ABSTRACT

Squamous cell carcinoma (SCC) is the most common neoplasm of the conjunctiva, and various studies have recently reported higher prevalence of HIV seropositivity among patients presenting with squamous cell carcinoma of the conjunctiva (SCCC). Elsewhere, some authors have shown an association of SCCC with high risk human papillomaviruses, in which the virus is thought to play a critical role in the oncogenesis through altered expression of key molecules regulating cell cycle and tyrosine kinase pathways. Thus, $p16^{INK4A}$ over-expression has been shown to reflect infection with high risk human papillomavirus genotypes in squamous cell carcinomas. Similarly, inverse expressions of EGFR and its phosphorylated form (p-EGFR) have been reported to contribute to pathogenesis of HIV/AIDS –associated SCCC. In fact, while elevated expression of $p16^{INK4A}$ protein has been used in some studies as a surrogate marker for high risk HPV infection, the expressions of the epidermal growth factor receptor (EGFR) and its activated form, phosphorylated epidermal growth factor receptor (p-EGFR), have been correlated with poor prognosis.

The aim of this study was to investigate the levels of expression of molecular biomarkers $p16^{INK4A}$, EGFR and p-EGFR and to correlate them with HPV genotypes in HIV/AIDS-associated SCCC.

Fifty-one HIV positive SCCC formalin fixed paraffin embedded (FFPE) tissue blocks were retrieved from the archives at the Department of Human Pathology, UON/KNH for analysis. Ethical approval to conduct the study was obtained from KNH-ERC. The criteria for inclusion as HIV positive case included information from the clinician in the patient's histological request form indicating on follow-up at the comprehensive care centre, on HAART treatment, ARV treatment, immunnosuppression, p24 marker reactive, retrovirus disease and HIV positive.

Sections were made from the tissue blocks and stained with haematoxylin and eosin to confirm the previous diagnoses and to grade the tumor. This was followed with analysis for the expressions of $p16^{INK4A}$, EGFR and p-EGFR using the technique of

immunohistochemistry. For the detection of high risk Human papillomavirus (HPV) and the genotypes, semi-nested PCR was performed, followed with DNA sequencing of the amplicons on ABI PRISM 310 Genetic Analyzer using A1/A2 primers, and the sequences compared with the GenBank database. Data was then analysed for significant statistical co-relations using SPSS (Version 16).

Out of fifty-one cases of SCCC, twenty-five (49%) had well differentiated (grade 1), eighteen (35.3%) moderately differentiated (grade 2) while eight (15.7%) had poorly differentiated (grade 3) tumours. Immunohistochemistry assay was carried out on all the fifty-one cases, of which thirty-two cases (62.7%) were positive for $p16^{INK4A}$ staining, forty-two cases (82.4%) for EGFR and forty-six cases (90.2%) showed positivity for p-EGFR. PCR results showed that HPV was present in 4 SCCC cases (10%); HPV 16 was the only HPV sub-type detected. Significant association was found between HPV detection and $p16^{INK4A}$ expression, $p=0.000$, at 99% confidence interval and EGFR expression, $p=0.028$, at 95% C.I.

This study points to a strategy in early screening for the presence of high risk HPV in patients with AIDS-associated SCCC for targeted therapeutic approach, by use of combined molecular biomarkers $p16^{INK4A}$ and EGFR.