A-01: TNF allele polymorphism and disease severity of *P.falciparum* malaria in Sri Lanka

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Malaria infections, particularly those caused by *P.falciparum*, lead to a wide spectrum of clinical disease ranging from mild uncomplicated to severe/complicated and life-threatening conditions. Factors involved in the pathogenesis in *P.falciparum* malaria are likely to include host genetics which determine the final outcome of the disease. Specifically, it has been shown that an allelic polymorphism associated with TNF genes correlates with severity of malarial disease. The objective of this study was to investigate the association between TNF allele polymorphism and the outcome of malarial infections in Sri Lanka patients.

The subjects comprised malaria patients detected in the malaria endemic Provinces of Anuradhapura and Moneragala. Based on a clinical valuation and monitoring programme, all patients were clinically assessed using standardized questionnaires and based on clinical and laboratory investigations. A numerical scoring system (the severe and complicated score [S & C score]) previously validated was used to classify patients into uncomplicated (S & C score of <6) and severe/complicated (S & C score >6) categories.

Human DNA was extracted from finger-prick blood samples taken from patients, using the phosphate lysis method. TNF alleles 1 and 2 from the promoter region of the TNF-alpha gene were amplified using oliogonucleotide primers by the Polymerase Chain Reaction (PCR).

PCR-amplified products were subjected to TNF allelic typing by probing with ^{32}P end-labelled allele specific oligonucleotides specific for TNF1 and TNF2 using the dot blot and hybridization techniques. Probe specificity was visualized by autoradiography using X-ray Omat films. The frequencies of the 2 TNF alleles in the patient population were calculated using the Hardy-Weinberg equation. The results were statistically analysed by a t-test.

TNF allele typing was carried out in 118 patients with uncomplicated malaria and 21 patients with life-threatening severe/complicated malaria; the latter group included cerebral symptoms, convulsions, severe anaemia and liver cell dysfunction/liver failure.

Of the 139 patients, 109 were homozygous for the TNF1 allele, 30 were heterozygous (TNF 1 & 2), and no TNF2 homozygotes were found. The allelic frequencies of TNF1 and TNF2 in the entire patient sample were thus 0.88 and 0.12 respectively.

The homozygous state for TNF was found to be 1.4 times more frequent in the uncomplicated patients ($n=118$) than in severe/complicated patients ($n=21$), although a level of statistical significance was not reached. This trend suggests that the TNF1 allele may be associated with uncomplicated malaria.

When the intensity of severe and complicated malaria was considered, however, patients who were homozygous for TNF1 had a higher severe and complicated score than those who were heterozygous ($p<0.01$).

The frequency of the 2 alleles in our sample of patients so far, does not support the findings of other workers that the TNF2 allele is associated with a higher risk of severe/complicated malaria. The higher frequency of the TNF1 allele in the uncomplicated group suggests such a trend, but this was not statistically significant. More importantly, and to the contrary in the severe and complicated group of patients, there was a statistically significant association between homozygosity for TNF1 and a higher intensity of complications.