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Prevalence rate of chloroquine resistance to plasmodium falciparum malaria among sudanese populations in malaria endemic areas in khartoum state and sennar state

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Abstract

Objective: The study was carried out to assessment or to evaluate chloroquine Resistance of Plasmodium falciparum among Sudanese populations (10–85 years) ,malaria endemic area in Sennar region (Sennar state in South East Sudan) and Khartoum State (the capital).

Design: The study was carried out on 190 samples of all of the study areas between March and December 2007, corresponding to the period of high malaria transmission. All samples were tested for chloroquine Resistance to Plasmodium falciparum by using in vitro micro tests. These study sites are located in an irrigated agricultural scheme, where transmission is seasonal.

Setting: AL-Neelain University – College of Medical Laboratory Sciences – Sudan – Khartoum.

Results: By using in vitro micro tests: In Sennar state the resistance to chloroquine was found to be 78.1% and about 21.9% were sensitive. Out of 105 patients only 23 patients(21.9%) were sensitive and 82 patients(78.1%) did not respond, 29 patients(27.6%), 19 patients (18.1%) and 34 patients (32.4%) of a total of 105 patients were early R1, RII and RIII levels of resistance respectively. In Khartoum state the the resistance to chloroquine was found to be 68.2% and about 31.8% were sensitive. Out of 85 patients only 27 patients (31.8%) were sensitive and 58 patients (68.2%) did not respond, 35 patients (41.2%) and 23 patients (27.1%) of a total of 85 patients were early R1 and RII levels of resistance respectively.

Conclusions: By using in vitro micro tests: In Sennar state the resistance to chloroquine was found to be 78.1% and about 21.9% were sensitive while in Khartoum state the the resistance to chloroquine was found to be 68.2% and about 31.8% were sensitive.

Keywords: Malaria resistance, Chloroquine, Malaria tests

INTRODUCTION

Malaria is a complex disease that varies widely in epidemiolog and clinical manifestation in different parts of the world. This variability is the result of factors such as the species of malaria parasites that occur in a given area, their susceptibility to commonly used or available antimalarial drugs, the distribution and efficiency of mosquito vectors, climate and other environmental conditions and the behaviour and level of acquired immunity of the exposed human populations.

Drug resistance has also played a significant role in the occurrence and severity of epidemics in some parts of the world. Population movement has introduced resistant parasites to areas previously free of drug resistance. The economics of developing new pharmaceuticals for tropical diseases, including malaria, are such that there is a great disparity between the public health importance of the disease and the amount of resources invested in developing new cures.[1,2] Chloroquine was discovered in 1934 by Hans Andersag and co-workers at the Bayer laboratories who named it "Resochin". It was ignored for a decade because it was considered too toxic for human use. During World War II, United States government-ponsored clinical trials for anti-malarial drug development showed unequivocally that chloroquine has a significant therapeutic value as an anti-malarial drug. It was introduced into clinical practice in 1947 for the prophylactic treatment of malaria.[3]

It has long been used in the treatment or prevention of malaria. After the malaria parasite *Plasmodium falciparum* started to develop widespread resistance to chloroquine,[4][5] new potential utilisations of this cheap and widely available drug have been investigated. Chloroquine has been extensively used in mass drug administrations which may have contributed to the emergence and spread of resistance.[5]

Plasmodium falciparum which is resistant to chloroquine—a pharmacologic 'staple' used to treat and as prophylaxis for visitors to malaria endemic regions of Africa Mechanism of resistance Resistant strains of P falciparum do not concentrate chloroquine; resistance is reversed by verapamil, a calcium channel blocker.[6]

Sudan is the largest country in Africa, covering over 8% of the entire continent. The total population is estimated to be 30.3 million inhabitants, of which 75% live in rural areas. There are 7.5 million malaria cases and 35 000 deaths every year due to malaria. The problem appears to have worsened in recent years, due to increasing levels of *P. falciparum* resistance against the two most commonly used antimalarials: CQ and Fansidar.[7]

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Malaria is a major health problem in about 100 countries. More than 40% of the world population live in malaria endemic areas, and almost half of them in Sub-Saharan Africa where about 90% of the clinical cases occur. There are nearly 500 million clinical cases of malaria worldwide each year and 1.1 to 2.7 millions die annually, the majority of whom are children under 5 years in Africa.[8, 9]

Chloroquine is a 4-aminoquinoline derivative of quinine, first synthesized in 1934 and has since been the most widely used antimalarial and the treatment of choice for uncomplicated malaria and for prophylaxis as well. Chloroquine resistant *P.falciparum* malaria has been reported in all areas where the parasite is transmitted except for malarious areas of Central America, the island of Hispaniola, and limited areas of the Middle East and Central Asia.

As the malaria parasite digests haemoglobin, large amounts of a toxic by-product are formed. The parasite polymerizes this by-product in its food vacuole, producing a non-toxic haemozoin (malaria pigment). It is believed that resistance of *P. falciparum* to chloroquine is related to an increased capacity for parasite to expel chloroquine at a rate that does not allow chloroquine to reach levels required for inhibition of haem polymerization.[10]

This chloroquine efflux occurs at a rate of 40 to 50 times faster among resistant parasites than sensitive ones [11]. Further evidence supporting this mechanism is provided by the fact that chloroquine resistance can be reversed by drugs which interfere with this efflux system[12]. It is unclear whether parasite resistance to other quinolone antimalarials (amodiaquine, mefloquine, halofantrine, and quinine) occurs via similar mechanisms [10].

Sudan is the largest country in Africa, comprising more than 8% of the entire continent. The total population is estimated to be 39.2 million inhabitants, of whom 75% live in rural areas. Malaria is a leading cause of morbidity and mortality, resulting in 3.1 million cases and 2,500 deaths annually[13].

MATERIALS AND METHODS

This study was carried out on 190 samples (two study area) of patients infected by plasmodium malaria by collected from Khartoum state and Sennar region. The samples was tested for chloroquine resistance of plasmodium falciparum by using in vitro micro tests. removing the parasites from the host and placing them into a controlled experimental environment. In the microtechnique most frequently used, parasites obtained from venous blood sample and finger-prick blood sample are exposed in microstate plates to precisely known quantities of drug and observed for inhibition of maturation into schizonts.[14]

In vitro test

The micro-technique test (Mark III: supplied by WHO Division of Control of Tropical Disease 2001, England) was used to evaluate *in vitro* sensitivity to CQ of *P. falciparum* malaria parasites. One hundred and ninety malaria cases were selected for this study, according to the *in vitro* protocol described in Mark III-WHO[15]. The test was performed in tissue culture plates pre-dosed with drugs in increasing concentrations; briefly, 0.9 mL of RPMI 1640 medium were taken into the sterile falcon tube and 100 µL from well-mixed blood mentioned previously were added. All wells of appropriate columns were dosed with 50 µL of blood-medium mixture using the 50 µL fixed volume Eppendorf pipette and a disposable sterile tip, as provided with the test kit. Plates were incubated at 37.5°C in a candle jar for 24 to 30 h. At the end of incubation, blood from each well was harvested and a thick film was prepared; thick films were stained for 30 min in a Giemsa stain at a dilution of 1% (vol/vol) in buffered water of pH 6.8. After drying, films were examined through a light microscope with oil immersion lens, and the number of schizonts with three or more nuclei of a total of 200 asexual parasites (i.e., schizonts and trophozoites) was counted. For an acceptable test, schizont maturation in control must be 10% or more (i.e., 20 schizonts with three or more nuclei per 200 asexual parasites). Average results for EC50, EC90, EC95, and EC99 (i.e., drug concentrations producing 50%, 90%, 95%, or 99% inhibition of schizont maturation, respectively) were calculated using the WHO log probit program (www.who.int/csr/drugresist/malaria/en/probit.xls) in all isolates.

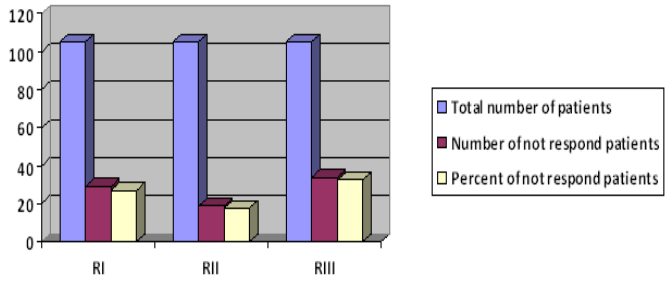
RESULTS

By using in vitro micro tests: In Sennar state the the resistance to chloroquine was found to be 78.1% and about 21.9% were sensitive. Out of 105 patients only 23 patients(21.9%) were sensitive and 82 patients(78.1%) did not respond, 29 patients(27.6%), 19 patients (18.1%) and 34 patients (32.4%) of a total of 105 patients were early R1, RII and RIII levels of resistance respectively.

In Khartoum state the the resistance to chloroquine was found to be 68.2% and about 31.8% were sensitive. Out of 85 patients only 27 patients(31.8%) were sensitive and 58 patients(68.2%) did not respond, 35 patients(41.2%) and 23 patients (27.1%) of a total of 85 patients were early R1 and RII levels of resistance respectively.

Table 1.Chloroquine resistance to plasmodium falciparum in Sennar state:

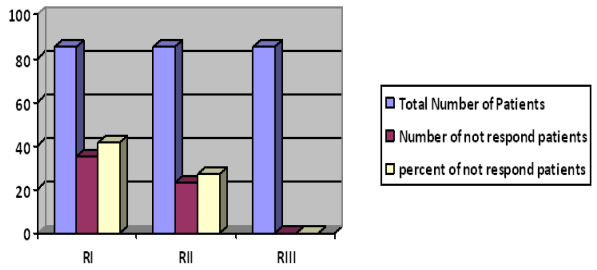
Level of resistance / Patients	RI	RII	RIII
Total number of patients	105	105	105
Number of not respond patients	29	19	34
Percent of not respond patients	27.6%	18.1%	32.4%



Chloroquine resistance to plasmodium falciparum in Sennar state

Table 2. Chloroquine resistance to plasmodium falciparum in Khartoum state

level of resistance / Patients	RI	RII	RIII
Total number of patients	85	85	85
Number of not respond patients	35	23	--
Percent of not respond patients	41.2%	27.1%	--



Chloroquine resistance to plasmodium falciparum in Khartoum state

DISCUSSION AND CONCLUSIONS

Antimalarial drug resistance is widely monitored using in-vitro susceptibility testing. There are sentinel sites throughout the malaria affected world monitoring for drug resistance. A variety of methods have been developed and the results have provided valuable information in the assessment and mapping of antimalarial drug resistance [16].Evaluation of stored isolates in reference centres allows proper standardisation of methodologies and repeated tests on single isolates. But most of this testing in the field is a "one-off" microtest on freshly obtained blood samples. When antimalarial drug susceptibility tests are reported the highest observed values observed are naturally of greatest interest as they may represent emerging drug resistance. Drug regimens should aim to cure all infections, and thus provide concentrations exceeding the inhibitory concentrations for the most resistant prevalent parasites. If only a single concentration range is evaluated in an in-vitro susceptibility assay using serial dilutions then, by definition, the true EC_{50} of the most resistant isolates must lie above or between the largest concentration differences tested. Thus, unless the parasites are retested with a higher concentration range (which they usually cannot be), the precision of the estimated EC50 or EC90 value of the most resistant isolate will usually be the poorest of all the isolates assayed. Furthermore as curve fitting or probit analysis takes no account of other isolates in the series tested, then if there are two adjacent points with extremely different values (often zero and 100% inhibition), a curve will be fitted as lying symmetrically between the two.

Antimalarial drugs will continue to be needed in the future despite the progress in malaria research at all fronts relevant to the parasite, the vector and the human host, in particular the areas of vector control, epidemiology, molecular biology, immunology and therapeutics. As long as drugs are used, chances of developing resistance are there, especially for *P. falciparum* the most serious and widely spread strain of human malaria parasites. Currently, the development of resistance is more rapid than the pace in the discovery of new effective and affordable drugs. Thus, the challenge is how to make maximum use of available drugs and to prolong the useful lifespan with the hope that new drugs or other methods of control will be developed and implemented. Firstly, there is need to improve prescription procedures and stop over-counter purchase of antimalarials.

In addition, there is need to upgrade focused malaria training and to raise awareness on the magnitude of the problem at all levels of health personnel. Improved access to and use of definitive diagnosis-based treatment is essential.

Furthermore, prevention of drug resistance can be attained by reducing drug pressure through more selective use of drugs particularly combination therapy which is inherently less likely to foster resistance or have properties that do not facilitate development or spread of resistance. There is need to identify strategies to improve acceptance of and compliance with combination therapy at all levels-official and non-official healthcare systems, private sector, and community.[17]

Because of the realities of health care infrastructure and the influence of the private sector, approaches to malaria therapy, especially in sub-Saharan Africa, will probably favour increased access to drugs (and, therefore, loss of control over how they are used) over restricted access (and, therefore, more control over how they are used).

If this proves to be true, while only minor advances against antimalarial drug resistance can be expected, short-term reductions in malaria morbidity and mortality may be achieved.

Long-term success of this strategy, however, will *depend* on a continuous supply of new and affordable drugs and on the development of effective and implementable control measures to reduce overall burden of disease. A more cautious approach would be to avoid placing too much faith in future scientific advances and technology and to invest in methods to improve the way people and antimalarial drugs interact in an environment of essentially uncontrolled use. The objective of this investment would be to prolong the useful life span of drugs enough to increase the likelihood that new drugs or other methods of malaria control will indeed be developed and implemented.

Significant advances against antimalarial drug resistance is probably unlikely without major change in health infrastructure leading to higherquality services that are more readily available.[18]

A strategy that has received much attention recently is the combination of antimalarial drugs, such as mefloquine, sulfadoxine/ pyrimethamine (SP), or amodiaquine, with an artemisinin derivative. [19]

Another approach that has not been widely adopted is the close follow-up and re-treatment, if necessary, of patients. The success of this approach is dependant on availability of reliable microscopy (to diagnose the illness initially as well as to confirm treatment failure), and either an infrastructure to locate patients in the community or a community willing to return on a given date, regardless of whether they feel ill or not. With this system, patients who fail initial treatment, for whatever reason, are identified quickly and re-treated until parasitologically cured, decreasing the potential for spread of resistant parasites.[20]

PRIORITIES

A. Investigate combination therapy

1. Fast-track a chlorproguanil/dapsone/ artesunate fixed dose formulation. From a theoretical basis, this would offer the best combination of overall efficacy, synergy between the antifolate-sulfa components, short half-life, reasonably well-matched pharmacokinetics, and probable cost. Because of growing use of and resistance to SP, an urgency exists to field this promising agent.
2. Investigate effectiveness of combination therapy in terms of robustness of strategy in face of high levels of self-treatment and unofficial use of component drugs (or related compounds) as monotherapy and in various epidemiological contexts (especially hightransmission areas).
3. Investigate how a combination therapy strategy could be

financed. This strategy, if proven cost-effective, will nonetheless be more expensive than current strategies. What mechanisms might be developed to assist countries in adopting this strategy?

B. Invest significantly in identifying strategies to improve acceptance of and compliance with drug regimens, especially a combination therapy strategy, at all levels of official and unofficial health care systems, private sector, and community. Similarly, investigate to teach concepts of judicious use of antimicrobials (including antimalarial drugs) to health care providers.

C. Investigate ways to improve effectiveness of drug regulatory systems and ability to control introduction of new antimalarials within endemic countries. This is required to avoid uncontrolled use of new antimalarials resulting in development of resistance before they are needed which could significantly compromise their efficacy when they are needed.

D. Support new drug development. Investigate new approaches to drug delivery, such as timereleased formulations or novel delivery systems that would allow use of short half-life drugs while optimizing compliance. Investigate drugs (or vaccines?) that have transmission-blocking effect that could be used in combination with drugs active against blood-stage parasites.

E. Improve access to and use of definitive diagnosis-based treatment.

F. Support more widespread use of insecticidetreated materials or other appropriate vector control strategies to reduce frequency of clinical illness (and therefore, treatment) as well as overall malaria transmission.[18]

Because overall drug pressure is thought to be the single most important factor in development of resistance, following more restrictive drug use and prescribing practices would be helpful, if not essential, for limiting the advent, spread, and intensification of drug resistance. This approach has gained support in North America and Europe for fighting antibacterial drug resistance.[21, 22]

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