Risk Factors for Esophageal Cancer in a High-incidence Area of Malawi

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Abstract

Purpose To explore associations of nutritional, infectious, and lifestyle factors with esophageal cancer (EC) occurrence in a high-risk area of Malawi.

Methods This case–control study was performed with 227 patients. Data on clinicopathological characteristics and risk factors were collected using a questionnaire developed for this study specifically. Ninety-eight blood samples were collected and the prevalence of antibodies against human immunodeficiency virus (HIV), herpes simplex virus (HSV), cytomegalovirus (CMV), Epstein–Barr virus (EBV), varicella-zoster virus (VZV), and *Helicobacter pylori* were determined serologically. Fisher's exact test was used for nominal variables and the Mann–Whitney *U* test was used for continuous variables. Binary linear regression was performed with variables that were significant in the Fisher and Mann–Whitney tests.

Results The tumor and control groups comprised 157 and 70 patients, respectively. Patients with tumors were significantly older than controls ($P < 0.001$). EC was associated with smoking ($P < 0.001$) and alcohol consumption ($P = 0.020$), but 43% of patients with tumors did not smoke or drink. EC was associated with the consumption of hot food and tea ($P = 0.003$) and smoked fish ($P = 0.011$). EC was not associated with any serologically investigated infectious agent. In logistic regression analysis, age [odds ratio (OR), 1.042; 95% confidence interval (CI), 1.019–1.066; $P < 0.001$] and hot food and tea consumption (OR, 2.331; 95% CI, 1.167–4.656; $P = 0.016$) were significant.

Conclusions Apart from alcohol consumption and smoking, the consumption of hot food or tea and smoked fish are associated with EC in Malawi.

Introduction

Esophageal cancer (EC) is the eighth most common cancer diagnosis, with an incidence of about 604,000 cases worldwide. It shows great geographical variation, with a high frequency of esophageal squamous cell carcinoma (ESCC) occurrence in areas deemed to be at high risk, including the "EC belt" in south-central Asia and in parts of southern and eastern Africa [1]. Malawi has one of the highest incidence rates of EC worldwide, with 24.2 cases per 100,000 inhabitants, in contrast to 6.6 and 3.2 cases per 100,000 inhabitants in the United States and United Kingdom, respectively [2].

Causative factors for this high incidence have not been identified conclusively. Tobacco use and alcohol consumption, the main risk factors in Western countries [3], have been associated with EC in African countries [4]. Thus, we investigated the association of alcohol and tobacco use with EC in Malawi, although the consumption in this country is similar to or lesser than that in other African countries with much lower EC incidence rates [5, 6]. We also investigated nutritional risk factors, which are common in the population of Malawi, where hot food and tea are commonly taken straight from the fire. In addition, in contrast to other African countries with lower incidence rates of EC, maize consumption is high in Malawi [7].

Other diseases with high incidence rates in Africa may be risk factors for EC in Malawi. Chronic infections are known to cause more than 15% of malignant neoplasms worldwide [8–11]. Seventy percent of patients with human immunodeficiency virus (HIV) infection live in sub-Saharan Africa. HIV is known to be associated with the occurrence of lymphomas and cervical and anal carcinomas [12]. High prevalence rates in Africa have also been reported for cytomegalovirus (CMV), herpes simplex virus (HSV), and *Helicobacter pylori* infection [13–15]. Human papillomavirus (HPV) has been found in only a subset of patients (15% of those with ESCC) in Malawi [16].

Thus, data on risk factors for EC in sub-Saharan Africa and Malawi are limited. The aim of this case–control study was to investigate the associations of EC occurrence in Malawi with nutritional, infectious, and lifestyle factors. As no curative treatment for EC is currently available in Malawi and most low- and middle-income countries worldwide, information about risk factors is needed to develop prevention strategies.

Materials And Methods

This study was performed with data from patients who underwent upper-gastrointestinal endoscopy at Zomba Central Hospital and Queen Elizabeth Hospital, Blantyre, Malawi, in 2010 and 2014–2016. All patients provided written consent (by signature or fingerprint) prior to endoscopy. The study was approved by the Research and Ethics Committee of the College of Medicine Blantyre (no. P.04/10/930). Data on patients' clinicopathological characteristics and risk factors were collected by a questionnaire developed by our group for this study specifically. Questionnaire categories were general characteristics (e.g., age, sex, weight, height), symptoms (e.g., dysphagia), history of infectious diseases (e.g., tuberculosis), nutritive factors (e.g., consumption of fish, smoked fish, hot food and tea, maize porridge), and smoking and alcohol use. Daily maize consumption was measured as the consumption of *nsima* maize porridge (in 250-g increments), and the consumption of vegetables and fish was measured as the number of times consumed per week. Patients with tumors were asked to indicate the amounts of maize porridge, vegetables, and fish consumed before their symptoms had started.

Endoscopy was performed on all patients with dysphagia, and on patients with other symptoms. EC was diagnosed macroscopically during endoscopy and confirmed histologically in a subset of 40 patients. Patients with EC were assigned to the tumor group, and those without tumor were assigned to the control group.

Serological analysis

For serological analysis, blood samples were collected from 98 patients before endoscopy. They were analyzed at the Department of Medical Microbiology, University Hospital of Jena, Germany.

Serological status for HIV, HSV, CMV, Epstein–Barr virus (EBV), and varicella-zoster virus (VZV) was determined using commercially available enzyme-linked immunosorbent assays [ELISAs; Enzygnost® HIV Integral II, anti-HSV/immunoglobulin (Ig)M and anti-HSV/IgG, anti-CMV/IgM and anti-CMV/IgG, anti-

EBV/IgM II and anti-EBV/IgG, anti-VZV/IgM and anti-VZV/IgG; Siemens, Erlangen, Germany] in a BEPIII system (Siemens) according to the manufacturer´s protocol. Anti–*H. pylori* IgG ELISAs were also performed. In brief, sera were diluted 1:201 with dilution buffer. Then, 100 µl diluted serum and 100 µl undiluted calibrator were placed in a plate and incubated for 30 minutes at room temperature on a microplate shaker. The microtiter plate was washed three times with wash buffer, 100 µl IgG conjugate was added, and the plate was covered and incubated at room temperature for 30 minutes on a microplate shaker, followed by three washes. Then, 100 µl Tetramethylbenzidine was added to each well of the microtiter plate and the plate was incubated 10 minutes at room temperature on a microplate shaker. Finally, 100 µl stop solution was added and extinction was measured by photometer.

Statistical analysis

To assess relationships between the epidemiological and clinical data, Fisher's exact test was used for nominal variables and the Mann–Whitney *U* test was used for continuous variables. Medians and interquartile ranges (IQRs) were calculated to describe the variance of the metric variables. Binary linear regression and calculation of odds ratios were performed with variables that were significant in the Fisher and Mann–Whitney tests. P values < 0.05 was considered to be significant. The statistical analysis was performed with IBM SPSS Statistics (ver. 22; IBM Corporation, Armonk, NY, USA).

Results

In total, 227 patients (157 in the tumor group, 70 in the control group) were included in this study. ESCC was diagnosed histologically in biopsy samples from all patients in the tumor group. Some patients of both groups could not provide responses for all items. The study results are summarized in Table 1.

Table 1
Clinico-pathological characteristics and risk factors: Comparison of tumor patients (n=157) and controls (n=70). Data are presented as absolute numbers with percentages in brackets or medians with interquartile ranges in brackets. EC - esophageal cancer.

	EC patients	Control patients	*P* value
General			
Age (years)	55.50 (22.00)	43.50 (34.00)	<0.001
Male	93 (59.24%)	38 (54.29%)	0.469
BMI (kg/m^2)	16.27 (3.73)	20.78 (4.04)	<0.001
Smoking and alcohol			
Smoker (former and current)	75 (50.68%)	16 (24.62%)	<0.001
Self-made cigarettes	40 (28.78%)	8 (12.31%)	0.022
Alcohol (former and current)	60 (41.38%)	16 (24.62%)	0.020
Locally produced alcohol	33 (23.74%)	7 (10.77%)	0.037
Beer	41 (30.83%)	10 (15.38%)	0.024
Spirits	29 (21.80%)	9 (13.85%)	0.249
Nutritive factors			
Hot food or hot tea	101 (72.66%)	33 (50.77%)	0.003
Spicy food	51 (38.64%)	20 (30.77%)	0.344
Fish (times per week)	2.00 (2.00)	3.00 (2.00)	0.104
Smoked fish	119 (86.23%)	44 (69.84%)	0.011
Vegetables (times per week)	7.00 (4.00)	7.00 (3.50)	0.536
Maize porridge (per 250 g)	4.00 (2.00)	4.00 (1.25)	0.077
Infectious agents			
History of			
Oral thrush	23 (16.43%)	7 (10.77%)	0.396
Tuberculosis	5 (13.16%)	1 (1.89%)	0.078
Herpes zoster	8 (5.76%)	0 (0.00%)	0.057
Serology			
HIV positive	5 (12.20%)	13 (22.81%)	0.200

H. pylori positive	36 (87.80%)	49 (85.96%)	1.000
HSV IgG positive	41 (100.00%)	57 (100.00%)	*
HSV IgM positive	2 (4.88%)	5 (8.77%)	0.696
CMV IgG positive	41 (100.00%)	57 (100.00%)	*
CMV IgM positive	2 (4.88%)	3 (5.26%)	1.000
EBV IgG positive	41 (100.00%)	57 (100.00%)	*
EBV IgA positive	7 (17.07%)	9 (15.79%)	1.000
VZV IgG positive	41 (100.00%)	54 (94.74%)	0.262
VZV IgA positive	17 (41.46%)	15 (26.32%)	0.131
* Exact fisher test cannot be performed as the variable is a constant.			

General characteristics

Ninety-three (59.2%) patients in the tumor group were male; sex was not associated with tumor presence ($P = 0.469$). Patients in the tumor group were significantly older than controls [median (IQR), 55.5 (20.0) vs. 43.5 (34.0) years; $P < 0.001$]. The ages of patients in the tumor group ranged from 22 to 90 years; 28 (18.7%) of these patients were aged ≤ 40 years. The body mass index was significantly lower in the tumor group than in the control group [16.3 (3.7) vs. 20.8 (4.0) kg/m^2, $P < 0.001$]. In 31.2% of the patients with tumors, symptoms had begun more than 6 months before attendance and diagnosis at the endoscopy unit. Fifteen (13.9%) patients with tumors were unable to swallow saliva, 22 (20.4%) were able to drink liquids, 45 (41.7%) were able to eat soft foods with difficulty, 23 (21.3%) were able to eat soft foods with no difficulty, and 3 (2.8%) reported normal swallowing.

Smoking and alcohol consumption

Compared with no exposure, smoking exposure (former and current smoking) was associated significantly with the presence of EC [50.7% (tumor group) vs. 24.6% (control group), $P < 0.001$]. Significantly more patients with tumors than controls smoked self-made cigarettes (28.8% vs. 12.3%, $P = 0.022$). Among smokers, patients in the tumor group had smoked significantly longer than those in the control group [20.0 (30.0) vs. 10.0 (26.2) years, $P = 0.035$]. The numbers of cigarettes smoked [5.0 (4.0) and 5.0 (7.8), $P = 0.887$] and pack-years [5.0 (6.8) and 2.5 (9.2), $P = 0.227$] did not differ significantly between patients with tumors and controls.

Compared with the control, the presence of EC was associated significantly with the consumption of alcohol (24.6% vs. 41.4%, $P = 0.020$), locally produced alcohol (10.8% vs. 23.7%, $P = 0.037$), and beer (15.4% vs. 30.8%, $P = 0.024$), but not spirits (13.8% and 21.8%, $P = 0.249$). Among alcohol consumers, the duration of consumption did not differ significantly between the tumor and control groups [15.0 (25.0)

and 5.5 (11.0) years, $P = 0.054$). Sixty-four (43.5%) patients with tumors consumed neither tobacco nor alcohol.

Nutritive factors

Compared with the control, the presence of EC was associated significantly with the intake of hot food or tea (50.8% vs. 72.7%, $P = 0.003$) and smoked fish (69.8% vs. 86.2%, $P = 0.011$), but not with the intake of spicy foods (30.8% and 38.6%, $P = 0.344$) or weekly fish [3.0 (2.0) and 2.0 (2.0) times, $P = 0.104$] or vegetable [7.0 (3.5) and 7.0 (4.0) times, $P = 0.536$] intake. Patients with tumors did not consume significantly more maize porridge than did controls [4.0 (2.0) and 4.0 (1.2) 250-g portions, $P = 0.077$].

Infectious diseases

Compared with the control, the presence of EC was not associated with a history of oral thrush (10.8% and 16.4%, $P = 0.396$), tuberculosis (1.9% and 13.2%, $P = 0.078$), or herpes zoster (0.0% and 5.8%, $P = 0.057$). Serological analysis performed in a subset of patients with tumors and controls revealed no association of EC presence with HIV (12.2% and 22.8%, $P = 0.200$), *H. pylori* (87.8% and 86.0%, $P = 1.000$), HSV IgM (4.9% and 8.8%, $P = 0.696$), CMV IgM (4.9% and 5.3%, $P = 1.000$), EBV IgA (17.1% and 15.8%, $P = 1.000$), VZV IgG (100.0% and 94.7%, $P = 0.262$), or VZV IgA (41.5% and 26.3%, $P = 0.131$) positivity. All individuals included in this analysis showed HSV IgG, CMV IgG, and EBV IgG positivity.

Binary logistic regression results

The binary logistic regression model included age, smoking, and the consumption of alcohol, hot tea and food, and smoked fish. Only age [odds ratio (OR), 1.042; 95% confidence interval (CI), 1.019–1.066; $P < 0.001$] and the consumption of hot food or tea (OR, 2.331; 95% CI, 1.167–4.656; $P = 0.016$) were significant in this analysis.

Discussion

Malawi has been designated as a country whose population is at high risk of EC development [1, 2]. The reason for this elevated risk has not been determined, and data on risk factors in sub-Saharan Africa are limited. As EC treatments will not be available soon in low- and middle-income countries, the exploration of risk factors is needed to identify subpopulations at high risk of EC development, and to develop prevention strategies to reduce morbidity and mortality from this disease in the long term. In this study, we investigated associations of EC occurrence in Malawi with nutritional, lifestyle, and infectious factors.

We collected data on risk factors in 227 patients, of whom 157 presented with endoscopically proven EC. All biopsies that were taken showed histopathological characteristics of ESCC; no adenocarcinoma was found. We observed 100% accordance between the macroscopic appearance and histological diagnosis. These findings are consistent with those of another study conducted in Malawi, in which no esophageal adenocarcinoma was found in 82 EC biopsy samples taken between 2004 and 2008 [17].

In this study, the presence of EC was associated with smoking, age and the consumption of alcohol, hot food or tea, and smoked fish. In the logistic regression analysis, age and the consumption of hot food or tea remained significant.

The association of age with the presence of EC is not surprising and is consistent with other data from Africa. ESCC incidence rates increase with age, and approximately 80% of people with EC in eastern Africa are at least 50 years of age [4]. On the other hand, 18.7% of patients in the tumor group in this study were aged ≤ 40 years. Thus, it is unlikely that the high occurrence rates of EC in Malawi can be explained primarily by age.

In Europe and the United States, alcohol and tobacco consumption are the predominant risk factors, with a strong synergistic effect on ESCC risk [18]. Smoking and alcohol consumption were also identified as major risk factors for EC in a high-incidence area in South Africa [19]. In this study, alcohol consumption and smoking were associated with EC, but 43.5% of patients with tumors consumed neither tobacco nor alcohol. Similarly, the consumption of alcohol and tobacco is less common in high-incidence areas in Asia than in Western countries, and seems to play a lesser role in the etiology of ESCC [18]. Tran et al. [20] reported relative risks of 1.34 for cigarette exposure (ever-smoking) and 0.92 for alcohol consumption in a high-risk area in China. In a high-risk area in Iran, the history of alcohol consumption was negligible (1%) and the use of cigarettes was limited (27%) among patients with ESCC [21]. Thus, other environmental factors also should be considered in high-risk areas.

Associations of ESCC with other risk factors have been discussed in the literature. Environmental factors such as the consumption of hot food and beverages and poor diet have been associated with elevated risks of EC and ESCC in high-risk areas [18]. The association of EC occurrence with the consumption of hot tea and food in this study is similar to the increased risk of EC associated with this factor in high-risk areas in Iran [22], South America [23], and China [24]. Hot beverages can harm the esophageal epithelium via thermal effects and chemical constituents [25]. Local hyperthermia impairs the barrier function of the esophageal epithelium [26], and mild and moderate esophagitis has been associated with the consumption of boiling-hot beverages [27]. In this study, we found no association of EC with the consumption of fish or vegetables. Studies conducted in high-risk areas in Iran and China revealed an inverse association of ESCC with the consumption of fresh vegetables [28–30] and fish [31]. However, our results are consistent with those of a case–control study conducted in Zambia, which revealed no significant difference in the consumption of fruits, vegetables, and fish between EC cases and controls [32].

We investigated additional factors that are common in southern Malawi, such as the consumption of smoked fish and maize porridge; the presence of EC was associated with smoked fish consumption. Carcinogenic substances such as polycyclic aromatic hydrocarbons (PAHs) have been found in smoked fish, especially fish smoked heavily in traditional kilns [33]. High exposure to PAHs in the general populations of high-risk areas in Iran [34, 35] and China [36] have been reported. PAH-DNA adducts have been detected in esophageal biopsy samples [37], and greater PAH exposure of the non-tumoral

esophageal epithelium was detected in patients with ESCC than in controls in a high-risk area in Iran [38]. Foods containing protease inhibitors, such as beans, which are frequently consumed in southern Africa, have been associated with EC [39]. Furthermore, EC was associated with the consumption of purchased maize in South Africa [40], possibly due to nutritional deficiencies [2], fungal contamination [41], or increased intragastric production of prostaglandin E_2, which leads to increased cell proliferation [42, 43]. However, we found no association between the consumption of maize porridge and EC in this study.

HIV, CMV, HSV, and *H. pylori* infection are prevalent in Africa [12–15]. Our serological investigation revealed no association of EC presence with positive HIV serology, consistent with the finding of another study from Malawi [44], or with positive serology for CMV, EBV, HSV, or VZV. CMV is known to have onco-modulatory properties [45], and EBV has been detected, for example, in patients with nasopharyngeal carcinoma [46]. Consistent with our findings, no association between CMV and EC was found in studies of tissue samples from China [47, 48]. Various prevalence rates of EBV in tissue samples have been reported; EBV DNA was found only in subsets of ESCC tissue samples [47, 49–51]. Similarly, various prevalence rates of HSV in EC tissue samples in high-risk areas of China have been reported [47, 48, 52]. The role of *H. pylori* in gastric malignancies has become evident [46], but its role in ESCC remains unclear [53]. We found no association between antibodies against *H. pylori* and the presence of EC in this study, in contrast to the decreased risk of ESCC in individuals with *H. pylori* infection observed in other case–control studies [55, 56].

The lack of association between tumor development and the infectious agents investigated in this study may be attributable to high seroprevalence among control patients and low prevalence among patients with tumors, or to the small number of blood samples investigated. The latter can be considered a study limitation. Another technical limitation is that we investigated associations of infectious agents with EC only serologically, and not using tumor tissues. A further limitation of our study is that we used a self-generated questionnaire and not a validated questionnaire, as no such instrument was available for the Malawian setting in 2010, when the study began.

In our sample, many control patients reported the same nutritional habits as did patients with tumors. High PAH levels in urine samples from healthy subjects in high-risk areas in China, Iran, and Brazil have been reported [18]. Thus, genetic susceptibility also needs to be considered in future studies. The risk of ESCC was elevated among patients with family histories of upper-gastrointestinal cancer and EC in a high-risk area of China [56]. Furthermore, EC is more common in certain ethnic groups in high-risk areas, such as Kazakhs in China [57, 58], and the genetic background of migrants in China appears to influence the incidence of ESCC [59]. Variants in several chromosome regions were associated with an increased risk of EC in China [60]. Genetic diversity appears to be greater in African populations than in populations living on other continents [61]. Data on the genetic susceptibility to EC in Africa are limited. In South Africa, certain variants of alcohol dehydrogenase were found to be associated with an increased risk of EC development [62]. Bye et al. [63] suggested that the genetic contribution to ESCC differs in South African and Chinese populations due to differences in genetic architecture. A recently published transcriptomic analysis conducted with patients with ESCC in Malawi revealed genetic aberrations similar

to those found in Asian and North American cohorts, such as TP53 mutations [64]. Mutational signature analysis showed common signatures associated with aging and cytidine deaminase activity; a third signature was of unknown origin, and signatures of inhaled tobacco use, aflatoxin, and mismatch repair were notably absent. Thus additional factors that are characteristic in Malawi should be investigated and the genetic susceptibility to EC should be considered in further studies.

The results of this study should be viewed as only one component of a systematic understanding of risk factors contributing to the high incidence of EC in southern Malawi. Investigations of fungal co-infection and toxins, maize contamination, and the association with HPV are in progress or have been published [16]. The performance of epidemiological studies in low-resource settings is always challenging. Thus, our results should be interpreted with caution, and more standardized investigations with larger patient samples are needed. However, our results suggest that more attention should be devoted to the development of primary prevention strategies for non-communicable diseases in sub-Saharan Africa. This effort may entail the addressing of other challenges, such as the shortage of healthcare workers; lack of infrastructure; insufficient access to early diagnosis, treatment, and palliative care; and poor public awareness of cancer [65].

Declarations

Funding

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Declaration of Conflicting Interests

The authors declare that there is no conflict of interest.

Availability of data and material

All data are accessable from the corresponding author.

Code availability

Not applicable.

Ethics approval

The study was approved by the College of Medicine Research and Ethics Committee (Nr P.04/10/930).

Consent to participate

All authors have been actively involved in the planning and conduction of the study and have assisted with the preparation of the submitted manuscript.

Consent for publication (include appropriate statements)

All authors have agreed to submit this proposal to Cancer Causes and Control.

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