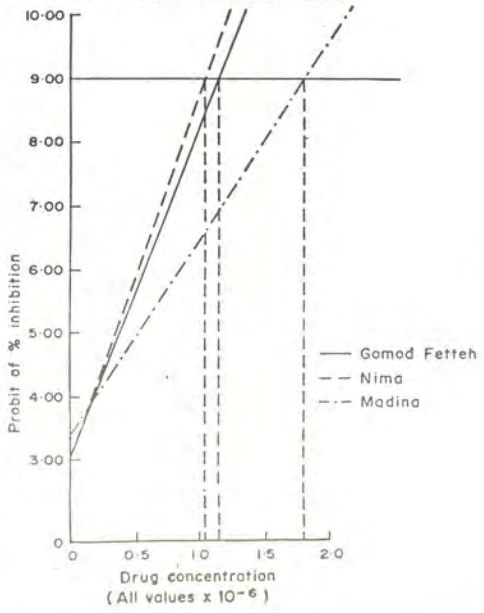
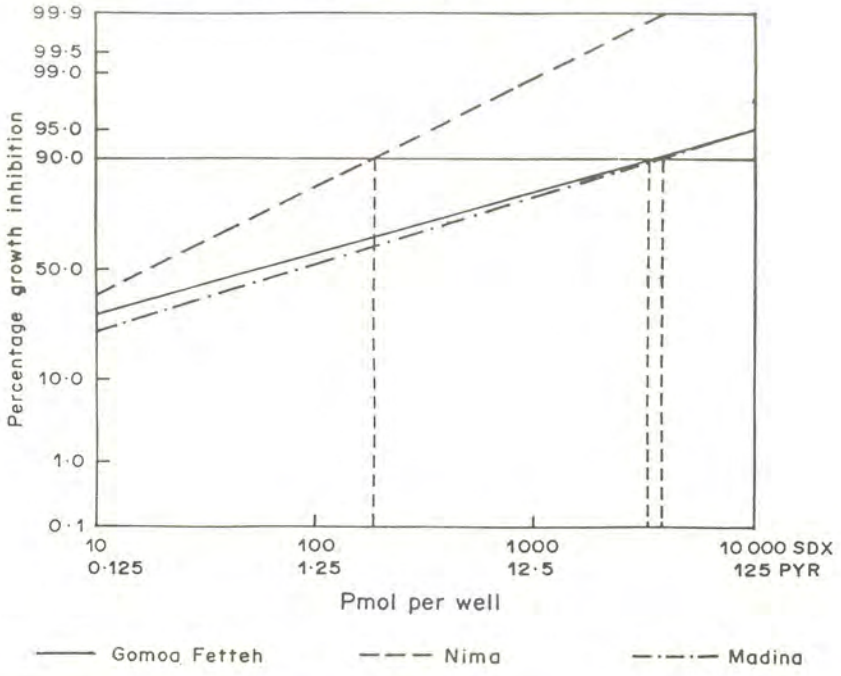


FIG.5: INHIBITION OF MATURATION OF *P.FALCIPARUM* IN DIFFERENT CONCENTRATIONS OF SULFADOXINE AND PYRITHAMINE.



for continual monitoring of any changes in parasite sensitivity to antimalarial drugs in the country and also calls for the careful use of the alternative antimalarial so as to avoid the rapid development and spread of drug-resistant parasites.

REFERENCES

1. Harinisuta, T. Dixon K.E., Warrel D.A. and Doberstyn E.B. *Recent advances in malaria with special reference to South East Asia.* S.E. Asian J. Trop. Med. Pub. Hlth. 1982; 13: 1-34.

2. Wesbster H.K. *Antimalarial drug resistance: Problems and prospects.* Thai J. Pharmacol. 1982; 4: 171-191.

3. Boudreau E.F. Wesbster H.K. Pavanand K. and Thosingha L. *Type II mefloquine resistance in Thailand.* Lancet 1982; 2: 1335.

4. Salako L.A. and Aderounmu A.F. *In vitro chloroquine and mefloquine resistant P. falciparum in Nigeria.* Lancet 1987; i: 572-573.

5. Rieckman K.H. *Drug sensitivity of Plasmodium falciparum and-in vitro microtechnique.* Lancet 1978; 1: 22.

6. Dorberstyn E.B. *Resistance of Plasmodium falciparum Experimentia.* 1984; 40: 1311-1317.

7. Smarkovski L.L. Buck R.L. Alcantara A.K. Rodriguez C.S. and Ulyangco V. *Studies of resistance to chloroquine, quinine, amodiaquine and mefloquine among Philipine strains of Plasmodium falciparum.* Trans. Roy. Soc. Trop. Med. and Hyg. 1985; 79: 37-41.

8. Spencer H.C. Masaba S.C. Chulay J.D. and Nguyen-Dinh P. *Field evaluation in Kenya of a 48-hour in vitro test for Plasmodium falciparum sensitivity to chloroquine.* Am J. of Trop. Med. and Hyg. 1983; 32: 916-921.

9. Hogerzeil H.V. Hogewoning A.A. Van Doorn J.W. Wernsdorfer W.H. and Van Deer Kaay H.J. *In vitro assessment of sensitivity of Plasmodium falciparum to chloroquine and mefloquine in Ghana.* Trans. Roy. Soc. Trop. Med. and Hyg. 1985; 6: 808-811.

10. Neequaye J. *In vivo chloroquine-resistant falciparum malaria in Western Africa.* Lancet 1986; i: 153.

11. Ofori-Adjei, D. Adjepon-Yamoah K.K. Commey J.O.O. and Ofori-Adjei, E. *In vivo sensitiviaty of P. falciparum to chloroquine in Accra, Ghana.* Ghana Med. J. 1988; 22: 11-14.

12. Afari, E.A., Akanmori, B.D. Nakano, T. Ofori-Adjei, D. Owusu-Adjei, S Gyan, B and Adjei, A.A. *In vivo and In vitro sensitivity status of P. falciparum to chloroquine in three communities in Ghana.* Ghana Med. J.

13. Gardiner C. Biggar R.J. Collins W.E. and Nkrumah F.K. *Malaria in urban and rural areas of Southern Ghana: a survey of parasitaemia, antibodies and antimalarial practices.* Bull WHO 1984; 62: 607-613.

14. Ofori-Adjei, D., Commey, J.O.O. and Adjepon-Yamoah K.K. *Serum chloroquine levels in Children before treatment for malaria.* Lancet 1984; i: 1246.

15. Le Bras J. Lancet 1985; ii: 1071.

16. Hess et al. 1983. Am J. Trop. Med. and Hyg. 32: 217-220.

17. *Severe and complicated malaria.* Trans. Roy. Soc. Med. Hyg. 1986; 80: (supplement) 1-50.