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Molekularbiologische Charakterisierung von Malaria-Infektionen in einem ländlichen Gebiet in Burkina Faso

Molecular assessment of malaria infections in a rural area in Burkina Faso

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The aim of this work is to evaluate the feasibility of recent molecular techniques as an epidemiological tool concerning human *Plasmodia* infections in malaria endemic areas, and to asses how these methods could be informative in detecting this infection, versus standard microscopic examination of blood films. A further aim is the detection of PfCRT K76T point mutation as a molecular marker for chloroquine resistant *P.falciparum* strains.

The study was conducted with sample collection from inhabitants of a rural area, endemic with malaria infection, in the north-west of Burkina Faso during the rainy (high transmission) season. This was followed by laboratory work done in the Parasitology department lab of Heidelberg-University, Germany.

Detection and species identification was done by microscopic examination of Giemsa-stained (thin and thick) blood films for each person.

For molecular techniques to be applied, the *Plasmodia* DNA was extracted from blood spots on filter paper, using 20% Chelex-100 method. Afterwards a nested PCR assay was performed, using 1st genus-specific pair of primers followed by 2nd species- specific pair of primers. *Plasmodia* species-specificity was confirmed by cloning each *Plasmodia* species PCR product in a vector and followed by sequence analysis. *P.falciparum* merozoite surface proteins *msp1* & *msp2* genes were chosen to detect multiplicity of *P.falciparum* strains.

Detection of *Plasmodium falciparum* strains resistant for chloroquine was accomplished by applying a technique for Nested PCR assay for *pfcrt* fragment amplification, followed by restriction enzymatic digestion reaction for detection of *pfcrt* gene K76T point mutation as a marker for chloroquine resistance.

This study showed absolute sensitivity of PCR assay in detection and species determination of human *Plasmodia* parasite from peripheral blood spotted on filter paper. So the PCR assay was an effective method in detection of malaria infection from preserved samples. In addition PCR assay showed more sensitive results than microscopy, revealing **15.3%** (29/190) of malaria-

endemic inhabitants had sub-microscopical parasitaemia, and **39.3 %** (71/181) of plasmodial infections were subpatent infections.

PCR assay was more efficient in human *Plasmodia* species identification, by conventional microscopy of Giemsa stained blood smears: prevalence of *Plasmodium* species, *falciparum, malariae and ovale* infections was **79.4%** (151/190), **3.7%** (7/190), **0 %** respectively compared to nested PCR method performed on blood samples spotted on filter paper for the detection of *Plasmodium* species-specific ssrRNA gene it was **95.3 %** (181/190), **14.7 %** (28/190), **10 %** (19/190). This would show that microscopy failed to detect three quarters of *P.malariae* infections, and all *P.ovale* infections.

Subsequently PCR assay was more effective in diagnosing mixed double and triple human *Plasmodia* species infections than microscopy, with microscopy **96%** (146/152) and **3.9%** (6/152) were single and double *Plasmodia* species infections, and no triple *Plasmodia* species infection could be detected by microscopy. However with PCR assay **77.9%** (141/181), **18.2%** (33/181) and **3.9%** (7/181) were single, double and triple *Plasmodia* species infections respectively. Detection of mixed infection is of medical importance as some *Plasmodia* species, when not effectively managed, runs the risk of delayed relapses, or others that have risk of serious remote complications.

Detection of *Plasmodium falciparum* multiplicity by PCR analysis revealed a high multiplicity rate of **78%** (141/181), which decreased with age, possibly reflecting specific anti-parasite immunity acquired with age in holoendemic malarious areas

Detection of *P.falciparum* chloroquine-resistant strains using PfCRT K76T mutation as a marker was successfully applied on preserved samples, and revealed a high prevalence of Chloroquine resistant strains **45.3%** (82/181) in this endemic area, providing a very efficient chloroquine resistance detector, thus avoiding the old tedious drug efficacy assessments and inefficient chloroquine therapy trials.

PCR assay was able to provide more accurate indicators about dynamics of malaria infection in this endemic area. It showed clearly that children up to 10 years old were a very high risk group; with *P.falciparum* prevalences up to **100%**, highest peaks of mixed *Plasmodia* infections, highest *P.falciparum* multiplicity **92.6%** (36/68), and highest prevalence **59%** of chloroquine resistance marker PfCRT K76T. Also PCR assay provided clues of gradual decrease in *Plasmodia* infection prevalences with age, which may support the theory of gradual acquisition of partial immunity against the disease in endemic areas.