

Genetic Diversity of Exon-20 of the IIS6 of the Voltage Gated Sodium Channel Gene from Pyrethroid Resistant Anopheles Mosquitoes in Sudan Savannah Region of Jigawa State

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Abstract : Malaria is a disease with global health significance. It is caused by parasites and transmitted by Anopheles mosquitoes. Increase in insecticide resistance threatens the disease vector control. The strength of selection pressure acting on a mosquito population in relation to insecticide resistance can be assessed by determining the genetic diversity of a fragment spanning exon- 20 of IIS6 of the voltage gated sodium channel (VGSC). Larval samples reared to adulthood were identified and kdr (knock down resistance) profile was determined. The DNA sequences were used to assess the patterns of genetic differentiation by determining the levels of genetic variability between the Anopheles mosquitoes. Genetic differentiation of the Anopheles mosquitoes based on a portion of the voltage gated sodium channel gene was obtained. Polymorphisms were detected; sequence variation and analysis were presented as a phylogenetic tree. Phylogenetic tree of VGSC haplotypes was constructed for samples of the Anopheles mosquitoes using the maximum likelihood method in MEGA 6.0 software. DNA sequences were edited using BioEdit sequence editor. The edited sequences were aligned with reference sequence (Kisumu strain). Analyses were performed as contained in dnaSP 5.10. Results of genetic parameters of polymorphism and haplotype reconstruction were presented in count. Twenty sequences were used for the analysis. Regions selected were 1- 576, invariable (monomorphic) sites were 460 while variable (polymorphic) sites were 5 giving the number of total mutations observed in this study. Mutations obtained from the study were at codon 105: TTC- Phenylalanine replaces TCC- Serine, codon 513: TAG- Termination replaces TTG- Leucine, codon 153, 300 and 553 mutations were non-synonymous. From the constructed phylogenetic tree, some groups were shown to be closer with Exon20Gambiae Kisumu (Reference strain) having some genetic distance, while 5-Exon20Gambiae-F I13.ab1, 18-Exon20Gambiae-F C17.ab1, and 2-Exon20Gambiae-F C13.ab1 clustered together genetically differentiated away from others. Mutations observed in this study can be attributed to the high insecticide resistance profile recorded in the study areas. Haplotype networks of pattern of genetic variability and polymorphism for the fragment of the VGSC sequences of sampled Anopheles mosquitoes revealed low haplotypes for the present study. Haplotypes are set of closely linked DNA variation on X-chromosome. Haplotypes were scaled accordingly to reflect their respective frequencies. Low haplotype number, four VGSC-1014F haplotypes were observed in this study. A positive association was previously established between low haplotype number of VGSC diversity and pyrethroid resistance through kdr mechanism. Significant values at ($P < 0.05$) of Tajima D and Fu and Li D' were observed for some of the results indicating possible signature of positive selection on the fragment of VGSC in the study. This is the first report of VGSC-1014F in the study site. Based on the results, the mutation was present in low frequencies. However, the roles played by the observed mutations need further investigation. Mutations, environmental factors among others can affect genetic diversity. The study area has recorded increase in insecticide resistance that can affect vector control in the area. This finding might affect the efforts made against malaria. Sequences were deposited in GenBank for Accession Number.

Keywords : anopheles mosquitoes, insecticide resistance, kdr, malaria, voltage gated sodium channel

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