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Epidemiological distribution of high-risk human papillomavirus genotypes and associated factors among patients with esophageal carcinoma at Bugando medical center in Mwanza, Tanzania

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Abstract

Background Esophageal carcinoma is a growing concern in regions that have a high incidence of human papillomavirus (HPV) infection such as East Africa. HPV, particularly the high-risk genotypes, is increasingly recognized as a risk factor for esophageal carcinoma. We set out to investigate the prevalence and associated factors of high-risk HPV in formalin-fixed paraffin-embedded (FFPE) tissue blocks with esophageal carcinoma at Bugando Medical Center, a tertiary referral hospital in Mwanza, Tanzania, East Africa.

Methods A total of 118 esophageal carcinoma FFPE tissue blocks, collected from January 2021 to December 2022, were analyzed. Genomic DNA was extracted from these tissues, and multiplex polymerase chain reaction (PCR) was performed to detect HPV using degenerate primers for the L1 region and type-specific primers for detecting HPV16, HPV18, and other high-risk HPV genotypes. Data were collected using questionnaires and factors associated with high-risk HPV genotypes were analyzed using STATA version 15 software.

Results Of the 118 patients' samples investigated, the mean age was 58.3 ± 13.4 years with a range of 29–88 years. The majority of the tissue blocks were from male patients 81/118 (68.7%), and most of them were from patients residing in Mwanza region 44/118 (37.3%). Esophageal Squamous Cell Carcinoma (ESCC) was the predominant histological type 107/118 (91.0%). Almost half of the tissue blocks 63/118 (53.3%) tested positive for high-risk HPV. Among these, HPV genotype 16 (HPV16) was the most common 41/63 (65.1%), followed by HPV genotype 18 (HPV18) 15/63 (23.8%), and the rest were other high-risk HPV genotypes detected by the degenerate primers 7/63 (11.1%). The

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factors associated with high-risk HPV genotypes were cigarette smoking (p-value < 0.001) and alcohol consumption (p-value < 0.001).

Conclusion A substantial number of esophageal carcinomas from Bugando Medical Center in Tanzania tested positive for HPV, with HPV genotype 16 being the most prevalent. This study also revealed a significant association between HPV status and cigarette smoking and alcohol consumption. These findings provide important insights into the role of high-risk HPV in esophageal carcinoma in this region.

Keywords Molecular patterns, High-risk HPV genotypes, Esophageal carcinoma

Introduction

Esophageal carcinoma is globally ranked eighth in cancer incidence and is one of the most serious tumors due to its rapid development and fatal prognosis [1, 2]. Worldwide, there are significant regional variations in the incidence of esophageal carcinoma. According to 2022 data from the International Agency for Research on Cancer (IARC) through their Global Cancer Observatory, Africa has the second highest incidence of esophageal cancer with an age-standardized rate of 3.3 per 100,000 people [3]. Additionally, some studies have reported that over 80% of mortalities due to esophageal squamous cell carcinoma (ESCC) worldwide occur in Africa, particularly in the region stretching from East to South Africa, which is referred to as the African Esophageal Squamous Cell Carcinoma (ESCC) corridor, where ESCC is the most common histological type [2, 4, 5]. ESCC is also the predominant histological type worldwide, followed by esophageal adenocarcinoma (EAC) [6]. Systematic review and meta-analysis on etiological studies of esophageal carcinoma in Africa reported risk factors for developing ESCC include tobacco consumption (smoking), heavy alcohol consumption, drinking hot tea, consuming red meat, poor oral hygiene, low intake of fresh fruit and vegetables and low socioeconomic status [7]. Esophageal mucosa dysplasia from acid reflux and Barrett's esophagus is a risk factor for developing EAC [7]. Also, a study done by the Food and Drug Administration (FDA) in the United States shows the prolonged use of bisphosphonates is related to Adenocarcinoma (EAC) and Esophageal Squamous Cell Carcinoma (ESCC) [8].

Few studies have assessed the potential effect of polymorphisms of susceptibility genes andthe association between selenium intake and esophageal squamous cell carcinoma (ESCC) [9]. Selenium has been found to possess anticarcinogenic and chemo-preventive properties. Selenium-containing enzymes, such as glutathione peroxidase, play an important role in polycyclic aromatic hydrocarbon metabolism and detoxification [10]. Genetic susceptibility plays a role in carcinogenesis, with the p53 gene being critical in DNA transcription, cell cycle regulation, tumor suppression, DNA damage repair, and apoptosis [11]. Mutations and polymorphism of the p53 gene at codon 72 are considered as a risk factor for the human papillomavirus-associated cervical neoplasia and ESCC [12]. Hot tea consumption at temperatures greater than 60°C and volumes more than 700 ml/day has been linked to more than 90% increased risk of ESCC [13]. Exposure to polycyclic aromatic hydrocarbons (PAHs) is another potential risk factor for esophageal squamous cell carcinoma (ESCC) [14]. Additionally, the risk of developing EAC is increased in conditions like Barrett's esophagus and gastroesophageal reflux, as well as the use of lower esophageal sphincter-relaxing drugs [15–17]. According to a case-control study done in Zambia, HIV infection and exposure to domestic and cigarette smoke are risk factors for ESCC [18]. Tanzania is among the nations in the African ESCC corridor, a region characterized by a relatively high incidence of esophageal carcinoma, early age of onset, delayed presentation, as well as poor outcomes and survival [5, 19]. However, little is understood about the cause of such a unique pattern.

Exposure to high-risk Human papillomavirus (HPV) may cause both cutaneous and mucosal cancers including skin carcinomas related to HPV subtypes 5 and 8 [20]. The mucosal subtype of high-risk Human papillomavirus (HPV) infection, especially genotypes 16 and 18, is reported as a risk factor for various cancers such as cervical cancer, oropharyngeal squamous cell carcinoma, penile carcinoma [20] and esophageal cancer in high-risk areas [21–23]. The estimated prevalence of HPV infection in East Africa is among the highest globally (33.6%) including Tanzania. However, a clear picture of HPV prevalence is not well established in Tanzania's general population [24]. Screening and detection programs on HPV infection and vaccination in Tanzania mainly focus on cervical cancers in young school girls and HIV-positive populations who have a high risk of developing cervical carcinoma [25]. Routine detection of HPV DNA on mucosal-related lesions of HPV is not established. However, several molecular detections of HPV DNA identification were done in screening programs of cervical carcinoma due to the high prevalence of diseases in Tanzania [25]. Likewise in esophageal carcinoma, there is an increasing probability of detecting HPV DNA in ESCC tissue from male patients in a younger age group below 55 years [26, 27]. The role of HPV as a risk factor for esophageal carcinoma in Tanzania is not well established,

leading to existing preventive efforts against HPV focusing on females to prevent cervical cancer only. Therefore, we assessed the magnitude of high-risk HPV genotypes among FFPE tissue blocks from patients with esophageal cancer to provide evidence-based justification for the burden of HPV in cases confirmed with esophageal cancer in our setting. This will offer baseline information essential for further causal relationship studies between esophageal cancer and HPV in Tanzania, that will contributing to HPV preventive measures for both males and females.

Materials and methods

Study population, study area, and inclusion criteria

A laboratory-based study involving 118 esophageal carcinoma FFPE blocks was conducted between May and July 2023 investigating the samples collected for a period of two years from January 2021 – December 2022. The blocks diagnosed with esophageal carcinoma were retrieved from the histopathology laboratory of Bugando Medical Center (BMC). The demographics, clinical, and all associated risk factors information were retrieved from the medical records. Laboratory procedures were conducted at the Histopathology laboratory of BMC and the Molecular biology laboratory of the Catholic University of Health and Allied Science (CUHAS). Only blocks double-verified by pathologists and with complete demographic and clinical information were included. Ethical clearance was granted by the joint CUHAS/BMC Research and Ethics Review Committee (CREC) with reference number: 2592/2023.

Laboratory procedures

Histopathological procedures

The retrieved tissue blocks were cut into thin sections of 2–3μ by using a Rotary Microtome. The tissues were then stained by routine Hematoxylin and Eosin staining methods. Deparaffinization was carried out by hot air oven at 60ºC then the slides were placed in two containers of xylene for 10 min in each, then, followed by dipping the tissue slides into the series of absolute ethanol (99.8%) and 95% ethanol for 10 min in each container. After this, the section was hydrated in clean tap water for 5 min, immersed in Hematoxylin for 10 min, and then blued in clean running tap water for 5 min. The section was immersed in eosin stain solution for 5 min followed by dehydration ofthe section in two different concentrations of ethanol at concentrations of 95% and 99.8% for 10 min each. After this, the tissue slides were immersed 10 times into two containers of xylene to clear the ethanol [28]. Lastly, the slides were mounted by Dibutyl-phthalate Polystyrene Xylene (DPX) for histological evaluation by a pathologist using an Olympus CX21 light Microscope to confirm the diagnosis of esophageal carcinoma.

DNA extraction

DNA was extracted from FFPE tissue blocks as previously reported [16]. Deparaffinization was carried out by adding 1 ml of xylene to the microtubes containing tissue sections followed by vortex mix for 15 s then spinning at 14,000 rpm for three washes, followed by two washes in absolute ethanol to remove the xylene vortex and spin for 10 min at 14,000 rpm air dry the pallet for 5 min. Tissues were dried at 55ºC and digested overnight at 55ºC in 100 µl of TEN buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0, 20 mM NaCl) containing 20 mg/ml proteinase K). Proteinase K was inactivated at 95ºC for 10 min. Finally, undigested tissue remnants were pelleted by centrifugation at 14,000 rpm for 10 min and the supernatant containing extracted DNA was transferred to a new microcentrifuge tube. Assessment of DNA quality was performed using 2% agarose gel electrophoresis [29].

PCR amplification

In the detection of high-risk HPV genotypes, a known positive control for all high-risk genotypes for the L1 region, HPV 16 and 18 was used. Also, the distilled water sample was used as a negative control. The targeted region was the L1 conserved region of the HPV genome for the broad spectrum and the E6/E7 region of the HPV genome for HPV 16 and 18 genotypes respectively using consensus primer and type-specific primer for 16 and 18 as previously reported [30].

For L1 region MY09 forward primer 5'- CGTCC(AC)A(AG)(AG)GGA(T)ACTGATC-3' and MY11 primer 5'-GC(AC)CAGGG(AT)CATAA(CT)AATGG-3' yielded amplicon size of 450 bp; detecting HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, and 81) for HPV 16 E6/7 forward primer 5'-TTGCTTTTCGGGATTTATGC-3' and reverse primer 5'-AGATCAGTTGTCTCTGGTTGCA-3' with amplicon size of 390 bp for HPV 18 forward primer 5'-AAGGATGCTGCACCGGCTGA-3' and reverse primer 5'-CACGCACACGCTTGGCAGGT-3' with amplicon size of 216 bp. Multiplex PCR was performed as previously reported [30]. Where 10 µl of extracted DNA was added to Eppendorf tubes containing a readily reconstituted master mix to make a final volume of 50 µl reaction mixture under the following PCR condition: incubation at 94 °C for 7 min followed by 40 cycles of 1-minute denaturation at 94 °C, 2-minutes annealing at 55 °C, and 2-minutes elongation at 72 °C. The last cycle was followed by a final extension of 10 min at 72 °C. Finally, amplicon was detected for HPV by 2% gel electrophoresis in TAE buffer.

Gel electrophoresis

The PCR product was visualized under UV illumination on gel electrophoresis by using 2% ultra-pure agarose gel

(ThermoFisher Scientific, UK). Staining of the DNA fragments was carried out using red-safe dye. The gels were run at 80 V for approximately 45 min. A standard DNA molecular weight marker was used as a ladder.

Data analysis

All information obtained was recorded on the computer using Microsoft Excel. The Data were imported to STATA software version 15 for analysis. The frequency distribution tables and graph bar plots were obtained to determine the prevalence and distribution of genotypes of high-risk HPV. To determine the factors associated with the acquisition of high-risk HPV genotypes, we used Pearson's Chi^2 test or Fisher's Exact test where appropriate. We used the Student's t-test to compare the significance of the difference in mean age in years among patients with high-risk HPV and their counterparts. We also used Pearson's Chi^2 test to determine the significance of the difference in distribution between high-risk HPV infection and histology types. In all analyses, the significance level was set at 0.05.

Results

Socio-demographic data

The mean age was 58.3±13.4 years and 74/118 (62.7%) of the samples were from patients aged above 55 years. The ages ranged from 29 to 88 years. The majority, 81/118 (68.7%) of the FFPE blocks originated from males and 44/118 (37.3%) were from patients residing in Mwanza region (Table 1). Esophageal squamous cell carcinoma was the predominant 107/118 (90.7%) histological type (Fig. 1) and EACC was 11/118 (9.3%) (Fig. 2).

The prevalence of HPV among FFPE tissue blocks with esophageal carcinoma

Out of 118 esophageal cancer FFPE blocks 63/118 (53.4%) tested positive for high-risk -HPV genotypes whereas 55/118 (46.6%) tested negative.

High-risk HPV genotypes among HPV-positive FFPE tissue blocks with esophageal carcinoma

To identify the high-risk genotypes infecting the HPV-positive Esophageal carcinoma FFPE tissue blocks, type-specific primers targeting the E6/7 region of HPV 16 and 18 were used. These primers are specific and produce amplicon of 390 bp and 216 bp respectively. Of the 63 HPV-positive FFPE tissue blocks, 41/63 (65.1%) had HPV genotype 16, 15/63 (23.8%) had HPV genotype 18, the remaining, 7/63 (11.1%) had other HPV genotypes detected by the MY09/MY11 primer pair alone and produced an amplicon of 450 bp (Fig. 3). Most of the HPV-positive FFPE tissue blocks 55/63 (87.3%) had ESCC histological type and 8/63 (12.7%) had EAC histological type. Tissue blocks from patients diagnosed with EAC were more likely to have high-risk HPV genotypes than those from tissue blocks of patients diagnosed with

Table 1 Social demographic characteristics of patients with the FFPE tissue blocks

Patient characteristic	Frequency (*n*)	Percent (%)
Age in years		
≤ 55 years	44	37.3
> 55 years	74	62.7
Gender		
Male	81	68.6
Female	37	31.4
Residence		
Mwanza	44	37.3
Kagera	24	20.3
Kigoma	14	11.9
Geita	13	11.0
Simiyu	9	7.6
Tabora	8	6.8
Mara	6	5.1
HIV status		
Negative	108	91.5
Positive	10	8.5
Cigarette smoking		
No	43	36.4
Yes	75	63.6
Alcohol consumption		
No	45	38.1
Yes	73	61.9

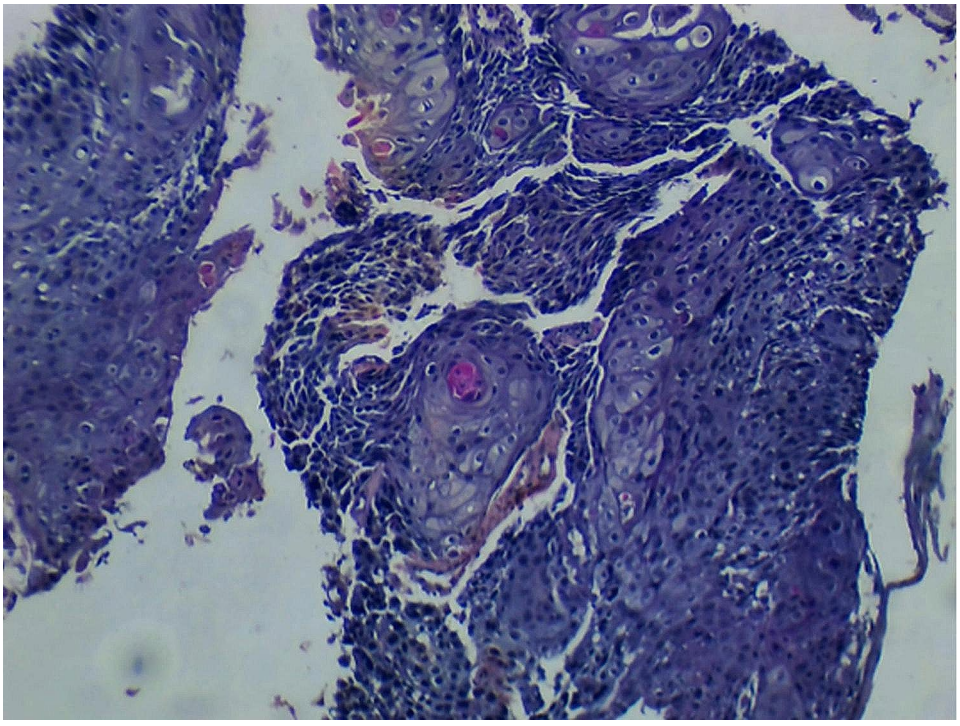

Fig. 1 Microscopic visualization of ESCC under x40 High Power Field (HPF) showing fragments lined with keratinizing squamous epithelial with trabecular nests of polygonal shaped cells, hyperchromatic with vesicular nuclei and keratin peals formation. (Hematoxylin & Eosin stain)

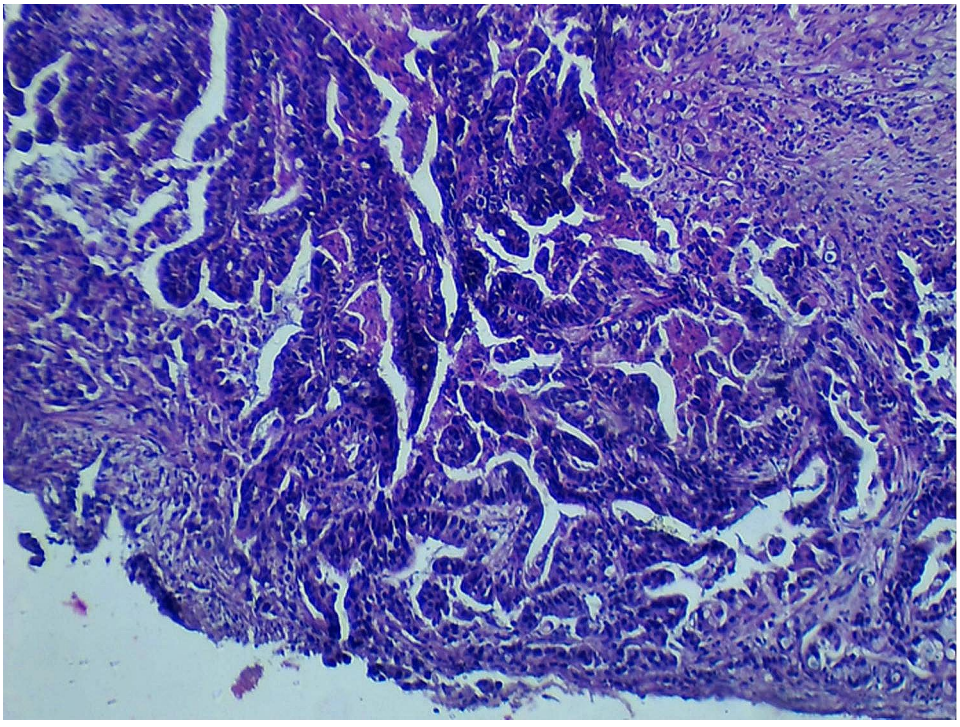

Fig. 2 Microscopic visualization of EAC under x40 High power field (HPF) showed neoplastic glands fused back-to-back and devoid basement membrane lined with epithelial malignant cells. (Hematoxylin & Eosin stain)

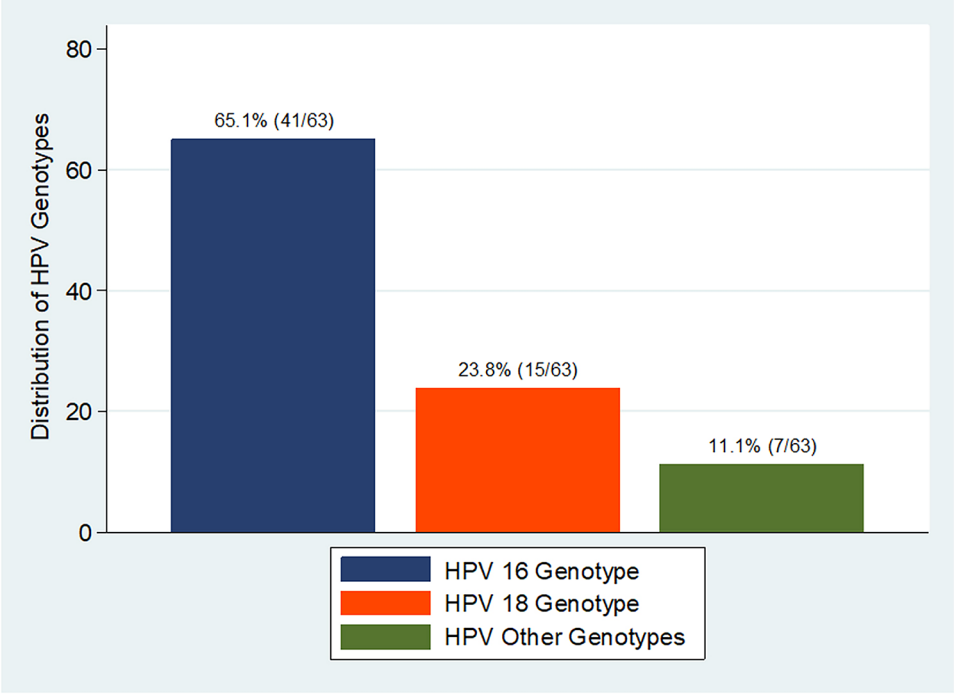

Fig. 3 Distribution of HPV genotypes among High-Risk HPV positive FFPE tissue blocks. HPV-16 genotype was the predominant 41/63 (65.1%) (histological type of HPV detected. It was followed by HPV-18 and the other HPV genotypes detected by the MY09/MY11 primer; 15/63 (23.8%) and 7/63 (11.1%) respectively

ESCC 8/11 (72.7%) versus 55/107 (51.4%). However, this was not statistically significant (p-value = 0.177, Pearson's Chi^2 test) (Fig. 4).

Factors associated with HPV infection among patients with esophageal carcinoma

In our analysis, cigarette smoking (p-value < 0.001, *Pearson's* Chi^2 *test*) and alcohol consumption (p-value < 0.001, *Pearson's* Chi^2*test*) were significantly associated with high-risk HPV acquisition (Table 2).

Discussion

Human papillomaviruses (HPV) are the first viruses to have been acknowledged to prompt carcinogenesis and are linked with cancers of the uterine cervix, anogenital tumors, and head and neck malignancies [31].The main mechanism of HPV-related malignancies is the presence of oncogenic protein products of the HPV virus E6 and E7; they act by modifying the control of the cell cycle and by regulating apoptosis [31]. The incorporation of viral DNA disrupts the activity of the E2 protein which is known to repress the transcription of E6 and E7, and thus its interruption causes dysregulated expression of these oncoproteins [31]. Other studies suggest that HPV oncogenesis is due to a direct association between HPV integration and host genomic instability [32, 33], where HPV integration drives chromosomal rearrangements which include deletions, translocations, and inversion in the genomic regions flanking HPV integrant [33]. Consequently, the integration of HPV leads to insertional mutagenesis which results in a specific change in gene expression at the site of integration [32].

Non-persistent high-risk HPV infection in some organs may result in non-tumorous lesions such as chronic otitis media (COM), chronic suppurative otitis media (CSOM), and chronic otitis media with cholesteatoma [34]. Persistent HPV infection and oncogenic exposure may result in the over-production of inflammatory mediators, overexpression of viral oncoprotein, and the development of squamous cell carcinoma in different organs like the middle ears, oropharyngeal, and sometimes ESCC 35).

Our study aimed to assess the magnitude of high-risk HPV genotypes among esophageal cancer lesions. Our findings indicate a higher prevalence (53.4%) of HPV-positive Esophageal Carcinoma than what has been reported previously from studies conducted in the African ESCC corridor [35]. Our study also highlights a high propensity for HPV infection among males. A previous study done in the Lake Victoria Zone in Tanzania on the clinicopathological patterns of esophageal carcinoma showed that males had a two times higher prevalence of esophageal carcinoma compared to females [6]. This explains the higher number of males with esophageal carcinoma and infected by HPV. The majority (68.6%) of esophageal FFPE tissue blocks archived at BMC belonged to male participants. These findings concur

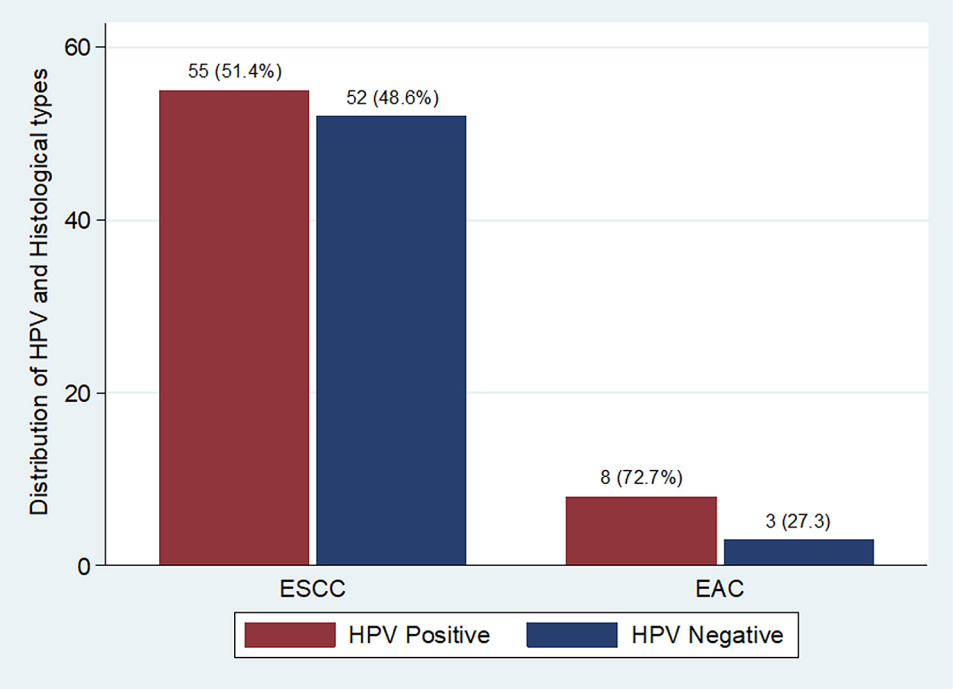

Fig. 4 HPV Status and Histological Types of Esophageal Carcinoma. There was no statistical difference in distribution between the acquisition of high-risk HPV genotypes and histological type (p-value = 0.177, *Pearson's Chi² test*)

Table 2 Factors associated with high-risk HPV among esophageal FFPE tissue blocks

Patients characteristics	HPV		Pearson's Chi² (Degree of freedom)	*p*-value
	Positive	Negative		
	n (%)	*n* (%)		
Mean Age in years	58.3 ± 11.4	58.2 ± 15.2	-#	0.968
Age group in years				
≤50 years	18 (48.7)	19 (51.4)	0.4869 (1)	0.485
>50 years	45 (55.6)	36 (44.4)		
Gender				
Male	40 (49.4)	41 (50.6)	1.6668 (1)	0.197
Female	23 (62.2)	14 (37.8)		
Cigarette Smoking				
No	5 (11.6)	38 (88.6)	47.4145 (1)	< 0.001
Yes	58 (77.8)	17 (22.7)		
Alcohol consumption				
No	14 (31.1)	31 (68.9)	14.5082 (1)	< 0.001
Yes	49 (67.1)	24 (32.9)		
PUD or GERD				
No	57 (57.8)	53 (48.2)	-*	0.185
Yes	6 (75.0)	2 (25.0)		
HIV				
Negative	55 (50.9)	53 (49.1)	-*	0.074
Positive	8 (80.0)	2 (20.0)		

-# The p-value was calculate using student's t-test

-* The p-value was calculated using Fisher's Exact test

Abbreviations: PUD: Peptic Ulcer Disease; GERD: Gastroesophageal reflux disease

HIV: Human immunodeficiency virus

with worldwide data in which, esophageal carcinoma more commonly affects males compared to females [6, 27, 36, 37]. Males and females have different exposures to lifestyle risks, such as cigarette smoking and alcohol intake, which together predispose males to a higher risk of developing esophageal cancer. Consequently, a large number (91%) of the FFPE tissue blocks studied are of the squamous cell carcinoma histological type, known to be predisposed by such risks. ESCC has also been reported as a common histological type of esophageal cancer in developing countries, especially in the African ESCC corridor [5, 6, 17, 19, 21].

Likewise, more than half of the FFPE tissue blocks were positive for HPV, the majority of which were of the ESCC histological type (87.3%). HPV DNA integration in the human genome has been implicated in leading to carcinogenesis in various body parts, including the esophagus. The expression of E6 and E7 viral proteins is a key step in this carcinogenesis [24]. E7 inhibits the host retinoblastoma tumor suppressor protein (pRb), leading to its proteasome-dependent degradation, and E6 targets p53 degradation and upregulates telomerase expression. The overall effect results in uncontrolled cell proliferation by evading the cellular checkpoints and cell immortalization [38, 39]. The prevalence of HPV-positive Esophageal Carcinoma in this study is higher compared to the prevalence from previous studies conducted in other regions considered high-risk for esophageal cancer, such as South Africa (9%), Iran (23.13%), and China (38.7%) [27, 36, 40]. This high prevalence may be attributed to the fact that East African countries, including Tanzania, have among the highest prevalence of HPV globally (33.6%) [24]. It may also be due to the different methodologies used in the detection of HPV infection, with the enzyme-linked immunosorbent assay technique used by Qi et al. [27] versus the nucleic acid detection technique used in this study. The two methods have shown statistically significant differences in sensitivity, with nucleic acid detection techniques being more sensitive [24, 41].

In this study, HPV 16 was the most common genotype of HPV detected in two-thirds (65.1%), followed by HPV 18 (23.8%), and the remaining 11.1% had other genotypes detected by the MY09/MY11 degenerate primer pair. This agrees with similar studies conducted in different geographical regions [27, 40].

Finally, high-risk HPV status among esophageal carcinoma patients' FFPE tissue blocks was associated with cigarette smoking and alcohol consumption as risk factors for esophageal carcinoma [18, 26], The integration of HPV DNA into the host genome is a cornerstone in the carcinogenesis process [24, 42, 43]. Tobacco induces DNA damage, for example, DNA strand breaks [44], a process that creates fragile sites for HPV integration. Alcohol has pro-oxidative effects, leading to oxidative DNA damage and, secondarily, through reduced folic acid levels, induces the expression of fragile sites for HPV integration [45]. Therefore, the risk of developing esophageal carcinoma is significantly higher in people infected with HPV who have a history of cigarette and alcohol consumption. These findings partly concur with those from a study in Zambia on high-risk HPV status among esophageal carcinoma patients' FFPE tissue blocks where esophageal carcinoma was associated with cigarette and domestic smoke, alcohol consumption, and HIV infection rather than HPV infection [18].

This study is limited by the fact that the data were collected from medical records, and some important identifiable risk factors for high-risk HPV among patients with esophageal carcinoma were missing. The information regarding alcohol and tobacco use as exposure risk factors were obtained from electronic medical records of FFPE tissue blocks of patients, therefore these exposures were not collected in the standardized way and may limit the accuracy. Additionally, a multivariate model was not performed in this analysis because we could not manage to obtain a full adjustment set due to the limited data abstracted from the patient medical records. The specific primers used in this study were not able to subtype all high-risk specific genotypes. Also, viral DNA genome sequencing and expression of oncoproteins or viral mRNA was not profiled. Immunohistochemical markers p16 and in-situ hybridization technique for identification of high-risk HPV was not done on tissue biopsy with a diagnosis of esophageal carcinoma during this study. However, the strength of this study hinges on being able to report the prevalence and genotype distribution of high-risk HPV among patients with esophageal carcinoma, the key information for vaccination and preventive public health.

In conclusion, a substantial number of esophageal carcinomas from Bugando Medical Center in Tanzania tested positive for HPV, with HPV genotype 16 being the most prevalent. This study also revealed a significant association between HPV status and cigarette smoking and alcohol consumption. These findings provide important insights into the role of high-risk HPV in esophageal carcinoma in this region. We recommend further studies to assess the activity of high-risk HPV in esophageal carcinomas, such as mRNA detection, p-16 Immunohistochemical detection, and in-situ hybridization on high-risk HPV to help establish if the virus indeed plays an etiological role in this fatal malignancy. Furthermore, studies with a large sample size investigating more identifiable variables to be conducted to determine the associated risk factors of esophageal carcinoma.

Abbreviations

BMC Bugando Medical Centre
CI Confidence interval

CUHAS	Catholic University of Health and Allied Sciences
DNA	Deoxyribonucleic acid
EAC	Esophageal adenocarcinoma
EDTA	Ethylenediaminetetraacetic acid
ESCC	Esophageal squamous cell carcinoma)
FFPE	Formalin-fixed paraffin-embedded
GERD	Gastroesophageal reflux disease
HIV	Human immunodeficiency virus
HPF	High power field
HPV	Human papilloma virus
OR	Odds ratio
PCR	Polymerase chain reaction
PUD	Peptic ulcer disease

Author contributions
OMO, PFR and BRK conceived, designed and coordinated the study. LMN, OMO, HDC and CAM coordinated and executed the study; OMO, CAM and BRK analyzed and interpreted the data; OMO, HDC and CAM wrote the initial draft of the manuscript, which was critically revised by all authors. All the authors reviewed and approved the final manuscript.

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Data availability
The datasets generated and analyzed in this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The study was approved by the joint CUHAS/BMC Research and Ethics Review Committee (CREC) with reference number: 2592/2023. The informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

Consent for publication
Not Applicable.

Competing interests
The authors declare no competing interests.

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