Original Research Article

Comparing the Parasite Clearance Rates of Chloroquine and Doxycycline in Malaria infection

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Abstract

Background: The emergence of artemisinin-resistant malaria parasites has generated great concerns. In order to explore other treatment options, the clearance rates of chloroquine (CQ) and doxycycline (DX) on malaria parasitemia were compared.
Methods: Participants were recruited at the central village square on consecutive basis. Thick peripheral capillary blood were obtained using finger pricking technique. Thick blood smears were prepared on laboratory slides using standard technique and visualized under 100X objective microscope per 100 high power fields (hpf). Of the 603 participants examined, 104 (17.2%) aged 10-≥60 years tested positive to malaria parasite and were randomly allotted into treatment Groups A (n=35) and B (n=35). The direct observed treatment method was adopted for the study. Group A was treated with the standard dose of chloroquine (CQ), while Group B was treated with 200mg daily dose of doxycycline (DX) for 7 days. Both groups were re-examined for parasite loads after treatment on day 8. Groups A and B were controlled with 17 participants per group who were treated with standard doses of multivitamin tablets for 7 days and re-evaluated for parasite load on day 8.
Results: The ≥60 age group recorded the highest (4.7mp/100hpf) Mean Parasite Density (MPD) in Groups A and B. The clearance rate of CQ (43.42%) was not significant ($p>0.05$), while DX recorded a significant ($p<0.05$) clearance rate of 100%.
Conclusions: The outcome of this trial highlights the viability of doxycycline as a potential partner drug to ACT in the treatment of malaria.
Keywords: Parasite; Clearance rate; Chloroquine; Doxycycline; Malaria.

Introduction

Malaria is a parasitic disease caused by *Plasmodium* parasites and spread through the bites of infected female Anopheles mosquitoes. It has been shown that the female *Anopheles gambiae* complex is responsible for most transmission of the disease in Africa [1]. There are four *Plasmodium* species which transmit malaria in humans; these include *falciparum, vivax, malariae* and *ovale*. It has been reported that *Plasmodium falciparum* and *P. vivax* are the most common, while *Plasmodium falciparum* is the most deadly [2]. Recently, *Plasmodium knowlesi* has been identified as the fifth specie which transmits malaria among monkeys and humans and occurs in certain forest areas of Southeast Asia [3].

Malaria is one of the most common diseases affecting humans worldwide [4] and the impact of the disease is mainly among the developing countries, with the heaviest burden in Africa [5]. It is estimated that about 2.6 million clinical episodes of malaria and 655,000 deaths occur worldwide, of these deaths, around 91% were seen in the African region, followed by the Southeast Asian region (6%) and the Eastern Mediterranean region (3%), while 86% of deaths globally were in children [6]. Furthermore, a study [7] opined that 3.3 billion people worldwide live in areas at risk of malaria transmission in 106 countries and territories. The economic impact of malaria has been estimated to cost Africa US$12 billion every year [8], this includes costs of healthcare, working days lost due to sickness, day lost in education, decreased productivity due to brain damage from cerebral malaria, loss of investment, tourism and diversion of household resources [9]. In Nigeria, the economic impact of malaria can be attributed to gross national income per capita (GNI) of US$260 [10].

Malaria parasite is a tiny intracellular parasite in the phylum Apicomplexa. The parasite has a unique structure known as the apical complex which contains secretary organelles; rhoptries, micronemes, and dense granules. These organelles sequentially secrete enzymes that allow the parasite to invade other cells. Dahl *et al.* [11] reported two lines of empirical evidences which showed that apicoplasts are essential in parasite survival; first, chemicals affecting apicoplast metabolism resulted in parasite death; secondly, parasites that were unable to replicate the apicoplast also died. Amazingly, in both cases, the parasites only died in the next generation. This means that

the parasite can survive without an apicoplast or with a chemically-damaged apicoplast while remaining in the infected host cell; however, the parasite is unable to establish a successful new infection [12]. This phenomenon is known as delayed death. Ralph *et al.* [13] hypothesized that the apicoplast synthesizes a molecule that is needed in the infection process and therefore stands out as an attractive target for known antibiotics and herbicides.

Doxycycline (DX) is a lipophilic bacteriostatic antibiotic and a translation inhibitor that specifically acts by blocking the expression of the apicoplast genome, resulting in the distribution of non-functional apicoplasts into daughter merozoites [14]. The loss of apicoplast function in the progeny of treated parasites leads to a slow but potent anti-malarial effect. Apicoplast–specific antibiotics such as doxycycline cause an unusual delayed death in the next progeny of the treated parasite, rather than the death of the treated parasites [15].

For many decades, CQ and sulphadoxine-pyrimethamine were the choice drugs for the treatment of malaria, until recently, it was discovered that the drugs no longer worked in most tropical countries and resistance was reported in Asia, South America and Africa [16]. As resistance to these drugs worsened, morbidity and mortality consequently increased. To curb the rising fatalities associated with resistance to these drugs, The World Health Organization (WHO) recommended the artemisinin-based combination treatments (ACTs) for uncomplicated *falciparium* malaria and replacement of the old treatment protocol.

However, the emergence of artemisinin-resistant malaria parasites in Southeast Asian countries, and the possibility of spreading to other endemic populations have been reported [16]. To avert resistance in ACT drugs, the artemisinin derivative and the partner drug must be present together at inhibitory concentrations. But for ACT drugs, artemisinin derivatives are eliminated rapidly, and the companion drugs are eliminated slowly; therefore, complete protection is provided only for the artemisinin derivative [17]. The combination still provides good protection against the emergence of resistance to the companion drug, but once resistance has developed, the residual concentrations of unprotected companion drug provide a selective filter enhancing the spread of resistance to the

artemisinin compound [18, 19]. Consequently, an ACT drug requires the presence of an additional partner drug with longer half-life and high parasite clearance rate during parasite interaction to forestall resistance. In the present study, the clearance rates of CQ and DX were compared to identify a potential strategy to expand the therapeutic lifespan of ACT drugs.

Methods

Study locale

The study was carried out in Ahani-Achi Community, Oji-River Local Government Area, Enugu State, Nigeria. The community has geographical coordinates of 6.1221°N 7.3609°E. It is marked with typical rain forest mosaic vegetation, characterized by mainly rainy seasons and traversed by many streams and fast flowing rivers such as Iyibenze, Nwoka, Ogba, Iyi Owerri, Iyi Agwo and Ngene Iyi Agu drained by a principal tributary known as Oji River. It has an annual rainfall of approximately 2,900-3,400 mm, with maximum precipitation occurring from June to August. The main occupations of the people are farming, fishing and trading.

2.2 Study design

The experimental design was adopted for the study.

Ethical consideration

The study was conducted in accordance with the 1967 Helsinki Declaration on Human Experiments (as modified in Fortaleza, 2013). Approval was obtained from the Ethical Committee of the Faculty of Science, Imo State University Owerri, Nigeria and the written informed consents of the participants were obtained.

Procedure for data collection

Six hundred and three (603) participants were examined on consecutive basis. The tip of the third finger was sterilized with a spirit swab; air dried and pricked using a sterile and disposable lancet to obtain a drop of blood placed on a microscopic slide. The drop of blood was turned in a circular pattern, 3-6 times with a glass rod to obtain a thick blood smear. Two (2) drops of field's stain A were applied on the dry blood smear. At 2 minutes post-application, the stain was gently run through water from a wash bottle and 2 drops of field's stain B were applied on the smear. At 3 seconds post-application, stain B was run through slow flowing water and the blood film was air dried. A drop of oil immersion was applied on the smear sample, air dried and directly visualized under a 100X objective microscope per 100 high power fields (Lumberg *et al*.) [20]. The

pre-treatment parasite load was quantified and recorded per 100 high power fields.

Of the 603 participants examined, 104 (17.2%) aged 10-≥60 years tested positive to malaria parasite, satisfied the inclusion criteria and were recruited into the study. The 104 participants were randomly allotted into treatment Group A (n=35) and Group B (n=35), as well as Control A (n=17) and Control B (n=17). Group A was treated with 1000mg loading dose of CQ, followed by 500mg after 6-8 hours, then 500mg each day for 2 days and the Control A was treated with the standard dose of multivitamin for 7 days. Group B was treated with 200mg daily dose of DX for 7 days, while Control B was treated with standard doses of multivitamin for 7 days. The post-treatment parasite densities for experimental and control groups were re-evaluated on day 8. All treatment protocols were based on the Direct Observed Treatment Method.

Statistical analysis

Data were analyzed with Z-test at 95% confidence interval and $p<0.05$ was considered statistically significant.

Results

The 60+ year age group recorded the highest pre-treatment MPD (4.7 mp/100hpf), while the 40-49 years group recorded the least pre-treatment MPD (2.4mp/100hpf). The total pre-treatment Mean Parasite Density (MPD) was 19.2mp/100hpf, while the total post-treatment MPD was 17.75mp/100hpf representing an insignificant ($p>0.05$) parasite clearance rate of 43.42% (Table 1). The ≥60 year age category had the highest MPD of 4.5 mp/100hpf. The total MPD was the same pre and post-treatment (Table 2). The total pre-treatment MPD was 18.6mp/100hpf, while the post-treatment MPD was zero representing a significant ($p<0.05$) clearance rate of 100% (Table 3). The 60+ year age group had the highest pre-treatment MPD (4.2mp/100hpf), while the 50-59 years age category had the least pre-treatment MPD (2.5mp/100hpf). The ≥60 year age group had the highest MPD of 3.5 mp/100hpf. The total pre-treatment MPD was 12.6 mp/100hpf and remained the same after treatment (Table 4).

Discussion

The outcome of chloroquine intervention in the present study provides a valid justification for the restriction of its use in the treatment of malaria due to the emergence of resistant plasmodium strains and cross-resistance to CQ analogs [21,22]. Although the repurposing potentials of CQ in treating other diseases have been identified [23-26], its application in the

treatment of malaria has been forestalled, notwithstanding some therapeutic advantages [27,28]. The study further demonstrates the insignificant ($p>0.05$) clearance action of CQ on malaria parasitemia and lends credence to the ACT initiative which has become the mainstay for the treatment of complicated and uncomplicated malaria in endemic and non-endemic communities [29-31]. However, molecular markers of decreased susceptibility to artemisinin have been reported [32].

The clearance rate of 43.42% generated by CQ indicates, to a significant extent, the enormity of the burden of CQ-resistant strains within the community and raises valid concerns about the wellbeing of the population where chloroquine dependency remains a significant challenge and could result in therapeutic failure, elevation of infection density, threat to life, economic loss, as well as poor quality of life. In addition, the poor parasite elimination rate of CQ recorded in the present study presents it as a non-viable option in the quest for a novel therapeutic paradigm and partnership with ACT in the treatment of malaria infection.

Furthermore, the MPD of participants in the present study did not inversely co-relate with age contrary to extant hypotheses [33-37] which suggest that children less than 5 years of age have higher pre-treatment MPDs than other groups, harbor more parasite load and are more likely to die of malaria due to immunological immaturity. The discrepant outcomes and numerical discordance may be predicated on ecological disparity and older ages of participants in the present study which could have elevated their immunological resistance to malaria infection.

The present study demonstrates the potency of DX on malaria parasites and supports the outcome of a previous study [38] where a comparable clearance rate was reported. It highlights the significant ($p<0.05$) action of DX on malaria infection which makes it attractive as a potential solution to ACT-resistance. Despite the remarkable treatment outcome of DX, evidence [39] has shown that it is contraindicated in children less than 8 years of age, and this appears to be a major set-back in resolving the ACT-resistance factor among children within this age category.

The present study and previous collaborative evidences [40,41] have demonstrated the waning effect of CQ on malaria parasites, while susceptibility to parasite recrudescence by patients treated with quinine has also been reported [42]. On the other hand, DX has shown a remarkable action against malaria parasites and has been reported to be safe and well tolerated [43] with longer half-life than artemisinin derivative [44]. Therefore, DX appears to proffer a potential solution to the ACT-resistance factor in the treatment of malaria infection.

Limitations

There was no preliminary parasitological profile to ascertain the specific strain of malaria parasite within the community prior to the study. This could raise valid ethical questions concerning the generalizability of the study outcomes and strain-specificity of drug actions. In addition, the lack of clarity on strain-specificity could lead to therapeutic failure and adversely affect the repeatability of the study in populations infected by variants of the parasite.

The biochemical compatibility of DX with ACT drugs was not investigated by the present study. This imposes limitations on the validity of DX tolerance when combined with ACT as an additional partner drug. Furthermore, previous evidences have shown that doxycycline is not well tolerated in children ≤8years and pregnant women; therefore, these populations were excluded from the study and data were not generated to demonstrate their suitability for this therapeutic strategy.

The presence of ACT-resistant strains in the community was not ascertained. Therefore, the study could not address the potential need for the application of an additional partner drug to ACT within the community. The general assumption of the emergence of ACT-resistant strains could be misleading without being population-specific and emphatic on the ecological discrepancies and parasitological variations among different populations.

Conclusion

The outcome of this trial highlights the inefficacy of chloroquine in partnering with ACT in the treatment of malaria and provides valuable insights into the remarkable clearance actions of doxycycline on malaria parasites. It further addresses the suitability of doxycycline in providing the therapeutic partnership required for the optimization of ACT drugs in the treatment of malaria.

Conflict of interest

The author declares that there is no conflict of interest.

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Tables:

Table 1: Mean Parasite Density pre and post-chloroquine treatment

Age (Years)	Pre-treatment MPD (mp/100 hpf)	Post-treatment MPD (mp/100 hpf) (% clearance)	p-value
10-19	3.2	2.97(7.19)	0.340903
20-29	3.7	3.43(7.30)	
30-39	2.7	2.51(7.04)	
40-49	2.4	2.23(7.09)	
50-59	2.5	2.38(4.80)	
≥60	4.7	4.23(10)	
Total	**19.2±15.49**	**17.75±14.36 (43.42)**	
Z-Score=	**0.41**		

Table 2: Mean Parasite Density pre and post-multivitamin treatment (Control A)

Age (Years)	Pre-treatment MPD (mp/100 hpf)	Post-treatment MPD (mp/100 hpf) (% clearance)
10-19	2.2	2.2 (0)
20-29	2.7	2.7 (0)
30-39	1.7	1.7 (0)
40-49	2.1	2.1 (0)
50-59	2.0	2.0 (0)
≥60	4.5	4.5 (0)
Total	**15.2**	**15.2 ((0)**

Table 3: Mean Parasite Density pre and post-doxycycline treatment

Age (Years)	Pre-treatment MPD (mp/100 hpf)	Post-treatment MPD (mp/100 hpf) (% clearance)	p-value
10-19	3.2	0 (100)	0.00001
20-29	3.1	0 (100)	
30-39	2.9	0 (100)	
40-49	2.7	0 (100)	
50-59	2.5	0 (100)	
≥60	4.2	0 (100)	
Total	**18.6±3.87**	**0±0 (100)**	
Z-score	**28.62**		

Table 4: Mean Parasite Densities pre and post-multivitamin treatment (Control B)

Age (Years)	Pre-treatment MPD (mp/100 hpf)	Post-treatment MPD (mp/100 hpf) (% clearance)
10-19	2.0	2.0 (0)
20-29	2.5	2.5 (0)
30-39	1.2	1.2 (0)
40-49	1.6	1.6 (0)
50-59	1.8	1.8 (0)
≥60	3.5	3.5 (0)
Total	**12.6**	**12.6 (0)**