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Genetic complexity of *Plasmodium vivax* infections in Sri Lanka

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Using *Plasmodium vivax* Merozoite Surface Protein-3α (*Pv*MSP-3α) marker, the existing genetic complexity of *P. vivax* infections was evaluated in individuals (N=146) from two malaria endemic areas, Kataragama (N=68), Anuradhapura (N=39) and from Colombo (N=39), a non-endemic region of Sri Lanka. A combination of polymerase chain reaction / restriction fragment length polymorphism (PCR/RFLP) techniques was used for this assessment.

Undigested PCR products showed a major size polymorphism. The product sizes were approximately 1900, 1500 and 1200 bp among which the former was the most predominant (72.8%), while the other two accounted for 25.8% and 1.4%, respectively. The predominance of the 1900 bp product was more significant [Chi square test: (with 1500 bp; $P<0.05$) and (with 1200 bp; $P<0.01$)] than the other two products in all three study areas. Despite the presence of only three size variations in undigested PCR products, RFLP analysis yielded highly diverse fragment sizes and banding patterns for all samples. The RFLP patterns of all isolates showed size conservation of the largest fragment obtained from *Hha* I digestion (~1,000 bp) and *Alu* I digestion (~500 bp), while smaller fragments showed considerable size variation. The summation of RFLP fragments of 27 samples (19%), was significantly greater than the size of the undigested product, indicating the presence of more than one *Pv*Msp-3α allele in these infections. The number of these multiclonal infections was significantly higher (Chi square test; $P<0.05$) in the two endemic areas as compared to the non-endemic area. From the samples in which single clonal infections were detected, where smaller fragments resulted from RFLP digestion, at least 10 different banding patterns from each restriction enzyme were detected, indicating the presence of a substantially high diversity at the nucleotide level.

These results demonstrate the prevailing genetic complexity among individual *P. vivax* infections in Sri Lanka, and also confirm the suitability of utilizing PvMsp-3α locus to analyze the degree of polymorphism as well as clonality of *P. vivax* infections in this geographical region.

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