TITLE: PLASMA, URINARY CHLOROQUINE LEVELS AND PLASMODIUM FALCIPARUM PARASITAEMIA IN CHILDREN TREATED FOR MALARIA AT KENYATTA NATIONAL HOSPITAL.

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DECLARATION;

I declare that this dissertation is my original work and that it has not been submitted elsewhere for purposesof obtaining a degree. It is in part fullfilment of the degree of master of Medicine (Paediatrics and Child Health) of the University of Nairobi.

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S U M M A R Y:

Between June 1984 and February 1985, 32 patients with Plasmodium falciparum malaria were studied. They were aged between 3 months - 12 years. The clinical response, parasitaemia levels, plasma and urinary chloroquine levels, and in vivo and in vitro chloroquine sensitivity were determined.

The parasite counts ranged from 39/300 w.b.c. - 9,630/300 w.b.c. On the third day 21(66%) of the patients showed clinical cure (i.e. symptomatic relief without necessarily having parasite clearance). Of these 6(19%) had cleared their parasites on chloroquine therapy, while 15(48%) were asymptomatic but still had parasitaemia. One patient was dropped from parasitaemia analysis because of incomplete counts. The other 10(32%) patients still had clinical malaria and parasitaemia on day 3.

Persisting symptoms on both days 3 and 7 were fever and vomiting in 10(32%) patients and 8(26%) patients respectively. They had associated parasitaemia.

A total of 13(42%) patients cleared on chloroquine during the study. The average clearance time on

chloroquine was 5 days,, The other 18(58%) patients failed to clear. Of these 10(32%) had their treatment changed on day 3 based on clinical and parasitological grounds. In the remaining 8(26%) patients treatment was changed between day 7 and 28 of follow up„ They all had clinical malaria.

The pattern of in vivo response was: Sensitive (S) - 13(42%) ; Resistant (R) at R 4(13%); R_{11} 8(26%) and R 6(19%).

There was therapeutic plasma chloroquine levels pretreatment in 19/22 (86%) of samples analysed. One of these had toxic levels of 0.7467 ng/ml_0 More patients attained toxic levels following administration of chloroquine in the hospital by the 2nd and 3rd day 8/22(36%). The therapeutic range for plasma chloroquine is O.Olng/mi -0.03Mg/ml The upper limit of tolerable level was taken as 0.4gg/mlo

In vitro sensitivity tests by Rieckmann micro-test were successifully done in 19 cases. The minimal Inhibitory Concentration (MIC) was taken as UlnM/L (0.03648ng/ml). Of the isolates 3/19(16%) were sensitive in vitro. They were

also sensitive in vivo. The other 16/19(84%) were resistant in $vitr_0$. Of these 7(37%) were resistant at concentrations equal to or above 640nM/L (0.2048Mg/ml). This was the highest attainable concentration on the plates used. There was disparity found between resistance in vitro and resistance in vivo. 6(32%) isolates were resistant in vitro but sensitive in vivo. Their MIC was mostly 640nM/L(0.2048Mg/ml) and over. All resistant in vivo cases were also resistant in vitro.

Urinary chloroquine assays showed (63%) of samples analysed had detectable chloroquine pretreatment.

There was no correlation between plasma chloroquine levels and parasite clearance by day 3.

All patients were from rural areas and were visiting Nairobi. Resistance in vivo and in vitro was demonstrated from Eastern, Nyanza, Western and Central provinces. There are pockets of resistant P. falciparum strains in endemic areas in Kenya as has been demonstrated at the coast and Kisumu in previous studies and now in Eastern Province in this study.

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INTRODUCTION:

Malaria is a serious disease in childhood. It is highly prevalent in children in East Africa, especially during the period when passive immunity is waning, and before active immunity is acquired (1). It is one of the top ten causes of morbidity and mortality in pre-school children (2). The same is true in Kenya where the disease is endemic in many parts of the country (3).

The diagnosis of malaria is confirmed by demonstrating malaria parasites in peripheral bbod films (4 , 5) „ The major causative species is P_0 falciparum in East Africa. Other types of malaria do occur C6). In some cases the outcome of the disease correlates with level of parasitaemia and the infecting species. A parasitaemia of 2% - 5% is usual in P. falciparum infection, a parasite that infects red-blood cells of all ages (7).

In Kenya the drug of choice for the treatment of malaria is Chloroquine. Local studies have shown in general, good sensitivity of the local P. falciparum steins to this drug (8,9,10).

Some cases of chloroquine resistance as defined by World Health Organisation (W.Ii.O.) (11),

especially in the nonimmune tourists visiting East Africa have been reported since 1978 (12, 13,14,15,16,17).

The first case of chloroquine resistance from Kenya was described in 1978 (14,15). Spencer and others have recently reported in vivo and in vitro Chloroquine resistance in indigenous Kenyans (18) (50). Kenya (6)and Spencer (19) have reported separately some cases of possible resistance or reinfection. There have been other reports of changing pattern of Chloroquine sensitivity both in vitro and in vivo in Kenya (20,21,26).

While working in the Paediatric Observation ward (POW; at Kenyatta National Hospital (KNH), the author-observed a number of cases of malaria which did not respond to the standard Chloroquine therapy. Fansidar, a reserve drug, had to be used in their treatment. Among the presenting symptoms in these patients were diarrhoea and vomiting. While diarrhoea does not affect blood chloroquine levels, vomiting does (22). It. is following these observations that an effort was made to study the clinical response of children with malaria treated with oral chloroquine at the standard dose of 25mg/kg body weight,with a view of correlating the parasitaemia with plasma and urinary chloroquine levels,,

AIMS AND OBJECTIVES:

1. To study the pattern of clinical response of children treated for malaria with oral Chloroquine» The following parameters were studied: Body temperature, frequency of diarrhoea, vomiting and convulsions.

2. To correlate plasma and urinary Chloroquine levels and parasite clearance,.

3 To determine the P. falciparum resistance pattern to Chloroquine among these patients.

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MATERIALS AND METHODS:

Study Area:

The study was carried out between June 1984 and February 1985 at the Paediatric Observation ward (POW) of Kenyatta National Hospital. This hospital is a regional Hospital to Nairobi and surrounding districts, a central referral centre for the whole country and a teaching hospital,

The outpatient department (OPD) of this hospital includes a Paediatric Filter Clinic (PFC) which handles all sick children using the hospital for the first time.

The clinical staff in PFC, which include Medical Officers (MO's) and Clinical Officers (CO's) with special training in Paediatrics, were approached for assistance. They were to request blood slides for malaria parasites for all clinically suspected cases of malaria. All positive P. falciparium cases were then to be referred to the author. Cases were screened throughout the week during the day time sessions. No patients were referred at night.

The author interviewed the parents or guardian of the referred patients individually. Patients of 12 years and below were considered for the study. The study protocol (appendix 1) and purpose, were explained. Consent was then obtained before the patient was admitted into the study. All referred patients were clinically examined and treated by the author.

Clinical diagnosis of malaria was made based on the presence of all or a combination of the following: a history of residence or recent travel in an endemic area; fever; diarrhoea; vomiting; chills and rigors, malaise, and convulsions. The clinical signs included fever (temperature), pallor, jaundice; splenomegaly and hepatomegaly.

An experienced technician from the Division of the Vector Borne Diseases(DVBD) made the slides. Two thick and two thin blood slides were made from finger prick for suspected cases. They were delivered to the Routine Laboratory where another D.V.B.D. technician stained the thick films with field stain A and B. He examined them under a light microscopeo Only cases of pure P. falciparum malaria were accepted in the study. The thin film was stained by Giemsa stain for species identification.

For the study patients the protocol (appendix 1) was filled following the clinical examination. The positive slide for malaria was verified. Only positive cases of P. falciparum malaria were included,, This was because:

i) P. falciparum has only one exoerythrocytic cycle. There would be no more re-seeding of parasites after the schizontocidal effect of chloroquine on the erythrocytic phase.

ii) Todate parasite resistance to chloroquine has been associated with P„ falciparum only.

iii) Chloroquine is not a tissue schizontocide. Only children whose parents or guardians certified no chloroquine ingestion at least two weeks prior to onset of illness were accepted into the study.

Excluded from the study were the very ill children with cerebral malaria or severe anaemia needing urgent transfusion. it was difficult to see them before start of drug therapy and other urgent measures. Patients who had malaria in addition to some other diagnosis such as pneumonia were excluded.

The study patients were admitted into the P.O.W. for three days for chloroquine therapy. A parent or a guardian stayed with each child on the ward. A daily clinical review and parasite count was done for each patient on the ward, also on the 7th day, and any other time within 28 days if patients reported ill. All patients reported back for review.

TREATMENT:

Drug administration:

All patients were weighed pre-treatment$_0$ The Tonedo Scale Model 1361 Sentinel from Tonedo Scales Tonedo Ohio, Division of Reliance electric company USA, was used for the under 2 years olds. The bigger children were weighed on an adult spring balance. Using the weight, $_R$ total . $_{dose}$, 25mg/kg body weight was administered over 3 days as follows:-

i) at zero hour - 10 mg/kg stat

ii) at 6 hours later - 5mg/kg stat

iii) at 24 hours later - 5mg/kg - O.D.

iv) at 48 hours later - 5mg/kg - 0.,D.

Syrup Chloroquine phosphate from the hospital pharmacy was used. The strength was 50mg/5ml.

Other supportive measures were given. These included oral rehydration for dehydrated children, tepid spongying for high-fevers, and anticonvulsants for convulsions.

Drugs were administered by a qualified nurse in POW treatment room. Where vomiting occured within 30 minutes of drug ingestion, a repeat dose was given. This was at the same measure as the one vomited. The vomitus was not measured.

Another antimalarial drug was given if the patient showed no improvement clinically and still had parasitaemia by the third day. If by the 7th day there was symptomatic malaria with a positive malaria slide, treatment was also changed. If within 4 weeks of initial treatment there was recurrence of malaria and a positive slide for malaria parasites, again another antimalarial was given.

The alternative drugs given to the chloroquine non-fossportders were: Quinine in combination with Fansidar

or Metakelfin. The dose of Quinine was lOmg/kg three times daily for ten days. Fansidar was given as a single dose:

\ tablet for under 3 year olds

1 tablet for 4-11 year olds

2 tablets for 12 year olds

Metakelfin was given in the same dosage as Fansidar (23).

t

SAMPLE COLLECTION:

URINE:

Pretreatment 20 ml of urine were collected by the clean catchment, method, transferred into a screw capped bottle and stored at -25°C until analysed. Similar urine specimens were tak€?n 2 hours following administration of 3rd and 4th doses of chloroquine.

BLOOD: Venous blood, 7.5 ml, were drawn by standard venepuncture procedure from a cubital vein, pre-treatment. 2ml was put in a sequestrene bottle and sent for full blood counts, peripheral blood film (PBF) and hemoglobin electrophoresis when indicated. From this sample duplicate thin and thick blood slides were made and sent to parasitology department for first day parasite counts. Another 2 mis were put in a sequestrene bottle , this blood was centrifuged

immediately and the plasma separated„ The plasma was then kept at -25°C until analysed„ 1.5ml was put in a heparinized container. It was kept cool to be used for the /vitro chloroquine sensitivity testing as soon as possible. The last 2mls were sent in a plain biochemistry bottle for urea and electrolytes (U/E) estimation.

A further 2ml of blood was drawn 2 hours after the 3rd and 4th dose$_s$ of chloroquine respectively. They were immediately centrifuged. The plasma was separated as above and kept at -25°C until analysed.

Duplicate slides were made from the sequestrenated samples taken after the 3rd and 4th doses of parasite counts.

ANALYSIS;

The analysis was done at the Traditional Medicines and drugs research centre of Kenya Medical Research Institute- Nairobi. Plasma and urinary chloroquine assays: Chloroquine extration was essentially the same as the one reported previously (24)$_0$ The only modifications were as follows: the interval- used was amodiaquine. The protein precipitation

step was replaced by saturation of the mixture with powdered ammonium carbonate.

HPLC*analysis: The procedure was as previously reported (24) with a few modifications. The aqueous phase, p Ho $2_0 3$, contained 0.5M$KH_2PC>_4$, O.IM triethanolamine. Acetonitrile was the organic modifier. The buffer acetonitrile mixture ratio was 58:42 respectively using a micro-processor controlled gradient mixer. The flow rate was 1.2ml/min.

Parasite Counts:

Thin and thick blood smears were stained with 4% Giemsa stain,, They were then examined under the light Microscope. Asexual parasites (Trophozoites) were counted against 300 white blood cells (W.BoC.) on the thick smears,

Haemogram: The full blood counts were done by the Coulter Counter Model S, from Coulter Electronics Co. Ltd. England.

Urea and Electrolytes: These were auto analysed by the Technicon Autoanalyser SMA II from Technicon (Ireland) Ltd. Swods Co. Dublin.

*HPLC -High pressure liquid Chromatography.

In vitro Sensitivity Tests:

The tests were done at the Clinical Research Centre of Kenya Medical Research Institute - Nairobi. Of the test blood, 0.1 ml, from the heparinised container was added to the culture medium RPMI 1640 with 25mM Hepes and 25mM $NaHC0_3$, giving a final dilution of 1:10. 50pl of the diluted blood was then added to the predosed chloroquine plates prepared and supplied by W.H.O. The plates had wells with increasing amounts of chloroquine to give the final concentrations as follows:-

Well	Concentration	nMol/L
A	0	Control
B	20	Test
C	40.	- "
D	80	- "
E	114	- "
F	160	- "
G	320	- "
H	640	- "

The culture material was added starting with the control and proceeding to the highest concentration using a micropip$_{etl(}$ introducer„

The plates were then covered, gently rocked to mix the culture and chloroquine. They were placed in a moist gas tight box. This was sealed. A gas jet was passed through for one minute and the valves closed to trap some gas in the box. The gas composition was: 3% CO_2, 5% O_2 and 92% N_2. The box was then placed in an incubator at 37.5°C for 24 hours. At the end of 24 hours the cultures were harvested,, The supernatant was t
discarded. Thick blood films were made, after mixing the sediment thoroughly, starting with well H and going up to well A. Slides were left to dry overnight then stained with 2% Giemsa for 1 hour. They were then examined under a light microscope.

Schizont maturation was assessed. A valid test was taken as growth of schizonts over 5% pre-treatment parasitaemia or at least a count of 20 schizonts in the control well. Schizonts were counted against 300 Wbc.

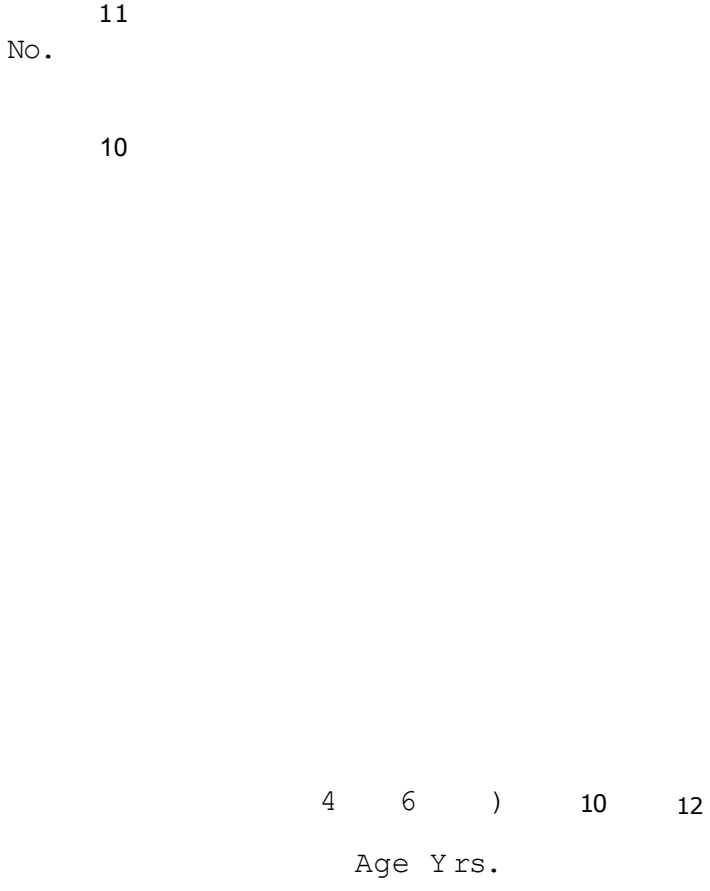
The minimal inhibitory concentration (MIC) was taken as the well which was associated with schizonts maturation of 1% of the control well or less.

R E S U L T S :

The total number of patients studied was 32. There were 18 males and 14 females. Their age distribution was as shown in figure I .

FIG 1. SHOWS THE AGE DISTRIBUTION OF 32 PATIENTS

Fig.. 1. Age distribution of 32 patients



A total of 63% were less than 6 years of Age

The mean age was 5 yrs. The age ranged from 3 months, to 12 years.

Weights:

All the patients were found to have normal weights for age. The average weight was 15.8kg. The range was 5.2 - 32 kg.

Symptoms:

Table I shows the number of observations of symptoms made on day 1, 2, 3, and 7.

TABLE I: SYMPTOMS IN 32 PATIENTS ON DAY 1, 2, 3, and 7:

Sytri ptoms	No of patients observed on day			
	1	2	3	7
Fever	30	18	11	8
Vomit ing	20	12	10	3
Chills & Rigors	17	12	1	0
Diarrhoea	9	9	1	0
Convulsions	5	1	0	0

There is a general reduction in the symptom frequency per day. On third day the main persisting symptoms were fever and vomiting. This was also noted on day 7.

Signs:

Table 2 shows the presenting signs at admission among the 32 patients.

TABLE 2: SIGNS IN 32 PATIENTS ON DAY 1

Sign	No.	%
Pallor	16	50
Splenomegaly	7	22
Jaundice	6	19
Hepatomegaly	5	16

Pallor was the commonest presenting sign followed by splenomegaly, Jaundice and Hepatomegaly„ There was no change in signs on day 1, 2, 3 and 7.

Haemoglobin levels:

In Table 3: is shown two age groups of patients, their mean Hb and the number found anaemic.

TART.F; 3: MEAN HI) AND ANAEMIA IN 32 PATIENTS:

Age group	No.	Av. Hb.gm/dl	No. anaemic
6M - 6 yrs	20	9.1	16
7 yrs -l2yrs	12	10.2	7

Anaemia was defined as:

lib < 10.5gm/dl - for age group 6M-6yrs.

Hb < llgm/dl - for age group 7yrs-12years (51),

Of the total patients 23 (72%) were found to be anaemic. 17(80%) of the age group 6M-6 yrs and 7(58%) of age 7 yrs - 12 years were anaemic.

Peripheral blood films showed the following picture: Normocytic normochronic (20), micro-cytic hypochronic (8); Normocytic hypochromic (3); Macrocytic normochromic (1).

Urea and Electrolytes:

U/E in all patients was within normal limits for sodium; potassium and BUN.

Parasite counts:

Table 4 shows frequency distribution of P. falciparum trophozoites per 300 wbc for 31 patients for days 1, 2, 3 & 7. Note one patient had incomplete data and was excluded.

TABLE 4: PARASITE DENSITY OF 31 PATIENTS ON DAY 1, 2, 3 & 7:

Day	Trophozoite Density/300 wbc Over 401	401 - 201	200 - 1	No. Patients (%) 0
1	15 (48)	6 (19)	10 (33)	0 (0)
2	13 (42)	5 (16)	13 (42)	0 (0)
3	8 (26)	6 (19)	11 (35)	6 (20)
7	2 (7)	0 (0)	5 (16)	24 (77)

Parasite densities were grouped into 4 groups for ease of reference.

Group 1 - had counts of 401/300wbc and above

2 - had counts of 400-201/300 wbc

3 - had counts of 200-001/300 wbc

4 - had counts of 0/300 wbc

There is a general decrease in the trophozoite density through to day 7 as seen in table 4 and figure 2. Only 6 patients (19%) of the patients had cleared their parasites by day 3. By day 7 24(77%) of the patients had cleared their parasites. This figures includes patients whose treatment was changed on day 3 to other anti-malairals other than chloroquine due to lack of clinical response.

Of the 25/31 patients (80%), who had not cleared their parasitaemia on day 3, 10(32%) had their treatment changed on day 3. Of these 2 had fever alone and 3 had vomiting alone, the rest had fever and vomiting. They had associated general malaise. Their range of parasite count was 39/300 wbc - 9,630/300 wbc. 4 of these had corresponding in vitro drug resistance showing an MIC range between 0,i024$_{M}$g/ml - 0 2048 kg/ml.

Cf the remaining 15 patients, 8 had recrudesced between day 7 and 28 post treatment. They had clinical malaria. Their treatment was changed. The rest (7) had cleared their parasites and did not recrudescence.

Therefore 13 (42%) cleared on chloroquine alone, 10 (32%) had their treatment changed on day 3, and 8 (26%) had other antimalarial drugs given between day 7 and 28„ In vivo response is therefore 42 % sensitive and 58% resistant as shown in table 5.

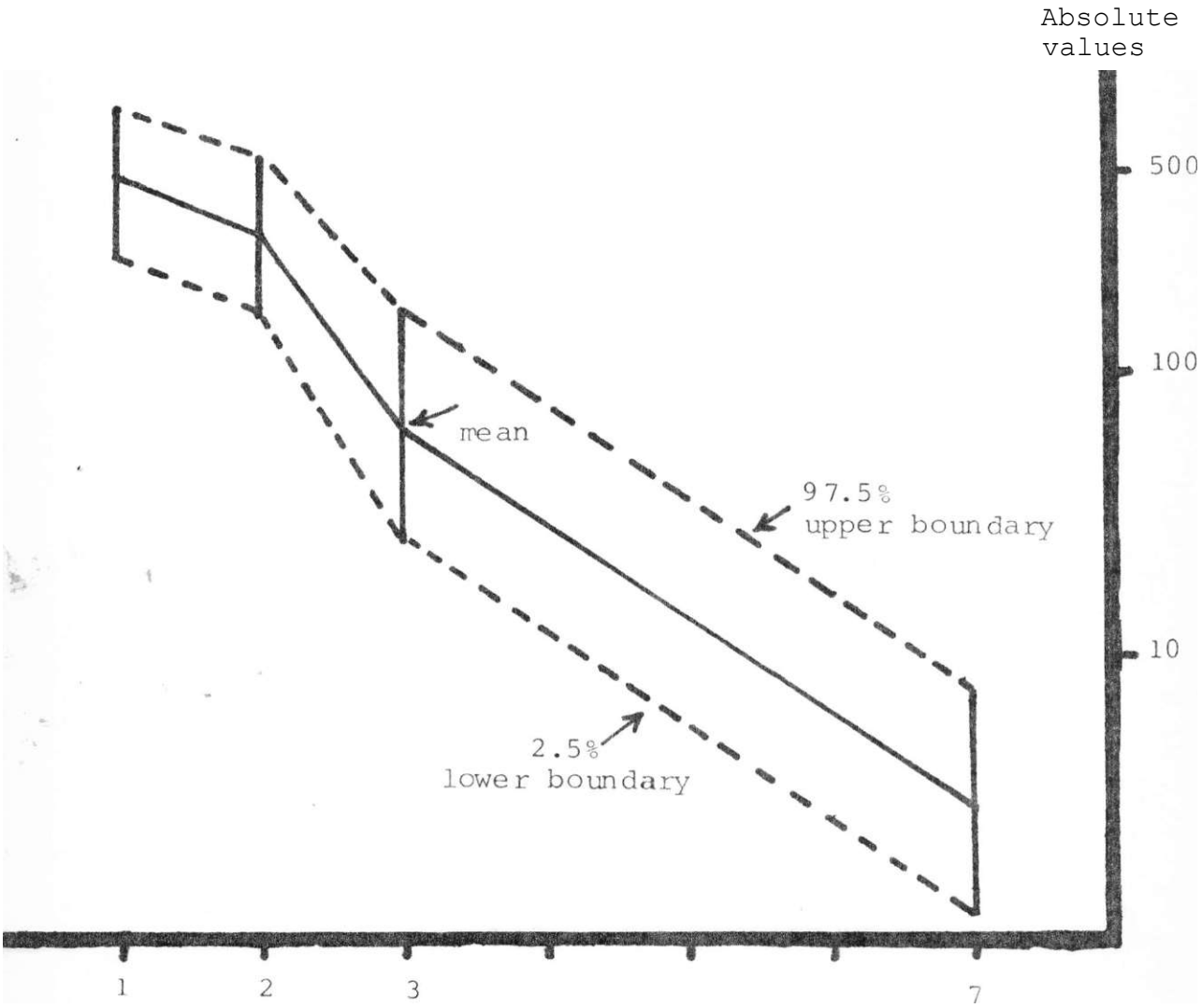
TABLE 5: IN VIVO RESPONSE OF 31 PATIENTS:

Response	No.	%
S (sensitive)	13	42
R^(Resistant)	4	13
R_2	8	26
R_3	6	19

58% of patients showed in vivo resistance.

IG. 2.' MEAN LEVELS OF PARASITAEMIA ON FOUR DAYS

1 2 3 and 7.



Chloroquine Levels:

Table 6 shows plasma and urinary chloroquine levels (pg/ml) in the samples analysed.

TABLE 6: FREQUENCY PLASMA AND URINARY CHLOROQUINE LEVELS Mg/ml

Time	No. analysec	Chloroquine .levels pg/ml - Plasma						
		>0.000 <0.200	>0.2 <0.4	>0.4 <0.6	>0.6 <0.8	>0.8	Average	Range
Ohr	22	18	2	1	1	-	0.2117	0.0000 0.7467
26hr	26	14	4	3	3	2	0 ,4079	0.0504 1„2705
50hr	22	10	4	6	-	2	0.4709	0.1243 1.8618
		U R I N E						
Time	No. analysed	0-20	20-40	40-60	60-80	>8C	Average	Range
Ohr	14	12	1	-	1		5.2821	0.0000 73.9498
26hr	18	6	3	3	1	3	47 .6645	3.3598 190.3258
50hr	15	7	2	9	3	1	32 . 3620	0 .0000 81.1574

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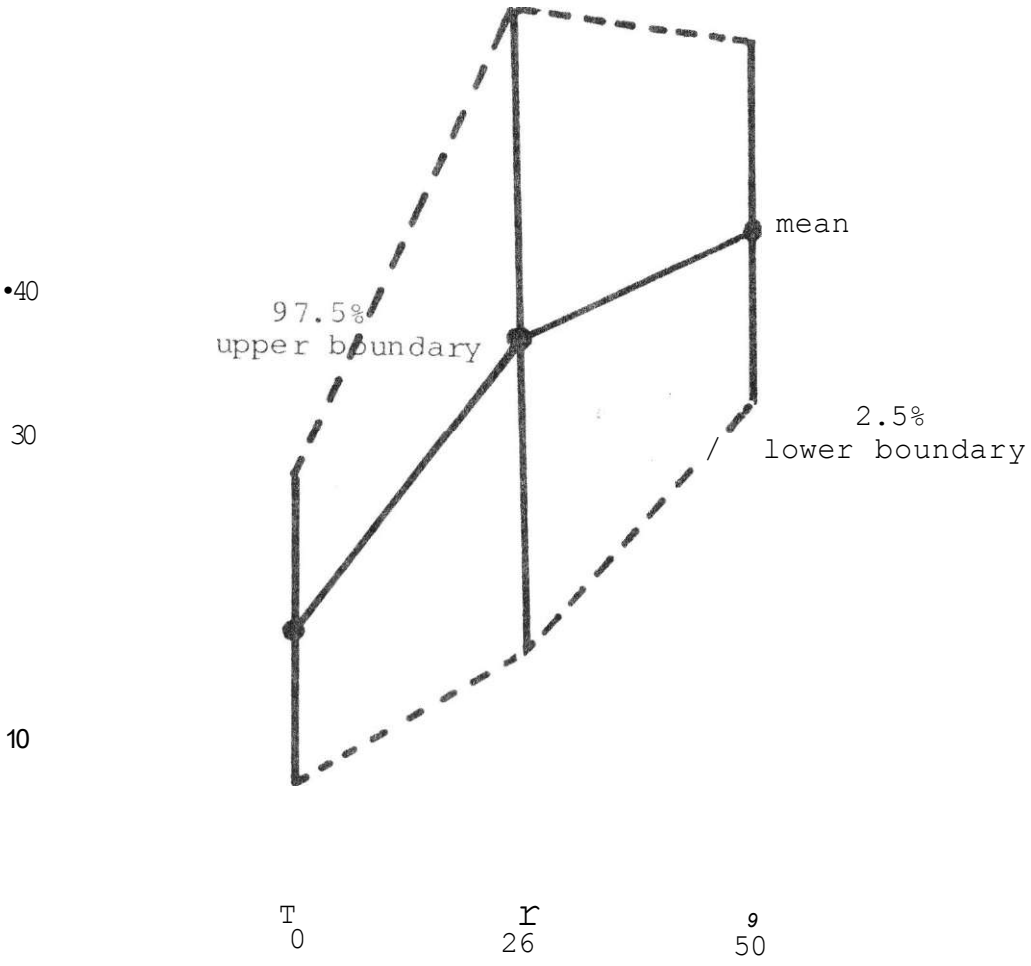
FIG. 3 MEAN LEVELS OF CHLOROQUINE CONCENTRATION IN PLASMA AT 3 POINTS IN TIME



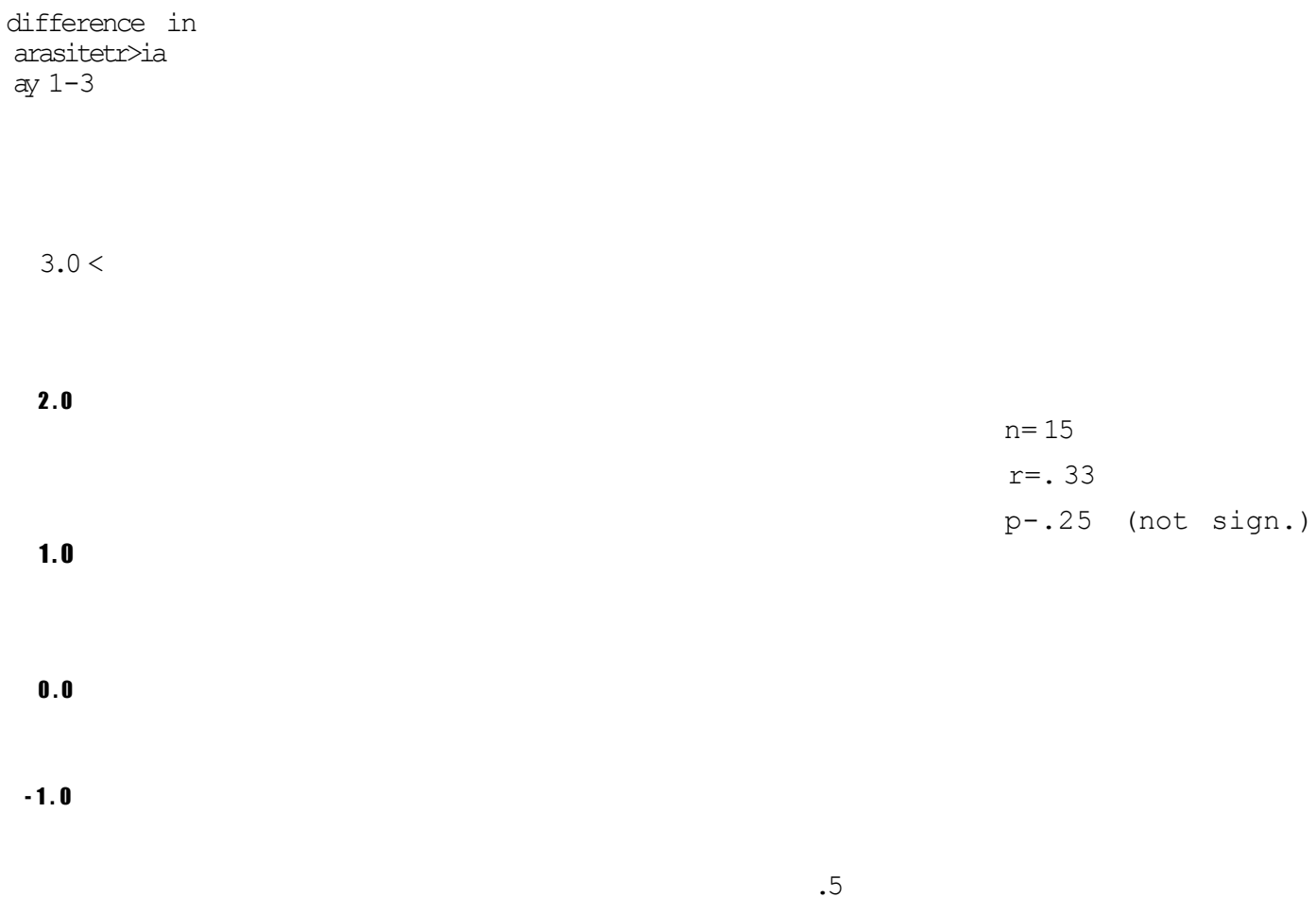
There was chloroquine in Ohr samples wich were pretreatment, as shown in table 6 and figure 3. Only 3 samples out of 22 had no chloroquine despite history of no chloroquine ingestion in preceding two weeks$_a$ Accepting the upper limit of tolerance as0.4|jg/ml than 17(77%) of samples had maximum tolerable range prior to further administration of chloroquine.

At time 26 hrs, following 3rd dose, 2 hrs later, 18 (69%) of samples analysed were at maximum tolerable level. 8(31%) had toxic levels. There was an increase in the number of patients attaining toxic levels.

Peak levels of chloroquine were attained 2 hrs following oral administration (42).

Two patients had no chloroquine in urine pretreatment. Ten samples had chloroquine at 0 hr. There is an increase in excretion after the 3rd dose and a fall after the last dose, There is adequate handling of chloroquine by the patients.
fchloxoqiline level and parasite clearance: There was ho correlation between chloroquine level and pai-usi to Clearance by day 3 as seen in figure 4. This is possibly due to the presence of chloroquine in plasma pretreatment.

FIG. 4. CORRELATION OF PARASITE COUNTS AND CHLOROQUINE CONCENTRATION BETWEEN DAY 1 - 3



Invitro Tests:

In vitro chloroquine MIC sensitivity response by Rieckmann microtest and their in vivo response of 19 patients is shown in table 7.

TABLE 7: IN VITRO CHLOROQUINE MIC RESPONSE AND IN VIVO RESPONSE IN 19 PATIENTS:

Pt No.	Age Yrs	MIC (jg/ml in vitro test	In vitro Response	In vivo Response
16	3	0.0128	S	S
10	2\	0 .0125	s	S
23	3/12	0 .0365	s	S
13	7	0.0512	R	S
21	8	0.1024	R	R
24	2	0.1024	R	R
32	2	0.1024	R	R
2	H	0.2048	R	R
17	12	0.2048	R	S
22	91	0.2048	R	S
34	7	0.2048	R	R
1	8i	Over 0.2048	R	R
5	6	Over 0.2048	R	s
6	13/12	Over 0„2048	R	s
9	9/12	Over 0.2048	R	s
27	li	Over 0.2048	R	R
30	45/12	Over 0 o 2048	R	R
31	7	Over 0„20 48	R	R
35	12	Over 0.2048	R	R

MIC - Minimal inhibitory concentration - This was taken as the well associated with - 1% trophozoite maturation to schizonts compared to the control well or less.

Of the 19 in vitro tests 16(84%) had an MIC over 114 nMol/L or 0$_{o}$0365 Mg/ml. Sensitive strains are inhibited at this concentration in vitro. Those growing in wells with higher concentrations were judged in vitro resistant.

Three isolates showing in vitro sensitivity also showed in vivo sensitivity. However 16 isolates showing in vitro resistance did not all show in vivo resistance. 6 fsolates out of the 16 were in vivo sensitive. The patients were aged 9 months and above.

Table 8 shows the cumulative frequency of in vitro response of P. falciparum isolates.

Table 8: CUMULATIVE FREQUENCY OF IN VITRO RESPONSE:

MIC	Cumulative (f)	
I4g/ml	N	%
0	0	0
0.0064	0	0
0.0128	1	5
0 .0256	2	11
0 .0365	3	16
0 .0512	4	21
0 . .1024	7	37
0 o 2048	12	63
Over 0-2048	19	100

At the maximum concentration attainable on the predosed plates (63%) isolates, were inhibited. 7(37%) were judged to be inhibited at MIC beyond 640nMol/L i.e. over 0.2048 ng/ml.

Probit Analysis:

Probit analysis of lOg/dose response was done by the method of Grab and Wernsdorper (unpublished WHO, Geneva - WHO/Mal/83. 990). The effective concentrations for 50% and 99% inhibition (EC^, ECgg) were 1.1 x 10^{-6}M/L and 10,1373 x 10^{-6}M/L respectively. These correspond to 0.32ong/ml and 32(jg/ml of plasma.

Area distribution:

Table 9 shows the distribution of 19 isolates in vitro and in vivo results according to the provinces.

TABLE 9: PROVINCIAL DISTRIBUTION OF 19 IN VIVO ISOLATES

Area Province	No	In vitro Resistant	In vitro Sensitive	In vivo Resistant	In vivo Sensitive
Eastern	7	6	1	3	4
Nyanza	6	4	2	4	2
Western	4	2	2	1	3
Central	2	2	-	2	-

The majority of resistant cases are from Eastern and Nyanza Provinces both in vitro and in vivo sensitivities. The numbers are too small though, to make any meaningful comparison.

Of the 6 in vitro resistant patients from Eastern province, 3 showed corresponding in vivo resistance. One isolate had in vitro resistance t
but in vivo sensitivity. Only one isolate from Western province showed both in vitro and in vivo sensitivity. Isolates from Central Province were (2), both showed in vitro and in vivo resistance. They were acquired outside the province. Isolates from Nyanza showed correlating in vivo and in vitro sensitivity and resistance.

D I S C U S S I O N :

The disease malaria, has been a prevalent disease, mostly in the tropics, but also in temperate lands for many years. The name Malaria was coined from an Italian words Mal-aria meaning bad air- from the swamps. Malaria was then believed to be caused by bad vapours blown from the swamps,

t

With the discovery of Romanowisky stains (1891) workers discovered the malaria parasite. Further work revealed the life cycle of the parasite (28).

The discovery of Quinine about 1630 AD brought new light in the treatment of malaria. This remained the main-stay of treament for 3 centuries. Onset of World war II 1934 - 44 increased the demand for quinine. Attempts were then made to discover alternative drugs. Synthetic drugs were discovered. The 4 - Aminoquinolines - of which chloroquine is the prototype showed more promise. Chloroquine has been the main stay of suppressive therapy as well as clinical cure for malaria for the pafet 40 years or so (29).

fhi§ plizzie of malaria and its treatment never seems to end. There has emerged resistance to antimalarials,

and more alarming to chloroquine in recent years (16, 29)Q In such areas as South East Asia chloroquine resistance is rampant (30).

Definition : Malaria is a protozoan disease caused by parasites of the genus Plasmodium. There are four species infective to man: P, falciparum; P. maiariae; P„ ovale, and P. vivax. Of these P. falciparum is the commonest and most serious in Africa.

V"

The disease affects people of all ages and sexes. The clinical manifestation depend on both host and parasite factors. Among the host factors is immunity to malaria. Parasite factors include the virulence of the parasite; ability to infect red cells of all ages; and level of parasitaemia.

Immunity may be passive or acquiredo Infants of immune mothers have some passive immunity upto six months of age. Thereafter those in endemic areas build up active immunity, premunition, from repeated malaria infections. The disease is worse in the non-immune. Children before they acquire active immunity may be regarded as a non-immune population,,

The incidence of malaria in endemic areas is highest in the infants and younger age groups. Indeed the splenic rates in children have been used to measure the endemicity of malaria (31) (52). In children malaria is a major cause of mortality, (3) in endemic

areas.

The study group:

The study population though select shows that the majority of the patients were below 6 years of age (63%). The weights in these patients were within normal limits for age. Malaria is less prevalent among malnourished children (32). This parameter was not looked for. Kwashiorkor children were admitted under malnutrition as a diagnosis. If they had clinical malaria, they are likely to have been missed out at the screening.

Clinical and Parasitological response:

The main symptoms observed were fever; vomiting; chills and rigors; diarrhoea and convulsions. Fever persisted in 10(34%) patients throughout to 3rd day. Fever in falciparum malaria may be quotidian. Studies elsewhere have shown that fever falls by the 2nd k 3rd day (33), Persistence of fever in presence of a positive slide for malaria parasites by the 3rd day of treatment suggests unresponding infection and possibly a resistant one.

Vomiting is a common symptom in febrile illness in children. It was however persistent in some patients by day 3 and 7. In itself, it may denote persisting m1 r i a be due to drug effects, possibly toxic effects (34)„ Other symptoms resolved rapidly on treatment. Chills and rigors are not common in P. falciparum malaria and more so in children (7). Con-vulsions have been associated with high rising fever.

In the study few patients had convulsions on day 1(16%). These had cleared by day 2 as patients settled. Only one patient, had a convulsion on day 2. This would seem to concur with the observation that the fever was associated with the convulsions. No patient had impaired level of consciousness suggestive of cerebral malaria. The patient who had convulsions on day 2 was 1\ years old and had no convulsion on day one.

The main clinical signs were pallor; splenomegaly hepatomegaly and Jaundice. Pallor and splenomegaly were the commonest. Anaemia was mainly normocytic normochromic by peripheral blood film done on day 1 of treatment (63%). There were some microcytic hypochromic cases (25%). Also there were some macrocytic hypochromic and normochromic pictures.

Reticulocytes were not determined., In malaria anaemia may be due to haemolysis„ As the bone marrow reacts to the onslaught there is production of some immature large cells. Hence there is a chance of having a mixed PBF picture. Microcytic hypochromic picture could be due to other causes e.g. hookworms or iron deficiency pet se.

Aiiaiemi**a** wftss noted in 23 (72%) of all the patients. The age group 6M - 6 years had 16 cases out of 20 (80%) while 7 year - 12 year group had 7 out of 12 (58%). Many children in the POW are seen with malaria and anaemia. In some transfusion is required. Anaemia is

a common finding in malaria (52).

Splenomegaly occurs rapidly from congestion then secondly from reticuloendothelial hyperplasia. (7,35) splenomegaly is a common sign in malaria.

/teexual parasites, trophozoites, were counted over 300 wbc. Parasitaemia was highest on day 1. Most patients had 401/300 wbc which is approximately 9357 trophozoites/ml of blood. The lowest count associated with clinical malaria was 6/300 wbc on day 2. The range of counts on day 2 was 6/300 wbc - 5450/300 wbc. Some patients had an increase in the counts by day 2 i.e. in 24 hours instead of the expected drop (36). In general there was a general decrease by 3rd day„ This would suggest parastological response. There was indeed associated clinical response in most patients 21/32 (66%). Only 10/32(34%) had not shown clinical response by day 3.

As regard parasite increase in 24 hours of treatment this has been observed before(36). Hence it is necessary to follow the parasitaemia for over 24 hours to evaluate response. Sensitive strains clear in mbgt Cases by 24 - 72 hours (33). WHO lias graded this SpbgtiHlhi of parasite response as S-sens.itive R-Resistant at R^ R_u R^ (appendix 2). R_1 resistance is the clearance of asexual parasitaemia within 7 days of inflation of treatment followed by recrudescence. Parasitaemia at 48 hrs is used to define resistance at HI1 and Rill (Hi)> RII is reduction in

sexual parasitaemia during first 48 hours of treatment to 25% or less of the original pre-test level, but without clearance of patent asexual parasites from blood.InRIII the parasite density decreases less than 75% or actually increases during the first 48 hours of treatment (16).

The study group showed that by day 3 of treatment the drop in patients with counts over 401/300 wbc is only 50%. Further 2 patients had high parasitaemia on day 7_0 This suggests that there were some patients who were not responding. There is a general decline in parasitaemia, but few cleared by 3rd day. Only G patients out of 32 had cleared. Workers in Kenya have observed a changing pattern of in vivo and in vitro response at the Coast and in Kisumu (36). Parasite clearance has increased to 5 days (37). In vitro response has changed from predominantly sensitive to resistant strains (9, 10,36,37,40).

In Kenya malaria is treated over 3 days. As expected or hoped, by the time of 4th dose i.e. 3rd day, the patient ought to be significantly improved and entering into convalescence. In some patients in this study high parasitaemia was noted to be associated with symptomatic malaria, mainly fever, vomiting and general malaise. Hence persistence of parasitaemia over 48 hours associated with symptoms may be initial clues of possible resistance. These two should be tak her.

Resistance has been defined as: the ability of a parasite strain to survive and/or to multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within the limits of tolerance of the subject (38,39).

The above definition is explicit. For the field worker and researcher there may be time to observe the response ir^vivo, in vitro and assay the drug levels. For the clinician and more so on an outpatient basis, identification of resistant infection is of prime importance. P. falciparum infection can be fatal in its own right, hence prognosis is even worse if it is of/^esistant strain. The clinician is required to monitor parasite counts and symptoms$_0$ The pattern of response of these parameters should guide him/her in the decision making. Parasite counts may not be done regularly on outpatient basis, leaving therefore, only the clinical state of the patient to go by„ In this study patients who on the 3rd day; 2 hrs following the last dose still had a positive malarial slide on peripheral smear, and clinical malaria were deemed non-responders and probable resistant malaria on clinical . and parasitolOgical groun$_{fls>}$ Treatment was therefore changed tihlbi'bquine to other antimalarials as indicated cn page 8 . There were 10 such cases.

The total number of patients who cleared on chloroquine alone in the study were 13 by day 7-28. By day 3 they were only 6_0 The average clearance time was 5 days. Other workers have observed the same (37). However in the study no parasite counts were done on day 4,5 &6. This may have affected the clearance time.

Out of 25 patients who had positive slides on day 3; V*

* 15 were a symptomatic. Immunity affects the response of patients. The non-immune is likely to show slower and less complete parasite and fever response, on the same drug and dosage whereas the partially immune subject may be quickly relieved of his symptoms even by a smaller dosage although parasites persist(39). Also parasites may persist in the blood in the absence of fever if parasitaemia patently (-^0 detectable parasitaemia level in blood), for fever is high (39)o This influenced the decision in the 15 patients who were asymptomatic but had patent parasitaemia. It is also known that parasite resistance and clinical symptoms are parallel only when the host defences are minimal; they diverge as host immunity supplements the therapeutic effects of medicine (11).

25 patients were positive by day 3. 10 of these had treatment changed as earlier explained leaving 15:

Nine of these cleared their parasites on chloroquine alone by day 7. Six had positive slides on day 7, One of those who were negative on day 3 had a positive slide on day 7. Therefore a total of 7 patients were positive on day 7 upon review,

A closer monitoring on day 4, 5, 6 could have identified these 7 cases seen on day 7, much earlier. Their parasite range was 8 - 1470/300 wbc. They had fever vomiting and general malaise. The average parasite count was 447/300 wbc which is approximately 10,000/ml of blood. From the foregoing those who did not respond to chloroquine alone were 18 (58%). One patient had incomplete data and was excluded. This means that there is a 50% chance or so of being a resistant case as seen at PFC - KNH. This is a worrisome situation.

Using the parasite counts at 48 hours of chloroquine therapy patients depicting RII and RIII resistance were 11. This leaves 20 patients who were either sensitive or resistant at RI. Seven patients had positive slides by day 7-28. This leaves 13 patients; as having cleared on chloroquine alone., Even from parasitolOgical point of view therefore, the pattern l-emetiiis essentially the same 13/31 (42%) as sensitive and 18/31 (58%) resistant at RI, RII and RIII. The decision made on day 3, 2 hours after 4th dose is justified both clinically and parasitologically.

Chloroquine levels:

Among the study patients there was chloroquine in pretreatment samples of plasma in 19 samples out of 22 analysed.

This has been the observation of Ofori-Adjei (1984) when he analysed chloroquine levels in serum of children before treatment (41). There was no history of drug ingestion in the preceding 2 weeks in most of his subjects - 5 cases (range 0 - 520 (jg/ml).

Plasma chloroquine levels may be equal to or higher than serum levels. It correlates well with the red blood cell levels during period of parasitaemia (42). The therapeutic range of plasma chloroquine is O.Olug/ml (29, 46). Levels upto 0.4(jg/ml are tolerable to patients. Beyond this toxic effects become manifest. A range of 0.4 - 0,8 pg/ml is associated with milder toxic effects, while levels above 0.8Mg/ml are associated with severe toxic effects (44).

It is clear that in the study population there was already chloroquine in blood pretreatment. Infact there were already therapeutic levels. This is due lb self medication for fevers, with chloroquine. Also malaria is often treated on clinical grounds in most peripheral units to which the patients may have gone before doming to Kenyatta National Hospital,

No parasitological confirmation is given in most cases. It is known that malaria can mimic many febrile illnesses.

Rees 1981 in Nairobi (43) has shown that malaria is over diagnosed. Upto 50% of cases of malaria had no parasitological confirmation when blood slides were done. This too may contribute to the presence of chloroquine in pretreatment samples. It is a common practice in endemic areas to give chloroquine even in presence of other diagnosis so as to cover for malaria. This is more so if no laboratory services are available, pressure of work prohibits screening for malaria routinely, or fever is high and has not been coming down.

A feature worth noting is the attainment of chloroquine plasma levels beyond the limits of tolerance. Holmberg 1979, working in Sweden; analysed serum chloroquine for rheumatoid arthritis In this particular study he noted the increasing frequency of toxic effects at concentration above 0.4 Mg/ml. Tolerable concen-

t ration were 0.4 Mg/ml. Toxic range was 0.4 Mg/ml - 0.8'Mg/ml. Beyond 0.8Mg/ml the effects were worse (44): There is a toxic "level in one patient pre-trtealtri'eht at 0»75 Mg/ml.

Following therapeutic dosages of oral chloroquine the plasma levels rise. The range at 26 hours samples was 0.0594 ug/ml - 1.2905 ug/ml. There is even a further rise at 50 hours. The range was 0.124ug/ml - 1.8618 ug/ml. These were levels attained acutely during additional therapy with chloroquine. Side effects e.g. visual disturbance headaches, bleaching of hair, vomiting, ototoxicity and retinopathy were not looked for. However noted was vomiting. This could Ibe due to drug effects other than the disease (34),

The therapeutic range for sensitive P. falciparum in man is 0.01µg/ml - 0.03 µg/ml (20, 46). All patients attained therapeutic levels, and above, of chloroquine. With repeat doses of chloroquine more patients move into the limits of tolerance and toxic levels. At 0 hr, 2/[pat]Wtf%oncentrations iP.4 Mg/ml. By 26 hours they were eight and by 50 hr[s]they were still 8. Also by 26 hr^the average chloroquine level was0.4079 Mg/ml and by 50 hrs it was 0.4709 ug/ml$_0$ Clearly drug accumulation rises with added chloroquine ingestion. Ofori-Adjei 1984 (41) has noted that there is a risk of chloroquine toxicity both at home and in the hospital. This practice may etihence the selection of resistant strains bj? Kiiiiftg dff sensit i ve strains and hence removing competition to the resistant strains. This allows them to establish themselves.

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Quantitation of urinary chloroquine levels would be much easier if it could be related to the plasma levels. Urine is easier to obtain. Qualitative tests exist e.g. Dil Glasko (45) to identify excretion and hence absorption of chloroquine. They do not reflect plasma levels attained.

Chloroquine excretion and half life is dose dependent and mainly renal (46), There was high excretion at peak levels. This falls off by the 4th dose. Chloroquine excretion is slow (46). A more careful study to measure plasma level and relate this to the urine levels is needed.

In vitro results:

In vitro tests were successful in 19 isolates. The MIC range was 0.0128 pg/ml - 0.2048 ng/ml Isolates of MIC. 114 nlvl/L, (0.0365)jg/ml) were considered in vitro resistant. In vitro sensitivity was 3 (16%) and resistance was 16 (84%)$_0$

There was discrepancy between the in vitro resistance ahd in vivo response. Thus 6 isolates showed in vitro resistance but in vivo sensitivity. Workers in Kenya have observed this (40) and elsewhere. This has been attributed to host factors, mainly immunity.

A point to note is possible effect of high chloroquine plasma levels. At the highest possible concentration on the plates. 0_U2048 Mg/ml only 63% of the isolates were inhibited. Thus 37% were judged to be inhibited, at concentrations higher than 0_o2048 Mg/ml° Since the patients attained higher levels than this it is possible that sheer drug pressure killed off even resistant strains in vivo thus making them appear sensitive., The pretreatment chloroquine levels did » not hamper parasite growth very much as parasites mature to schizonts in most tests. The added chloroquine did not amount to higher than well H at 0.2048ug/ml. It is only following ingestion of further chloroquine that toxic levels possibly lethal to some resistant parasites, were attained.

All isolates showing in vitro sensitivity were also in vivo sensitive. All isolates showing in vivo resistance were also in vitro resistant. This is a welcome observation in favour of the accurapy of the Rieckmann micro in vitro sensitivity test. In vitro resistance was noted in age range lj^ - 12 years. In vitro resistance descrepancy with in vivo sensitive response was noted in patients from $\frac{Q}{12}$ - 12 years, 5 out of total, were aged above 1 year. Presumably they had acquired some immunity. The range of chloroquine level was 0_o13 Mg/ml - 6.87 Mg/ml. The average was 0,39 Mg/ml; at the upper limit of tolerance.

The sensitive strains had plasma chloroquine range of 0.1339 ug/ml - 0.4283 ug/mlo The average was 0.28 ug/ml within tolerable range. The parasite counts for the 19 patients show that on the whole, they had relatively high levels with 8 above 200/300 wbc and 8 200/300 wbc by day 3. Average counts were 1013/300 wbc day 1; 747/300 wbc day 2; 973/300 wbc day 3. This is evident of resistant strains. The in vivo response were RI and RII.

The MIC cut off point 0.0365 yg/ml corresponds to the upper limit of therapeutic range. At this level only 16% of isolates were inhibited. The highest attainable concentration on the plate 0.2048 ug/ml is midway of upper limit of tolerance. Only 63% of the isolates were inhibited. It is possible that wells at 0.6 pg/ml will improve on the gradation of responses in vitro; to give a lee way margin beyong the limit of humanly tolerable chloroquine concentration.

Provincial Distribution:

Malaria in Nairobi is usually imported (49). Analysis of the epidemiological value of the results of in vitro tests showed most resistant cases came from Eastern grBVifl(2@: Mach&kos borders Nairobi on the East as one of the Eastern province districts. Areas of Kitui from where other patients came are within easy reach of Nairobi. Nyanza and Western province provided the bulk of the patients. There are many travellers

from these areas to Nairobi.

Work on rmlaria has been concertedly done by joint efforts of the Kenya Government and WHO researchers on a malaria eradication programme. Apparently no in vitro sensitivity work has been done in Eastern Province. It would be a good area to survey as it poses a greater danger to the city of Nairobi in terms of imported malaria. Malaria in Nairobi has increased in incidence. Though current work indicates no transmission cycle (48, 49) clinicians "strongly feel that this is not so. There could be transmission in Nairobi as factors in favour exist, namely the parasites, the vectors and the human hosts. The increasing population of human beings certainly epidemiologically favours transmission.

CONCLUSIONS;

1. There is parasitological and clinical evidence for resistant malaria in Kenya.

PiitJyrils had chloroquine within tolerance Ruiiie'y pi-/«trR»ttttit»rit „ This was possibly due to self medication.

3. There is in vitro evidence for resistant malaria in Kenya. By monitoring imported malaria in Nairobi and other urban areas, surveillance may identify rural endemic areas from where such malaria originates to non-endemic areas.

4. A possibility of self or iatrogenic chloroquine toxicity exists in the face of continued treatment of clinical malaria.

5. Strains of resistant malaria are possibly present in all endemic areas in the country. The patterncf resistance is RI, RII and RIII.

6. High drug levels may be masking partially resistant strains especially at RI.

RECOMMENDATIONS;

1. Patients should be reviewed clinically and parasitologically, with a view of detecting resistant malaria, at least on day 3 and 7.

2. Drug history, especially chloroquine ingestion, should be ascertained in all cases of malaria ffldre m if confirmed by blood slide. Differential diagnosis should be ruled out as much as possible.

3. Chloroquine levels should be assayed in plasma in problematic cases. Chloroquine abuse should be minimised.

4. In vitro sensitivity tests should be carried out in areas of suspected in vivo resistance.

5. Malaria therapy should be streamlined by policy. Evident resistant cases with absolute clinical parasitological proof should be treated by combination therapy e.g. Quinine/sulphadoxine/Pyrimethanine. Doses and duration of therapy must be adhered to.

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REFERENCES:

1. Bruce Chwatt, L.J,
Malaria Epidemiology. Brit- Med. J. 2. 91 1971.

2. King, M.Ao
Medical care in Developing Countries
Nairobi. Oxford University Press. E. Africa,, 1966 .

3„ Grounds, J.G„
Child Mortality under six years of age in
Government Hospitals in Kenya. E. Afr. Med. J„
41. 473. 1964.

4. Faich G. A. Marison J.
The prevalence and relationship of malaria anaemia
and malnutrition in a Coastal area of El. Salvador.
Amer. J. Trop. Med. Hygn. 24: 161 1975.

5. Draper C.C.
Malaria laboratory diagnosis
Brito Med. J„ 2 : 93 1971.

6„ Kenya P.R. Okelo , G.B.A., Kyobe, J,
Malaria endemicity in Central Division. Kitui
District Kenya. Proceedings of the fifth Annual
Scientific Conference of Kenya Medical Research Institute
and Kenya Trypanosomiasis Research Institute.1984.

7. Plorde, J.J.
Harrison's Principles of Internal Medicine.
MC GrOw - Hill Koya Kusha Ltd. Tokyo. Japan 1982.

8; MkSdba S»C,s> Spencer, H.C.
Sensitivity of Plasmodium falciparum to chloroquine
in Kisumu and Malindi, Kenya
Trans. Roy. Soc.. Trop,, Med, Hygn. 76: 314, 1982 .

9. Spencer, H.C., yasaba, S.C., Kiaharo, D.
Sensitivity of P. falciparum isolates to chloroquine in Kisumu and Malindi, Kenya.
Amer. J. Trop. Med. Hygn. 31: 902, 1982.

10o Spencer, HUC., Masaba, C., Chulay, J.D., Nguyen-Dinh, P,
Field evaluation in Kenya of a 48-hour in vitro test for P. falciparum sinsitivity to Chloroquine
Amo J. Trop. Med. Hyg. 32 . 91G, 1983.

11. Chemotherapy of malaria and resistance to antimalarials.
WHO Tech. Rep. Ser. 529, 1973.

12. WHO Expert committee on malaria. Sixteenth Report.
WHO Tech. Rep. Ser. 549, 71, 3-974.

13. Review of Research on malaria
Bull. Wld. Health. Org. 50. 151, 1974.

14. Chloroquine resistant malaria acquired in Kenya and Tanzania Communicable disease centre. Morbidity and Mortality Weekly report. 27. 463, 1978.

15. Kean, B.H.
Chloroquine resistant falciparum malaria from Africa.
JAMA. 241: 395, 1979.

16. Bruce, C., Bluberg, R.S., Campel, C.C., Jones, T.C., Mount, D.L., Friedman, S.M.
High chloroquine resistance P. falciparum acquired in Kenya.
. New Engl. J. Med. 307. 1560, 1982.

17 * Revised recommendations for malaria.
Ch emoprophylaxis for travellers to E. Africa.
Communicable disease Centre: Mortality and Morbidity Weekly Report 31: 328, 1982.

18. Spencer, H.C., Kariuki, D.M., Koech, D.K.
In vivo and in vitro chloroquine resistance in P. falciparum from Kenya infants,,
Amer. J, Trop. Med. Hyg. 32. 922, 1983.

19. Owaga, M.L., Wegesa, P. Kaliech, P.M.
Sensitivity test of P_0 falciparum to chloroquine Nangina Busia District Kenya. Proceedings of the First Annual Scientific Conference of the Kenya Medical Research Institute. Nairobi, Kenya 1981_0

20. Spencer, H.C., Kaseje, D.C.O., Koech, D.K.
The Kenya Saradidi Community malaria Project. 1. Response of P. falciparum isolates to chloroquine in 1981 and 1982„
Trans. Roy. Soc. Trop. Med. Hygn. 77. 689, 1983.

21. Sixsmith, D.G., Spencer, H.C., Watkins, W.W., Koech, D.K., Chulay, J,D.
Changing in vitro response to chloroquine of P. falciparum in Kenya,, Lancet 2 1022, 1983.

22. Rambo, L. , Bjorkman, A., Brohult, J., Iierdman, P.
Penison, P.O.: Serum and erythrocyte concentrations of chloroquine in patients with acute diarrhoea.
Ann. Trop./parasitology Med 76. 253, 1982.

23. Chemotherapy of malaria. 2nd Edition. Editor Bruce Chwatt„ WHO Geneva 1981.

24. Kofi Tsekpo, W.M., Mugambi, M., Were, O., Ochieng, M.E
The determination of some chemotherapeutic drugs and related compounds in Biological fluids<
Proceedings of the Fourth annual scientific conference of Kenya Medical Research Institute Nairobi Kenya. 198:

28c, Chloroquine resistance of P. falciparum in detected by a simple in vitro method
Bull. WHO. 45. 157. 1971

26. Bennett D.B., Watkins, W.W., Oloo, A.J.
« Sensitivity of P_u falciparum to chloroquine in Coastal and Western Kenya. Update. Proceedings of the 6th Annual Scientific Conference of Kenya Medical Research Institute. Nairobi Kenya 1985. (press)„

27. Ross, R.
On some peculiar pigmented cells found in some mosquitoes fed malaria blood. Br. Med. J. 2. 1796o

28„ Garnham, P.C.C.
Exo-erythrocytic Schizogony in Plasmodium Kochi Laveran. A preliminary note. Trans. R. Soc. Trop. Med. Hyg„ 40. 719, 1947.

29. Chemotherapy of malaria.
1st Edition. Editor Sir Gordon Covell.
WHO. Geneva 1955.

30. Drug - resistant malaria
The report of a meeting held in Kuala Lumpur Malasya 10-15th Aug„ 1981 Pg. 13 - 32.

31. Terminology of malaria and of malaria Eradication
WHO. Geneva 1963.

32. Malaria in Kwashiorkor children in Mulago Hospital.
Jitta, J.S. M.Med thesis (unpublished). 1978.

33. Hemed, A., Omar, S.
Response of P. falciparum in Sudan to oral chloroquine, Amer. J. Trop. Med. Hyg. 27. 853, 1977.

34 Kofi Tsekpo, W.W., Ngira, J.A.
Selective liquid chromatography chloroquine detection and quantitation of plasma and urine from Children under malaria treatment. Proceedings of the 6th Annual Scientific Conference of KEMRI/KETRI Nairobi, Kenya. Feb. 1985 (press).

35. Charters, A.D.
A clinical study of the spleen in black water fever, J, Trop. Med. Hyg. 38. 1, 1935.

36. Spencer, H.C., Kipingor, T., Agure, R., Koech, D.K., Chulay, J.D.: P. falciparum in Kisumu-Kenya: Differences in sensitivity to Amodiaguine and chloroquine In vitro. J. Inf. Dis. 148. 732, 1983.

37. Watkms, W.W., Spencer, II.C., Kariuki, D.M., Sixsmith, D.G., Boriga, D.A_0, Kipingor, T., Koech, D.K. Effectiveness of Amodiaquine as treatment for chloroquine resistant P. falciparum infection in Kenya. Lancet 1. 357, 1984.

38. Report of WHO scientific committee. Resistance of malaria parasites to drugs. WHO Tech Rep. Ser. 296. 29, 1965.

39. WHO Expert Committee on malaria 14th Report. WHO Techn. Rep. Ser. 382 18 1968.

40. Nguyen-Dinh, P., Trager, W.
P. falciparum in vitro determination of chloroquine sensitivity of three new strains by a modified 48-hr test. Am. J., Trop. Med. Hyg. 29. 339, 1980 .

41. Ofori-Adjei, D., Commey, J.O.O., Adjepon -Yamoah, K.K.: Serum chloroquine levels in children before treatment for malaria. Lancet 1 1246 1984.

42. Adelusi, S.A., Dawodu, A.H., Salako, L.A.: Kinetics of the uptake and elimination of chloroquine in children with malaria. Br. j. Pharmac. 14. 483. 1982.

43. Rees, P.H., Touquet, V.L., Allen, A.V.: Clinical malaria in Nairobi. E. Afr. Med. J. 48. 51, 1971.

44. Frisk-Holmberg, M.I)., Bergkvist, Y., Domeij-Nyberg, B„: Hellstrom L , Jansson, F. Chloroquine sc:rum concentration and side effects: Evidence of dose-dependent Kinetics J. Clin. Pharmacol. Ther. 25: 345, 1979.

45. Leiijveld, J., Kortrnann, H.: The eo sin colour test of Dill and Glasko: A simple field test to detect chloroquine in urine. Bull. WHO 42. 477, 1970.

46. The pharmacological Basis of Therapeutics. Goodman and Gillman. 5th Edition 1975. Page 1045 - 1080. New York Mcmillan.

47. Spencer, H.C., Oloo, A.J., Watkins, W.W. Sixsmith, D.G,: Churchil, F.C., Koech, D.K.: Amodiaquine more effective than chloroquine against Plasmodium falciparum malaria on Kenyan Coast. Lancet i 955 1984.

48. Rapuoda, B.A., Achola, D.: Studies on malaria and vectors in and the prevalence of the disease (unpublished).

49. Bhatt, K.M., Omani, J.M., Kyobe, J., Kanja, C.: Clinical malaria in Nairobi. E. Afr. Med. J. 61 303 1984.

504 Bhatt, K„M., Bhatt, S.M., Okelo , G.B.A., Watkins, W.W.: Chloroquine resistant Plasmodium flaciparum Malaria in a local Kenyan: A case report. E. Afr. Med. J. 61: 745, 1984.

51. Nelson Text Book of Paediatrics.
Editors: Victor C. Vaughan II M.D.
R. James McKay Jr. M.D.
Richard E. Berhrman M.D.
W.B. Saunders Company
Philadelphia. 11th Edition 1979.

52. Nakimwero, F.N.
Anaemia as seen in children admitted to the Paediatric observation ward of the Kenyatta National Hospital.
M.Med thesis 1981 (unpublished).

MALARIA STUDY

APPENDIX I:

NAME	PFC NO
AGE	IP. NO
SEX	Study No
Residence - Rural - Urban	Weight(kg)

Symptoms and Signs	Duration in days 1 2 3 7
Fever Diarrhoea Chills Rigors Convulsions Anaemia Vomiting Jaundice Splenomegaly Hepatomegaly Others	

MP's Slides

Day	Positive		Negative		Parasite type	Parasitaemia
1	Thick	Thin	Thick	Thin		level
2						
3						
7						

Chloroquine level

Blood Urino

] BlHik OHR

2; g.rd Ws<*} 2hrs later

3. 4th dose; 2hrs later

APPENDIX I (continued)

Parameters

Haemoglobin leveL	
U/E	
lib Electrophoresis	
Others	

Resistance Type

	S	RI	R2	R3	In vitro Test Result

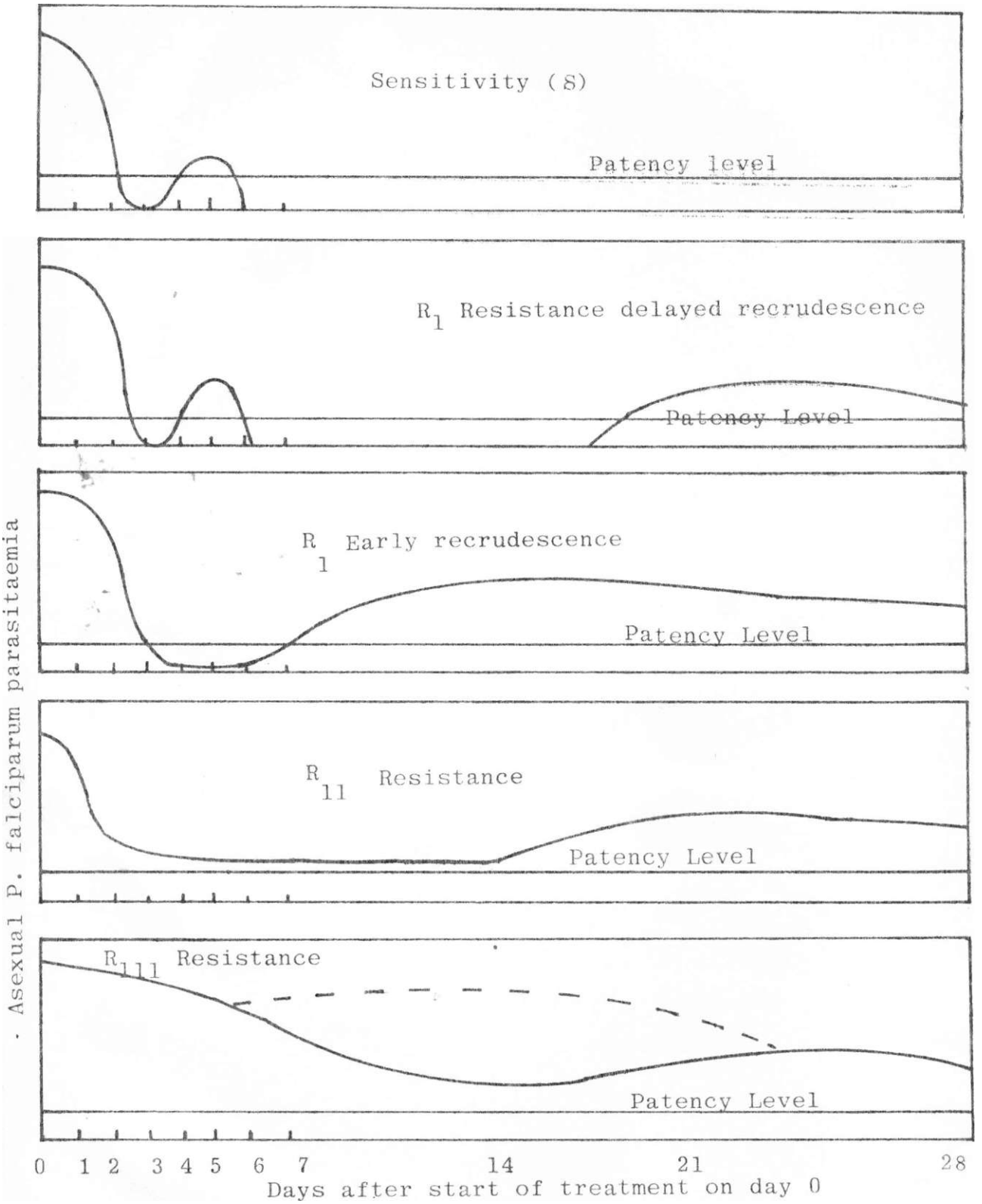
APPENDIX II:

Grading of Resistance - P. falciparum to Chloroquine

Response	Symbol	Evidence
Senstiti-vity	S	Clearance - asexual parasitaemia within 7 days of start of treatment without recrudescence.
Resistance	R_1	Clearance -asexual parasitaemia - followed by recrudescence
	R_{ir}	Marked reduction -asexual parasitaemia but no clearance
	R_{111}	No marked reduction of a sexual parasitaemia

APPENDIX II (continued)

Response to in vivo field test for P. falciparum



Standard Tests
7-day observation

Extended test
28 day observation

From WHO Tech Rpt Ser, # 529 1973 (AMENDED)