

ABSTRACT

The burden of malaria is enormous and control of the disease is constrained by the resistance developed by the parasite towards most antimalarials currently used to treat it, among other factors. Development of a vaccine which could be an alternative to antimalarial treatment have also suffered a setback because of antigenic diversity and gene polymorphisms and existence of many alleles in the same gene of the parasite. Typing of these genes, which is a pre - requisite in the development of an effective vaccine is currently hampered by lack of an effective method to analyze PCR products.

This study aimed at determining the detection limits of the radioisotopic method and agarose gel electrophoresis and by extension, finding out which method is more effective in determining the prevalence rates of **Fe - 27** and **le (3D7)** alleles of *Plasmodium falciparum*. The study also aimed at comparing the results from reverse or forward primers when used as probes and then selecting the suitable primers that can be used as probes in investigating the prevalence rate of these alleles.

Samples in this study were obtained from Western Kenya, a high transmission region and the Nubian region of Sudan, a low transmission region. A total of 64 samples and four positive controls were analyzed, 24 of the samples were from the Nubian region of Sudan and 40 from Western Kenya. The positive controls for **Fe- 27** allelic family were HB3 and T994 whereas for **le (3D7)** allelic family, the positive controls were **le (3D7)** and T996.

••

vii

The investigation involved collecting blood samples from malaria patients, extracting the DNA of *Plasmodium falciparum* from these blood samples, amplifying the extracted DNA using PCR method and finally analyzing the amplified products using either agarose gel electrophoresis or radioisotopic method to determine presence of the two alleles. Results of this analysis gave the prevalence rates of the two alleles in the population studied as determined by each method. To find out the detection limits of the two methods, a few selected samples were subjected to a serial dilution process. Lambda Hind III DNA was used as the standard in determining the detection limits. The detection limits for the two methods were significantly different ($p < 0.05$); the radioisotopic method was four times more sensitive than the agarose gel electrophoresis method. Their detection limits were 72.55 ng/ul for radioisotopic method and 290.7 ng/ul, for agarose gel electrophoresis method.

Studies on use of either forward or reverse primers as probes showed that forward primers hybridize better. Forward primer (M2-FCF) gave better signals with 65.6% of samples having FC - 27 allele compared to 9.4% for reverse primer (M2-FCR). For IC (3D7) allele, 25% of samples had this allele when forward primer was used compared to 1.6% of samples, which had this allele when reverse primer was used. A significance difference was noted for each allele ($p < 0.05$).

The study also focused on determining the prevalence rate of the two alleles in the population studied. There was a significant difference between the results for both FC - 27 and IC (3D7) families i.e. $p < 0.05$.

viii

The strength of association between the method used and the outcome i.e. whether positive or negative for the alleles, was determined. There was significant association for FC - 27 allele with a Pearson's chi - square value of 11.3 and phi - coefficient of

0.376 ($p > 0.05$). On the other hand, the association for the IC (3D7) allele was not significant ($p < 0.05$).

Further analysis on the ability of each method to detect the presence of both the alleles in the population was studied. Using radioisotopic method, a higher prevalence was obtained (29.7%) and this was significantly different from agarose gel electrophoresis (6.25%) i.e. $p < 0.05$. A t - test was also carried out on the mean percentage prevalence as determined by the two methods. A significant difference was noted ($p < 0.05$).

In conclusion, this study proved that radioisotopic method is indeed more sensitive than the agarose gel electrophoresis method. When used, radioisotopic method is likely to give a prevalence of these alleles that reflects their real distribution in the region of study. Also, the study showed that forward primers are better probes than reverse primers and therefore their use should be encouraged vis a viz reverse primers. Based on this therefore, I recommend that studies involving distribution of these alleles be carried out using radioisotopic method, as this will give the correct picture of the distribution of these alleles in the area of study. I further recommend the adoption of radioisotopic method in other similar studies .