THE EPIDEMIOLOGY OF MULTIPLE *PLASMODIUM FALCIPARUM* INFECTIONS IN CHILDREN LIVING IN A HOLOENDEMIC AREA

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In areas with high levels of transmission of *P. falciparum* malaria, a large proportion of individuals is always harbouring malaria parasites without any associated clinical symptoms. On the average, an unprotected individual in villages around Muheza would experience between 1 and 7 infective bites in one night. Acute attacks of *P. falciparum* malaria in semi-immune children living in endemic areas are probably associated with the acquisition of new infections, possibly of novel variant antigenic types. Modelling studies have proposed that within a certain number of parasite strains a small number of more virulent parasites exist. These parasites are thought to be less frequently transmitted, thus immunity against them develops much more slowly than it does against the other common mild strains.

With the new technology of polymerase chain reaction (PCR) based genotyping, Amani Centre scientists recently made further in-depth longitudinal analysis of the dynamics of *P. falciparum* multiple infections. The studies aimed at understanding the complexity of *P. falciparum* sub-populations harbored by constantly exposed asymptomatic individuals, the associated dynamics and changes as the host develops clinical symptoms. Observations were made on a cohort of initially asymptomatic children who were then followed daily for 31 days. During the observation period, over half of the children developed clinical malaria symptoms on one or more occasions. The collected samples thus enabled the team to study the population dynamics of the different parasite sub-populations (strains) before, during and after clinical malaria episodes. The studies were based on analysis of the highly polymorphic merozoite surface proteins 1 and 2 (*msp1* and *msp 2*) as well as glutamate rich protein (*glurp*) marker genes. Studies of this kind are the only ones expected to provide an understanding about the role of parasite allelic diversity in the progression from asymptomatic to symptomatic conditions.

Daily fluctuations in *P. falciparum* densities were observed, very often exhibiting a 48 hours periodicity of peak densities. On some of the days, the fluctuations were observed to display unpredictable sinusoidal patterns. In symptomatic children, peak parasitaemias were often associated with fever and other clinical symptoms whereas asymptomatic children could harbour high parasitaemias without developing fever or other clinical malaria symptoms. Parasite density was found to have a linear association to increased risk of developing clinical malaria symptoms.

Very dramatic day to day variations in the parasite allele profile between children and between samples collected from the same child on subsequent days were observed. All children were found to carry multiple *P. falciparum* infections. The mean number of alleles estimated for children belonging to asymptomatic or symptomatic groups were 9.3 and 5.4 alleles per child, respectively. Symptomatic children were found to carry an average of 4 genotypes or strains whereas asymptomatic ones were found to carry an average of 6 genotypes or strains. A significant difference in *msp1* allele frequency distribution between the symptomatic and asymptomatic groups was observed.

Both estimates for mean number of allele and mean number of genotypes per child indicate a higher level of *P. falciparum* multiplicity in asymptomatic children. These observations, like in the previous findings suggests reduced risk of clinical malaria in children infected with multiple strains (populations) of *P. falciparum*.

From the available information, it is unclear whether the observed low multiplicity in symptomatic children is a cause or consequence of clinical symptoms. Despite this limitation, the observed scenario conforms to the expectation with the strain specific immunity phenomenon. A child with high multiplicity is expected to have a broader spectrum of immunological memory than one with low multiplicity, therefore being more protected.

However, several other propositions could as well explain this observation. Firstly, since most fever episodes appear to be associated with parasitaemia peaks maybe such peaks could be resulting from expansion of just a few or even single genotype(s) that provide a disproportionately larger amount of DNA. This may in turn result in a single or a few particularly abundant genotype DNA's to outcompete and overshadow the less abundant genotype DNA's during amplification. This could result in identifying just a few

genotypes despite presence of more genotypes in the sample. Development of quantitative PCR is expected to resolve this paradox. Secondly it could be that, fever itself is known to have an antiparasitic effect that might result in elimination of several genotypes (strains) from the initial parasite pool. Lastly, there is a possibility that symptomatic children are more predisposed to such condition and may be more likely to be taking antimalarials more often thus eliminating a considerable number of genotypes (strains) from their parasite pool.

Presence of certain allele types (Mad20 or IC1) was found to be significantly predictive of development of clinical symptoms. This finding differ with previous observations where FC27 was associated with clinical symptoms in Papua New Guinea and in Uganda where Mad20 type has been recently associated with protection against clinical malaria. It appears that different studies in different locations are likely to associate different allele types with clinical symptoms possibly as result of variations in both parasite genotype composition and human host immunological experiences.

Contrary to our expectation, our attempt to investigate the effect of new alleles on clinical malaria outcome could not show any significant effect. Earlier studies in Senegal and Sudan showed that emergence of new alleles was associated with clinical malaria. There is a marked difference in the respective levels of transmission, and our study area has the highest level of transmission. With high endemicity, multiclonal infections and cross-matings are prevalent. The two are known to be potential mechanisms for promotion of diversity, resulting from a high level of novel genotypes. It therefore appears that the rate of acquisition of new alleles in our study area was extremely high and not all of them resulted in clinical infections. This may have led us to obtain such an insignificant association in our analysis. However, this observation do not necessarily contradict the earlier conclusions made on the association between emergence of new alleles and clinical symptoms in Sudan and Senegal.

One of our observation with major epidemiological implications is that; in holoendemic areas, a single blood sample is unlikely to be a true representative of the parasite population(s) infecting an individual. The finding that all isolates examined in this study had multiple infections is unprecedented. All these observations have very important implications in terms of interpreting and understanding the dynamics of *P. falciparum* sub-populations.