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Resistance to anti-malarials: Evaluation of mutations in the *Pfcrt* and *Pfmdr1* genes in isolates from Gabonese patients

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Objectives

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Major objective : to evaluate the prevalence of specific parasite mutations involved in the resistance to chloroquine (CQ), quinine, mefloquine (MF), halofantrine, as well as artemisinin-based combinations in isolates from Gabonese patients

Specific objectives:

-To analyse point mutation in *Pfcrt* (K76T) gene in isolates from children with uncomplicated or severe malaria

-To analyse point mutations in *Pfmdr1* (Asn86Tyr, Tyr184Phe, Ser1034Cys, Asn1042Asp, Asp1246Tyr) gene in isolates from children with uncomplicated or severe malaria

-To determine if there is any realtionship between the prevalence of these mutations and the severity of the disease.

Methods (1)


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Place: Albert Schweitzer Hospital in Lambaréné, Gabon (Central Africa)
Population: children > 6 months with uncomplicated or severe malaria.
Periods of recruitment of patients: (1) January 1995 - March 1996 during a matched pair case-control study, (2) January - March 2002 by using similar inclusion or exclusion criteria

***Blood collection*:** at inclusion after confirmation of *P. falciparum* infection and signed informed consent.
The gender, age, parasitaemia, haemoglobin level, any sign of severe malaria, treatment outcome were recorded.

Molecular characterization of parasites
After parasite DNA extraction using the QIAamp DNA blood mini Kit:
- *P. falciparum* parasite genotyping using *MSP-1* and *MSP-2*
- Analysis of point mutation in codon 76 of *Pfcrt* and those in *Pfmdr1* (Asn86Tyr, Tyr184Phe, Ser1034Cys, Asn1042Asp, Asp1246Tyr) by different nested PCRs and successive enzymatic digestions.

Resuts (1)

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Characteristics of patients at inclusion:

Year 1996: 46 severe *vs* 45 uncomplicated cases

- **Mean age** (months): 43.3 *vs* 45.9
- **Geometric mean parasite density** (p/µl): 220,849 *vs*10,545 (P<0.0001)
- **Mean haemoglobin rate** (g/dl): 8.2 *vs*10.5 (P<0.0001)
- **Multiplicity of *P. falciparum* infection**: 2.4 *vs* 2.2

Year 2002: 30 severe *vs* 30 uncomplicated cases

- **Mean age** (months): 22.6 *vs* 25.2
- **Geometric mean parasite density** (p/µl): 21,544 *vs*15,796
- **Mean haemoglobin rate** (g/dl): 4 *vs* 8.6 (P<0.0001)
- **Multiplicity of *P. falciparum* infection** : 3 *vs* 2.9

Prevalence of point mutations in the *Pfcrt* and *Pfmdr1* genes of clinical *P. falciparum* isolates:

- Presence of the *Pfcrt* K76T mutation in all isolates.
- *Pfmdr1* 86Tyr mutation: 85.5% and 98.7% in severe and uncomplicated group respectively.
- *Pfmdr1* 184Phe mutation: 70% and 76% in severe and uncomplicated group respectively.

Resuts (2)

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- No difference in the frequency of the *Pfmdr1* 86Tyr and 184Phe mutations between severe and uncomplicated group in both periods of recruitment.

- No *Pfmdr1* 86Asn wild-type allele in isolates from uncomplicated patients in 2002

- No mutations at the *Pfmdr1* 1034 and *Pfmdr1* 1042 position in all isolates.

- *Pfmdr1* 1246Tyr mutation: 8.8% and 10% in uncomplicated cases in 1996 and 2002 respectively.
- No *Pfmdr1* 1246Tyr mutation in isolates from severe malaria.

- Mixed genotypes at the *Pfmdr1* 86 position in the severe malaria group in one and two isolates from 1996 and 2002 respectively.

*** No specific mutation in the *Pfmdr1* gene associated with the severity of disease**
*** No change in the polymorphism of *Pfcrt* and *Pfmdr1* genes in *P. falciparum* isolates collected in 1996 and 2002, and the severity of the disease was not associated with specific mutations neither in the *Pfcrt* nor in the *Pfmdr1* genes in the study site.**

Capacity building

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- 2 *PhD* students trained in molecular biology of malaria parasites and immunology
- Acquisition of competencies in clinical research,
- Training in GCP and GLP

Networking activities

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- South- South : Strengthening collaboration between the CERVE (Brazzaville, Congo) and MRU (Lambarene, Gabon): exchange of students

- SMAC (Severe Malaria in African Children) funded by the National Institute for Health (USA) involves six Institutions from Gabon, Gambia, Ghana, Kenya and Malawi.

- ANTIMAL, a consortium for anti-malarial drug discovery and training of PhD student through which more than twenty African and European research centres are involved.

Discussion & Conclusion


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- The absence of difference of multiplicity of infection between uncomplicated and severe confirms previous results in Lambaréné. The high prevalence of the *Pfmdr1* 86Tyr, 184Phe and *Pfcrt*K76T mutation may reflect higher levels of CQ resistance

- The presence of the *Pfcrt* K76T mutation in all isolates was expected in Lambaréne with regard to the *in vivo* resistance to CQ.

- The absence of difference in the prevalence of *Pfmdr1* mutations between isolates from severe and uncomplicated group may confirm the lack of a close association between *Pfmdr1* point mutations and *in vivo* resistance to antimalarials like CQ and MF in our study site.

***These results may suggest that other changes in intrinsic parasite and/or host factors that enhance parasite multiplication and virulence may lead to the severity of the disease in our study site.**