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USE OF SALIVA FOR MONITORING ANTIMALARIAL DRUG RESISTANCE AT THREE SITES IN SOUTHERN COTE D'IVOIRE

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Abstract

Background: *The malaria diagnostic methods developed to date all require blood to be taken. However, blood sampling can be an obstacle because some people are reluctant to have their blood sample taken because of the sting of needle (especially when blood sampling is repeated) or even because blood is taboo. Saliva, which is minimally invasive to collect, offers an alternative way of overcoming this obstacle. The aim of this study was to use saliva to monitor Plasmodium falciparum resistance to pyrimethamine at three sites in southern Côte d'Ivoire.*

Methodology: *Blood and saliva samples were collected in three different localities from 94 patients over 2 years of age with microscopically confirmed uncomplicated Plasmodium falciparum malaria. P. falciparum genomic DNA was then extracted and amplified by nested PCR using primers specific to pfdhfr (Plasmodium falciparum dihydrofolate synthethase) gene. The amplification products were sequenced using the Sanger method. After sequencing, the prevalences of pfdhfr mutations (N51**I**, C59**R**, S108**N**) confirmed to be involved in pyrimethamine resistance in P. falciparum were determined. Data were analysed using R statistical software, version 3.2.2.*

Results: *After amplification, 153 DNA fragments of pfdhfr gene were sequenced including 86 fragments of blood DNA and 67 fragments of salivary DNA. In blood, 65 (75.58%), 66 (76.74%) and 81 (94.18%) DNA fragments were successfully sequenced, compared with 57 (85.07%), 58 (86.56%) and 63 (94.02%) in saliva for the N51**I**, C59**R** and S108**N** mutations respectively. Sequences analysis indicated that mutations in pfdhfr gene were observed at prevalences of 61.53% (N51**I**), 54.54% (C59**R**) and 74.07% (S108**N**) in blood compared with 49.12% (N51**I**), 63.79% (C59**R**) and 79.36 (S108**N**) in saliva. No significant difference was observed between the prevalence of gene mutations in the two biological products (p=0.44). Molecular analysis showed that the susceptible haplotype NCS (51N59C108S) was observed in 17 of the 153 isolates, with a prevalence of 13.96% (12/86) in blood compared with 7.46% (5/67) in saliva. The IRN (51**I**59**R**108**N**) triple mutant haplotype was observed in 48 of the 153 isolates with a prevalence of 31.4% (27/86) in blood compared with 31.34 (21/67) in saliva. The prevalences of the IRN triple mutant did not differ significantly in blood and saliva (p = 0.685).*

Conclusion: *Polymorphism study of pfdhfr gene showed that the prevalences of genotypes conferring resistance to pyremethamine (P) reached comparable levels in blood and saliva isolates. Thus, more than a decade after the adoption of sulfadoxine-pyrimethamine (SP) as intermittent preventive treatment (IPT) for pregnant women in Côte d'Ivoire, prevalence of the Asn-108 allele and the IRN triple mutant haplotype in blood and saliva were relatively high in Anonkoua-kouté, Port- bouët and Ayamé.*

Keywords:

Pfdhfr, Saliva, Côte d'Ivoire, Sulfadoxine-Pyrimethamine, Resistance, Antimalarial Drugs, Plasmodium Falciparum