Modifiable Risk Factors of Esophageal Squamous Cell Carcinoma in Southern Malawi: A Case-Control Study

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Collaborative research endeavors between German and Malawian entities have resulted in significant findings that have relevance not only with the African scientific community, but also to a broader healthcare landscape. These findings impact both patients and medical professionals. To maintain transparency, streamline information dissemination, and ensure accessibility and comprehensibility to a wider audience, the decision was made to present this body of research in English. This decision aligns with the goals of sharing knowledge and facilitating the utilization of research outcomes to impact other healthcare domains.

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Abstract

Background:

Malawi is affected by the highest incidence of esophageal squamous cell carcinoma (ESCC) in the world, for which several modifiable risk factors may play a key role. More information about these variables is needed to implement useful prevention steps.

Methods:

The study is conducted as a hospital-based case-control study in Zomba Central Hospital, Malawi. A control group was matched for age, income, and gender in a 1:1 ratio. An odds ratio (OR) and confidence intervals (CI) were calculated for all variables.

Findings

113 ESCC Patients were included. It was found that smoking raised the odds for ESCC by 2.6 times (CI 1.36:4.87), the exposition of smoke from cooking by 3.1 times (CI 1.80:5.33), and that of burning trash by 2.7 times (CI 1.80:5.33). Hot foods and drinks were also found to raise cancer risk by 2.4 times (CI 1.40:4.01) and 2.2 times (CI 1.35:3.57), respectively. The choice of unprocessed maize flour, such as m'gaiwa (OR 0.4, CI 0.19:0.66), instead of white flour (OR 1.1, CI 0.57:1.94), was shown to have a protective effect, even if combined in the diet (OR 0.5, 0.29:0.83). Other factors considered were not found to influence the odds ratio significantly.

Interpretation

The findings indicate that the incidence of esophageal squamous cell carcinoma (ESCC) in Malawi is influenced by several factors. Particularly, exposure to smoke and consumption of hot foods appear to promote the development of ESCC, while the consumption of unprocessed maize flour seems to have a protective effect. Additional research studies, including meta-analyses and longitudinal experimental trials, are required to further investigate these observations. Implementation of interventions aimed at reducing modifiable risk factors could potentially alleviate the burden of ESCC in the country.

Funding

Else Kröner-Fresenius-Stiftung, medizinisch-humanitäre Entwicklungszusammenarbeit

Abstract (Deutsch)

Hintergrund:

Malawi ist das Land mit der weltweit höchsten Inzidenz von Plattenepithelkarzinomen des Ösophagus, für welche einige beeinflussbare Risikofaktoren eine Schlüsselrolle spielen können. Mehr Informationen werden benötigt, um wirksame präventive Maßnahmen ergreifen zu können.

Methoden:

Die Studie wurde als hypothesengenerierende, krankenhausbasierte Fall-Kontroll-Studie im Zomba Central Hospital (ZCH) in Malawi durchgeführt.

Eine Kontrollgruppe wurde in Bezug auf Alter, Einkommen und Geschlecht im Verhältnis 1:1 erstellt. Ein Odds Ratio und 95%-Konfidenzintervalle wurden für alle Variablen berechnet.

Ergebnisse:

113 Tumorpatienten wurden in die Studie eingeschlossen. Es wurde gezeigt, dass Tabakkonsum die Wahrscheinlichkeit zu erkranken um das 2,6-fache (KI 1,36:4,87) erhöht, die Exposition zu Küchenrauch um das 3,1-fache (KI 1,80:5,33) sowie die Exposition durch brennenden Müll um das 2,7-fache (KI 1,80:5,33). Heiße Speisen und Getränke erhöhen das Krebsrisiko um das 2,4-fache (KI 1,40:4,01), bzw. um das 2,2-fache (KI 1,35:3,57). Der bevorzugte Verzehr von unverarbeitetem Maismehl, wie m´Gaiwa (OR 0,4, KI 0,19:0,66), anstelle von weißem Maismehl, zeigte eine Reduktion des relativen Risikos, sogar wenn dieses mit Weißmehl kombiniert wird (OR 1,1 KI 0,29:0,83). Andere Variablen zeigten keinen signifikanten Einfluss auf das Odds Ratio.

Interpretation:

Die Ergebnisse legen nahe, dass die Inzidenz von ösophagealem Plattenepithelkarzinom (ESCC) in Malawi von mehreren Faktoren beeinflusst wird. Insbesondere scheint die Exposition gegenüber Rauch und der Verzehr von heißen Lebensmitteln die Entwicklung von ESCC zu begünstigen, während der Verzehr von unverarbeitetem Maismehl eine schützende Wirkung zu haben scheint. Zusätzliche Forschungsstudien, einschließlich

Metaanalysen und longitudinalen experimentellen Tests, sind erforderlich, um diese Beobachtungen weiter zu untersuchen. Die Implementierung von Interventionen zur Reduzierung von modifizierbaren Risikofaktoren könnte die Belastung durch ESCC im Land potenziell verringern.

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Introduction

Esophageal Carcinoma

Cancer of the esophagus (EC) occurs relatively frequently across the globe. The World Cancer Research Fund reported an estimated 456,000 new cases in 2012. This corresponds to 3.2% of all cancer cases worldwide and makes it the eighth most common cancer. In terms of mortality, it was estimated to claim the lives of 400,000 patients a year, 4.9% of all cancer patients [1]. In accordance, a relatively poor survival rate of M:I 0.88 was determined with significant geographical differences [2]. Leading mortality rates are found in Eastern Asia (14.1/100,000) and Southern Africa (12.8) in men, and Eastern Africa (7.3) and Southern Africa (6.2) in women [2]. Less developed regions seem to suffer the greatest burden where about 80% of all cases can be found. In men threefold higher compared to women [2]. Nevertheless, it must be differentiated between the two main histologic types of cancer of the esophagus, the adenocarcinoma, and the squamous cell carcinoma.

Adenocarcinomas evolve from columnar glandular cells that line the junction of the esophagus and stomach while squamous cell carcinomas arise from the squamous epithelial lining of the upper part of the esophagus. In both cases, the epithelial cells lining the esophagus are exposed directly to carcinogenic substances, suffering damage by repetitive exposure. The resulting irritation and inflammation favor a genetic aberrance and as a consequence give rise to tumor development [1].

In the case of squamous cell carcinoma, irritation takes place due to ingested materials. For instance, damage by burns from very high-temperature drinks or from the direct contact of alcohol. Adenocarcinomas on the other hand have been found to be majorly influenced by gastroesophageal reflux and its resulting inflammation [1]. The globally more frequent squamous cell carcinoma accounts for 88 percent of esophagus cancer cases, yet the proportion of adenocarcinomas occurring in industrial countries is on the rise [1].

Considerable symptoms that lead to medical consultation typically only appear at an advanced stage, which contributes to a poor prognosis. The five-year survival rate of esophageal cancer in the United States is about 20%, in Europe, it is about 10%. In less

developed countries, however, where esophageal cancer is particularly common, survival rates are far worse [1].

Cardinal Symptoms of esophageal cancer are dysphagia, typically experienced as pain, and pressure sensation after ingestion of food. Yet, dysphagia is very unspecific and may be present for many other reasons. Furthermore, EC may cause an unusual loss of weight due to failure to sufficient nutrition input, loss of appetite, and a complex interaction of tumor and host factors. Tumor cachexia is the reason for death in most EC cases. Also, anemia may result as a consequence of EC. Tumor bleeding could then be observed as hematemesis or melaena.

Malawi: Country, Health and Economy

Malawi is a landlocked country located in the southeastern part of Africa, bordering Mozambique, Zambia, and Tanzania, and it has a population of about 19.5 million residents in 2020 [3]. This corresponds to 0.25% of the world population. According to the United Nations Development Report, Malawi ranked 172 among 189 countries in 2019 (Table 1) [4], reflecting the scarcity of resources available to the local population. In terms of gross national income per capita (PPP), it is considered the third poorest country in the world, only followed by Burundi and South Sudan [5]. In Malawi, challenges such as inadequate transportation, financial constraints, and lengthy travel to healthcare facilities create obstacles to accessing timely healthcare. Thirty-nine percent of male-headed households and 59% of female-headed households indicated insufficient financial resources to seek medical care at a hospital [6].

Tourism does not play a major part in Malawi´s economy. Most of the economy is based on agriculture and its exports. The local population depends hereby mainly on subsistence farming to survive. Especially maize produce contributes as a major basic foodstuff [7] . Most of the maize is dried and ground up to maize flour which serves as the main ingredient for N´sima, the national dish.

Table 1: Malawi´s rank in the United Nations Development Index (HDI) 2019 in comparison to Germany [4]

	Rank	Country	Human Development Index (HDI)	Life Expectancy at birth (yr)	Expected years of schooling (yr)	Mean years of schooling (yr)	Gross national income (GNI) per capita (PPP$)
	172	Malawi	0.485	63.8	11.0	4.6	1159
	4	Germany	0.939	81.2	17.1	14.1	46946

Electricity is only available in big cities and only about 11% of the population has access to electricity [8]. In most places, electricity is usually only on a schedule of a few hours per day. Preserving food in refrigerators or preparing food on an electric stove are therefore highly improbable for the majority of the population. Food is consequently often stored poorly and meals are generally prepared over open fire.

Hospital care is only available in the few district hospitals across the country where often only basic diagnostics and treatments are available. One of which is Zomba Central Hospital (ZCH), where this study was conducted. With 400 beds it is the fourth biggest hospital in the country and serves as a reference for about two million people in the area [9]. Besides the endoscopy department, the hospital offers facilities for internal medicine, surgery, orthopedics, gynecology, and pediatrics. The vast majority of medical work is performed by clinical officers (CO) [9], trained for specific treatment functions without the background of a medical degree from university.

Malawi: The Burden of Squamous Cell Carcinoma of the Esophagus

EC is endemic in southeastern Africa [1; 2]. In Malawi, it ranks first with a prevalence of 12% throughout all age groups [2]. The prevalence of EC in Malawi even exceeds the prevalence of HIV infection. According to Ferlay et al. [2], the incidence reaches even 22.7/100,000 followed by China with 20.1/100,000 making it the highest in the world.

The etiology of this protruding incidence of squamous cell carcinoma is unclear. Alongside toxins, also particular viruses or special dietary habits are discussed to be influential factors [10]. Due to the lack of medical resources, the people affected in this region have little perspective. Surgical options, let alone chemotherapeutic protocols, effectively do not exist or are prohibitively expensive in most regions.

In Malawi, which according to the UN Poverty Report of 2019 [4] is one of the poorest countries in the world (ranked 172/182), esophageal carcinoma is one of the most common malignancies in the country. With an incidence of over 20/100,000, it is equally distributed in almost all age groups. Men are about 3 times more likely to be affected than women and mainly in the +30 years of age [2].

Risk Factors

Alcohol

Among causing over 200 health conditions, alcohol has long been established to be a risk factor for various types of cancer, inter alia, squamous cell carcinoma of the esophagus (ESCC) [1; 11; 12]. The consumption of alcohol was attributed to a 3 to 5-fold increase in ESCC [13-15] .

The World Health Organization (WHO) estimated a total of 5.3% of all deaths worldwide due to the harmful consumption of alcohol, 12.6% of which are attributable to cancer [12]. The alcohol-related increased risk of esophageal cancer has particularly been described

for esophageal squamous cell carcinoma [16], whereas esophageal adenocarcinoma is mainly linked to the development of Barrett esophagus [17; 18].

The explicit biomechanics of how alcohol induces squamous cell carcinoma is still subject to controversial debate. While the dosage-response association has been described [11; 19], a threshold could not yet be identified. Light drinkers are only attributed considerable risk of esophageal cancer in the case of gene involvement in the catabolism of alcohol. Several studies have investigated the association between polymorphism in aldehyde dehydrogenase 2 (ALDH2) and neoplasm [20-23].

ALDH 2 is responsible for eliminating acetaldehyde, a carcinogenic metabolite of ethanol [24]. In the instance of non-functional ALDH2, acetaldehyde accumulation favors the development of cancer even in low alcohol blood levels. However, this mechanism has not yet been described for populations outside of Asia.

As is usual in many cultures, the consumption of alcohol is widely practiced in Malawi. Due to low incomes, many Malawians revert to cheap traditional locally brewed, or even illegally distilled beverages. The production process of these beverages is not monitored, resulting in potential health-afflicting practices of unknown extent. Among the most popular alcoholic beverages are “masese” and “kachasu”. Masese is a local beer, brewed from finger millet and maize and has an alcohol content of around 4% to 8%. Its production is common in African countries in a slight variation of the brewing method and has been associated with esophageal cancer development in a South African setting [25]. Kachasu, even though referred to as a local beer, results from the distilling process of masese and has an alcohol content of up to 55%, depending on the time of badge collection [26]. Here, all the distilling products are collected. The product may not merely include ethanol, but also methanol and a not yet described range of branched-chain alcohols, resulting in a highly effective alcohol cocktail of not yet investigated influence on human health.

Smoking

Just like alcohol, cigarette smoking is a well-established risk factor for a variety of cancers. It contains a large number of different carcinogenic compounds. Among the most potent

are polyacrylic hydrocarbons, N-nitrosamines, benzene, aldehydes, aromatic amines, 1,3-butadiene, and ethylene oxide. High levels are produced in the combustion process of burning tobacco [27; 28]. In particular, nitrosamines have been demonstrated to be very effective in inducing Squamous cell carcinoma of the esophagus (ESCC) in rat models, which are the most commonly used animal species for Esophagus cancer research [28; 29].

Not only has it been estimated that ESCC increases 3 to 7-fold in smokers, but also that the synergistic effect of alcohol drinking and smoking is more than additive [28; 30]. It is postulated that the pro-oxidative property of these carcinogens generates reactive oxygen species, which can initiate and promote carcinogenesis. The mechanisms of how this reaction influences the cellular damaging process due to acetaldehyde, the main carcinogenic metabolite in alcohol, is not yet clear [31]. A generally accepted hypothesis of the multiplicative effect of ethanol and cigarette smoking is that ethanol dissolves and facilitates the transport of tobacco carcinogens to cells. This could make the cells more susceptible to malignant degeneration [28].

Means of Cooking

In Malawi, as it is the case in many African countries, the lack of electrical infrastructure and availability of power drives most people to use open fire as a predominant source of energy. Wood burning is the prevalent means of cooking, with about 85% of households stating to use wood as fuel in the Malawian Demographic and Health Survey in 2011 [32]. Charcoal is used by those who can afford them while only a small minority of people make use of electric stoves or gas.

Burning of biomass such as fire logs or charcoal results in the emission of combustion particles such as polycyclic aromatic hydrocarbons (PAHs), benzene, and 1,3-butadiene, as well as other compounds [33; 34]. The combustion and subsequent release of these substances have been reported to influence the risk of ESCC in studies conducted in southern Africa, Iran, and Brazil [15; 35; 36]. Here, considerable risk factors were established to be exposure to wood stoves, smoking, and living in rural areas.

In Malawi, the vast majority of households cook inside enclosed kitchens which may or may not be separated from the main living areas and are poorly ventilated. This is the case, especially in rural areas, where most Malawians live [34]. The use of wood-burning stoves typically contributes to indoor air pollution and exposes the cook to high dosage. In the case of separated kitchens, women are traditionally more affected than men which shall be considered in this study.

Furthermore, it has been shown that wood burning was more strongly associated with ESCC than charcoal burning [10]. Even though both are considered dirty fuels, wood burning produces more particulate matter due to combustion by a naked flame, whereas charcoal decays by glowing embers. More particulate pollution is the result of incomplete combustion and results in products such as PAH. Previous studies by Ellegård et al. have shown that wood users were exposed to considerably higher levels of particulate pollution during cooking time (1200 micrograms/m3) than charcoal users (540 micrograms/m3) and users of modern fuels (LPG and electricity) (200-380 micrograms/m3) [37].

Indoor wood-burning with subsequent release of PAH have been demonstrated to be a risk factor for ESCC, not least in central and eastern Europe, where an association has been established [35; 38]. Nevertheless, there are also other potential risk factors to consider during the preparation of food.

Furthermore, high levels of PAH exposure has been described among black tea and maté consumers in other studies [39; 40]. These dried-leaf-based beverages may acquire some of these potentially carcinogenic contaminants during their dehydration processing [15; 41]. The reason was suspected to be the burning of firewood in the drying houses. PAHs are then released and absorbed by the tea leaves, resulting in a vast increase in concentration than measured in previous steps of the process [15].

Hot Food and Beverages

Thermal damage to the esophageal epithelium may be another important reason for increased risk for ESCC. Responsible factors are the temperature at which foods or beverages are consumed. Strong evidence suggests that frequent intake of hot drinks

such as mate, coffee, or tea causes ESCC has been provided in various investigations, including cohort studies [1; 15; 42-44] . Furthermore, it has been claimed that hot tea consumption is one of the leading three causes of ESCC, only topped by smoking and alcohol drinking [42].

While thermal effects clearly play a role in this assumption, the following factors may also matter but are still points of discussion. It has been reported that thermal injury may induce a range of biological responses, including inflammation and intracellular signaling, which could form mutant cell lines [15] . Additionally, thermal insults may directly facilitate the permeation of carcinogens, leading to an aberrant proliferation of esophageal squamous cells and hence potentially causing ESCC [15] .

On the other hand, some experimental studies in animals have suggested cancer-preventive activities for these beverages [45-47], due to increased plasma antioxidant activity [48]. Yet, this could not be confirmed in a large clinical trial in Linxian and Huixian, China. Here, green tea was not shown to have a positive effect in suppressing the development of ESCC after eleven years of follow-up [49]. An explanation has been offered that suggests that antioxidants, such as flavonoids, in high concentrations may act as prooxidants that can generate free radicals. These may lead to DNA damage and finally irreversible pre-neoplastic lesions [50].

Furthermore, investigations showed that the total contents of PAH in black tea after the drying stage were significantly higher than those in the tea leaves sampled after each processing step beforehand. Drying houses, in which pine wood is burned during the drying stage, air PAH levels were about 100 times higher than those measured outside. This leads to the conclusion that PAH released is absorbed by the tea leaves, resulting in a higher carcinogenic potential of the final product consumed [51].

The predominantly consumed hot beverage in Malawi is tea. Particularly black tea is widely available. Coffee on the other hand, even though a national product, is hardly found in Malawian households. Whether the cultural attachment to tea is due to previous colonization by the English or due to the high prices of coffee dictated by international business, is not complexly clear. Nevertheless, it can be assumed that most of the

potential influence of hot beverages in Malawi is due to the consumption of tea, rather than coffee or any other hot beverage.

Spicy Food

The relationship between spicy food and the cause is still controversially discussed. A relationship may be suspected due to high rates of gastrointestinal cancer in regions where spicy food is widely consumed such as China [52]. Publication in this aspect is heterogeneous. A recent meta-analysis identified spicy food as a risk factor for gastrointestinal cancer, yet not for cancer of the esophagus explicitly, due to limited numbers of such studies [53]. The results are limited by missing longitudinal data.

Nutrition

Nutritional influence on carcinomata is a general topic of concern. In the US, low intake of fruits and vegetables by urban African Americans was associated with ESCC [14; 54]. Yet, the reported protective effect was only moderate. More recent meta-analysis reports only limited evidence of protective properties by regular intake of fruits and vegetables. A dose-response meta-analysis failed to show a significant association between ESCC risk and vegetable consumption [1]. For the dose-response of fruit intake, however, there is limited data to indicate a moderate association with statistical significance [1].

Evaluation of a relationship between dietary factors on ESCC is complex and investigation of an influence of nutritional intake and ESCC is highly prone to confounding by healthy user bias. The present evidence is inadequate for claiming the cancer-preventive effects of the intake of fruits and vegetables in most studies.

Mycotoxins

Several fungi are known to produce mycotoxins such as aflatoxins and fumonisins, which are harmful to human health. Aflatoxins are common contaminants of staple foods in sub-

Saharan Africa which is a known promoter of hepatocellular carcinoma (HCC) [15; 55; 56] . The exposure and toxic effect of aflatoxins are said to negatively affect health factors and account for over 40% of the burden of disease in developing countries [57] .

But in particular, fumonisins have been associated with esophageal cancer [15; 58; 59] . Fumonisins are metabolites produced by *Fusarium verticillioides, Fusarium proliferatum*, and other related species, as well as *Aspergillus nigri* [59; 60] . These fungi are contaminating maize and maize-based products in regions where there are no regulations to control exposure or where such controls are not enforceable [11; 61; 62]. In countries such as Malawi, maize is a staple food and is consumed at all times of the day, especially in the form of n´sima, the national dish [7] . The contamination may here result in a serious health threat which is not well-studied in this region.

Fumonisins express their toxicity by induction of apoptosis, cytotoxicity, and alterations in cytokine expression [63] . The degree of plant infection is linked to location, climate, and susceptibility of the plants to fungal invasion, as well as crop stress due to factors such as insect damage [64] . Yet, also the way of processing maize-based products has an influence on toxin concentration [59] .

The spreading of fumonisin is not limited to maize, other grains such as rice, wheat, barley, maize, rye, oat, and millet, as well as grain products like tortillas, corn flasks, and chips, are commonly contaminated [62; 65; 66] . Additionally, high levels of this toxin have been reported in black tea and medicinal plants [41] ,contributing to the occurrence of ESCC alongside thermal damage and PAH levels through consumption

Hypothesis and Objectives

The objective of this study is to investigate the underlying causes that contribute to the high incidence of esophageal squamous cell carcinoma in Malawi. To this end, specific variables were assessed through the use of a questionnaire that is based on factors known to have potential carcinogenic effects or have been previously linked to cancer in other contexts. Given the potential multifactorial nature of the disease, it is unlikely that a single factor can fully account for the development of esophageal squamous cell carcinoma in the region. Therefore, the questionnaire also aims to quantify the extent of the suspected exposures, which may serve to establish potential associations.

Rational

Primary prevention is of great importance due to a poor prognosis by reasons of late presentation and insufficient resources for the diagnosis and treatment of this devastating disease. Yet, prevention requires an understanding of the modifiable causes of EC in Malawi. The here conducted study pursuits to collect reliable data to identify factors associated with the high prevalence of EC. The investigation is planned to be a hospital-based case-control study. Due to little previous data and the probability of a multifactorial cause of EC, this should be a hypothesis-generating study is to explore multiple potential risk factors using a survey.

Executive Summary

The type of research study: Hypothesis-generating, hospital-based case-control study

The problem to be studied: Collection of reliable data to identify factors associated with the high incidence of squamous cell carcinoma of the esophagus in Malawi.

The objectives: Contribution to the understanding of the modifiable causes of esophageal cancer in Malawi. An objective that is essential to impose proper primary prevention strategies to combat this disease.

Methodology: Multiple potential risk factors of esophageal carcinoma should be explored using a survey. This shall include a study population with endoscopic confirmed squamous cell carcinoma of the esophagus and a control group of healthy individuals.

Materials and Methods

Type of Research Study

This study was conducted as a case-control study. Patients with confirmed squamous cell carcinoma of the esophagus were eligible to participate during the study period. Data collection consisted of a questionnaire developed by Janosch Missbach to assess specified relative risk factors believed to have contributed to the onset of the disease as outlined in the introduction section of this research paper. The questionnaire was provided to all eligible patients who were not excluded by specific eligibility criteria. A corresponding number of control subjects were also enrolled from the pool of hospitalized patients with other health conditions, such as trauma patients. Participants were explicitly informed about the voluntary nature of their involvement and the objectives of the study. All individuals provided written consent prior to study participation.

Study Place

The study takes place at Zomba Central Hospital(ZCH). Here patients are being diagnosed and treated as part of the “Zomba Stent Project”. The hospital in Zomba is among the four largest hospitals in Malawi. 400 beds are available for internal medicine, surgery, orthopedics, gynecology and pediatrics. It serves as a reference hospital for about two million people in south Malawi [9]. The large catchment area suggests a representative study population and easy access to controls.

Data collection was conducted as part of a palliative care initiative at Zomba Central Hospital. Janosch Missbach and Mathias Grade aided in providing voluntary support for esophageal stenting in patients with ESCC. This collaboration brought expertise in both skills, knowledge, and medical resources to the initiative. Due to the voluntary nature of this research, all activities were provided during holiday vacations, spanning approximately two to six weeks each year. Data collection occurred during these visits, and patients seeking treatment were invited to participate in the study. Importantly, patients were not required to wait for these visits, nor were they invited to attend during specific time frames.

Study Population

Adult patients who visit Zomba Central Hospital due to follow-up on an already diagnosed squamous cell carcinoma of the esophagus (EC) or freshly diagnosed individuals. All qualifying patients have been offered to participate. Random controls were collected by inpatients suffering from other diseases and without symptoms of EC. Zomba Central Hospital is a catchment area of about two million people and is expected to provide good external validity for the Malawian population.

Inclusion criteria

The following inclusion criteria had to be met for the participation in the study:

- Legal age (over 18 years old)
- Capacity to declare consent AND voluntary approval
- Communication skills in English or Chichewa
- Visit to Zomba Central Hospital during the defined interval of data collection
- For case group: Presence of a histologically confirmed squamous cell carcinoma of the esophagus or its presence with high probability after endoscopic diagnosis if a histologic confirmation was not possible.
- Freshly diagnosis

Exclusion criteria

Individuals who did not meet the inclusion criteria or refused to sign the consent form were disqualified from participation.

Study Period

The study was approved by the University of Oldenburg Ethics Committee (2019-051), Germany, and the National Health Science Research Committee, Malawi (#20/07/2620).

After approval, the collection of data was conducted over a period from September 2019 to March 2021. Mathias Grade and Janosch Missbach conducted yearly in-person data collection and study surveillance in Zomba Hospital for several weeks during our visits. Unpredictable operating hours and inconsistent availability of resources within the endoscopy suite resulted in problems with specific and consistent time intervals for data collection. Probes were processed as rapidly as possible after extraction. Data analysis was conducted after data collection was completed.

Sample Size

The study ought to be of an explorative nature. Criteria that may cause EC shall be identified. Yet, due to limited available data on variance in the Malawian population, a sample size may only be approximated by elsewhere established risk factors and may bear the risk of great deviations from the truly needed case numbers.

Here we will approximate the case numbers needed by data available about the risk of smoking, as this is a commonly accepted and investigated risk factor for ESCC worldwide. Available information indicates that roughly 15% of the Malawian population smoke tobacco [67] . We used the conventional 80% chance of detecting whether the odds ratio is significantly different from 1 at the 5% level and accepted a risk increase of 2 to 3-fold as a sufficiently important difference between the two groups. A previous review on smoking claims even an actual risk increase of 3 to 7-fold [28]. Based on Kelsey's formula [68] ,

$$n_{cases-Kelsey} = \frac{\left(z_{\alpha/2} + z_{1-\beta}\right)^2 * p * (1-p) * (r+1)}{r * (p_0 - p_1)^2}$$

α = The probability of type I error

β = The probability of type II error

P_0 = The proportion of cases

P_1 = The proportion of controls

r = The ratio of case-control (1 case/r controls)

a case collection of n ≈ 77 to 209 would be needed (The calculation was performed by use of EpiInfo v5.5.6).

In the here reported study, it was aimed to acquire 100-200 patients over several visits to Malawi. Experience from studies that had been conducted in other settings required similar sample sizes and served additionally as reference points.

Data Collection

The questionnaire, a patient information sheet, and an informed consent form were provided in English or Chichewa, based on the patient´s language competence. The forms were delivered during the patient´s stay. The participants were informed verbally and by means of an information sheet. Enough time was provided to answer questions. A translator for Chichewa was present if required. Particularly, all patients were informed about the voluntary nature of participation and that the decision to participate may not influence the course of treatment. A refusal to participate would not result in any disadvantage for the patient.

The filled documents were stored in containers that were not accessible to third parties. The information sheet remained with the patient. The signed consent form and the cover page of the questionnaire were marked by an identical numeric code and were kept in separate locations. It was possible for a patient to revoke his consent at this stage by collating the numeric code. The data could then be destroyed. Yet, it was explained that tracing and destruction of the data will not be possible after the anonymization of the data set at a later point in time.

The anonymization process began before data analysis. The cover pages that included the numeric code were therefore separated from the questionnaires and destroyed.

Esophageal specimens were obtained by trained endoscopists using endoscopic forceps. Mathias and Janosch Missbach, along with local clinical officers, performed the

procedures gathering biopsy material that was placed in formalin-filled test tubes for later histologic analysis. Hematoxylin and eosin staining were performed by a board-certified pathologist in a specialized lab.

Data Management and Analysis

The anonymized data was collected and analyzed using R-Studio (Version 1.3.1093). The response rate was determined by comparing the number of participants to the total number of eligible patients during the specified time period.

The frequency of individual variables will be reported using scales that provide the greatest possible information. To achieve this, certain variables were grouped into categories, such as appropriate age ranges.

The relationship between potential risk factors and the development of the disease in patients versus exposed controls was assessed using odds ratios, with confidence intervals calculated for all influencing factors. Janosch Missbach analyzed the comprehensive statistical data by utilizing R programming and creating graphical charts to present the data.

As an exploratory case-control study based on observations, definitive conclusions about causality cannot be made. The Bradford Hill Criteria will be used to evaluate the collected risk factors and support their likelihood of contributing to the development of the disease. Nevertheless, the analyzed data may serve as valuable information for generating hypotheses and designing further prospective cohort studies.

Results

Histological Feedback

103 histological samples of highly suspicious esophageal carcinomas were successfully collected employing flexible endoscopy forceps. Each tumor was sampled at three different locations to ensure an accurate and representative assessment of the tissue.

Table 2: Histological results of cancer samples taken by esophagogastroduodenoscopy.

	Squamous Cell Carcinoma	**Adeno-carcinoma**	**Other Carcinoma**	**Negative Result**	**Lost Sample**
Total	70	5	1	26	1

The large majority of probes were found to be Squamous Cell Carcinoma (see table 2). Five patients were found to have Adenocarcinomas and were therefore excluded from the survey. One patient was found to suffer from a carcinoma of the stomach which seemed to prolapse into the esophagus. This patient was also excluded from the questionnaire. In 26 cases it was not possible to histologically confirm a cancer diagnosis and in one case the sample vial was damaged and lost on transport (figure 1). Included in the study were 27 patients without histologic confirmation of ESSC (table 2: Negative results or lost samples) based on a clear visual diagnosis, clinical presentation and the findings from barium swallows. Resampling was not possible.

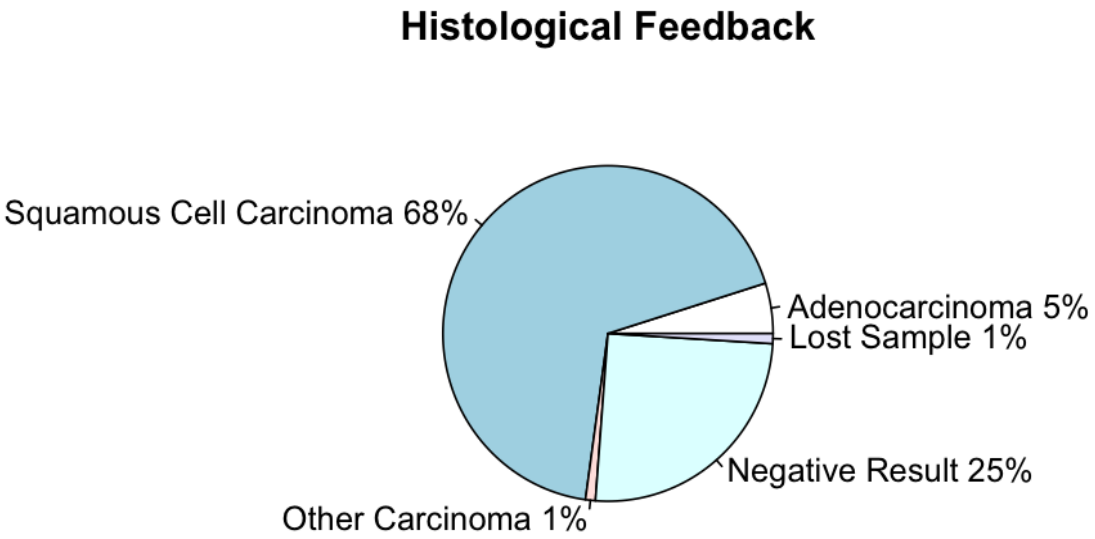

Figure 1: Proportion of histological results of cancer samples taken by esophagogastroduodenoscopy (n=103)

Sampling and Matching

In Malawi, patients typically present at a hospital with an advanced stage of cancer [69], where straightforward visual diagnosis is possible. The visual identification has been shown to have a pre-test probability of over 90% for diagnosis of ESCC in Eastern Africa [69][70]. It is the common method of diagnosis in countries including Africa where limited resources such as pathology services and computer tomography services are limited or not available.

In performing our research, we utilized a research approach commonly used in similar research studies [70][71]. 119 suspected cases were reviewed with exclusions including 6 patients whose histology ruled out ESCC (5 adenocarcinoma and 1 gastric carcinoma). 70 of the remaining 113 patients had a histological confirmation of an ESCC diagnosis with the remaining patients having had an endoscopic-visualized tumor. For 27 patients diagnosis was not possible due to inability to confirm a histologic confirmation. This was

due to sampling rejection (10 patients), insufficient resources that resulted in 6 patients not having a biopsy performed (figure 2). For these patients, visual diagnosis was accepted based on the highly suspicious clinical and endoscopic evaluations as obtaining another biopsy sample was not possible.

To optimize the control group in terms of age and gender, pre- and post-statistical matching techniques were employed (see figure 3 and 4 for both female and males data). The control group include almost twice as many males as females. Additionally, the questionnaire was distributed to individuals with ages comparable to those of the case group. Ultimately, 75 males (table 4) and 54 females (table 3) completed and submitted the questionnaire. The two groups were then matched statistically for age and gender to obtain a matched pair of equal numbers of males and females with the best age match (figure 5). Sixteen unmatched controls (table 3 and 4: 12 females and 4 males) were excluded from the study. These procedures allowed for valid comparisons to be made in the subsequent analysis.

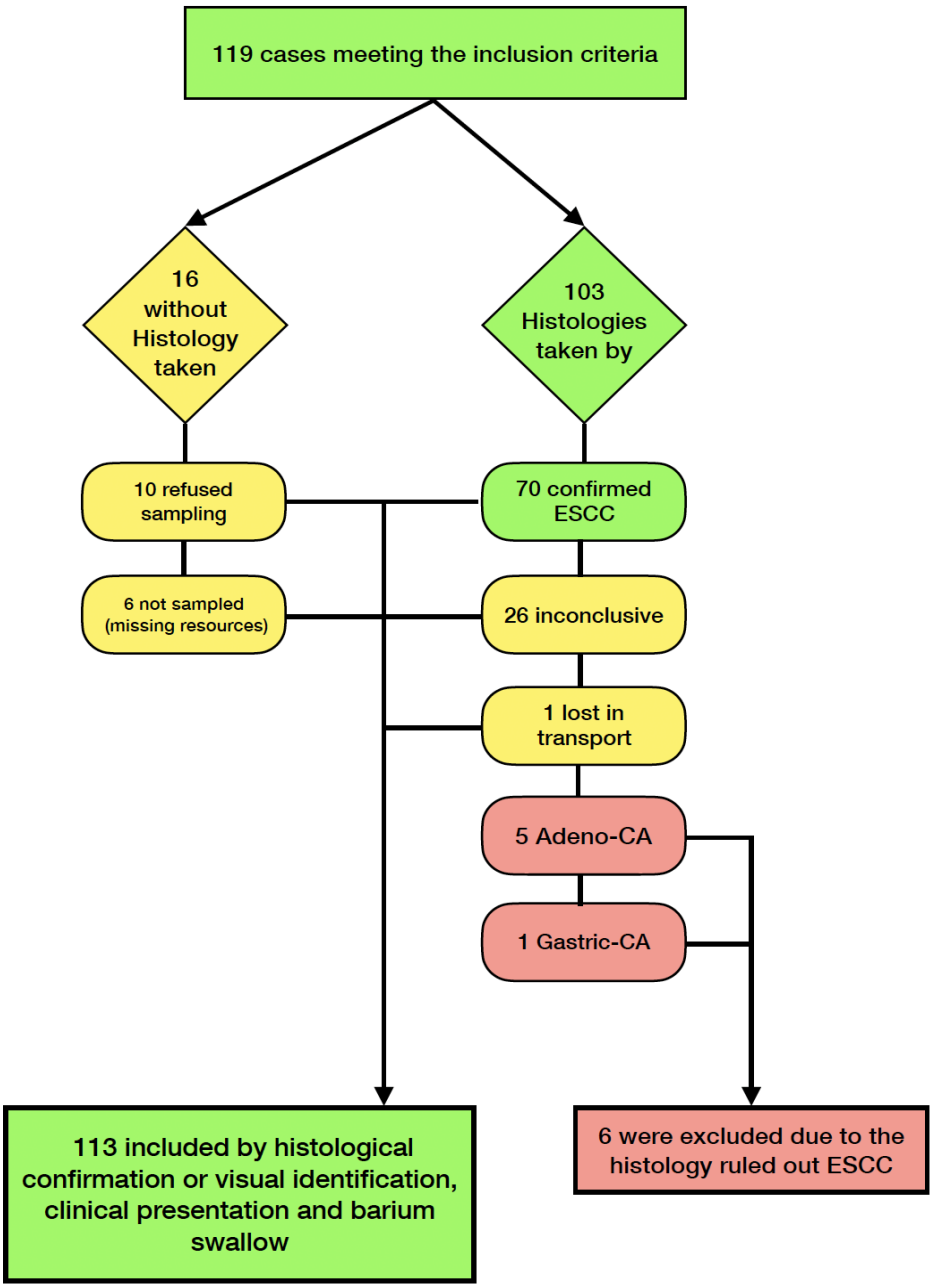

Figure 2: The flow diagram illustrates the selection process subsequent to the completion of questionnaires and endoscopy procedures by 119 cases that met the inclusion criteria. All 119 cases had undergone endoscopy. Histological sampling was performed in 103.

Females

Table 3: Female cases and controls were compared by age discrepancy after matching. The sample size of 54 female participants, resulted in 12 individuals being excluded to achieve an optimal age match for a 1:1 ratio of cases to controls.

Means Age Cases (Treated Units)	Means Age Controls	Std. Mean Diff.	Var. Ratio	Discharged Controls
58.2619	56.64	0.13	1.15	12

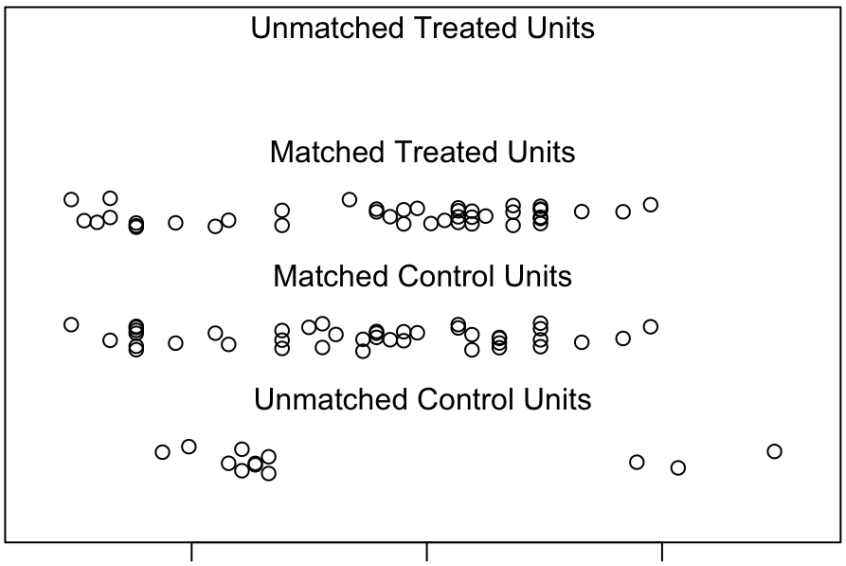

Figure 3: Stripchart of best fit pair-matching for age among female participants. Unmatched individuals were excluded from the study

Males

Table 4: Male cases and controls were compared by age discrepancy after matching. The sample size of 75 male participants, resulted in 4 individuals being excluded to achieve an optimal age match for a 1:1 ratio of cases to controls.

Means Age Cases (Treated Units)	Means Age Controls	Std. Mean Diff.	Var. Ratio	Discharged Controls
55.507	59.93	0.13	1.07	4

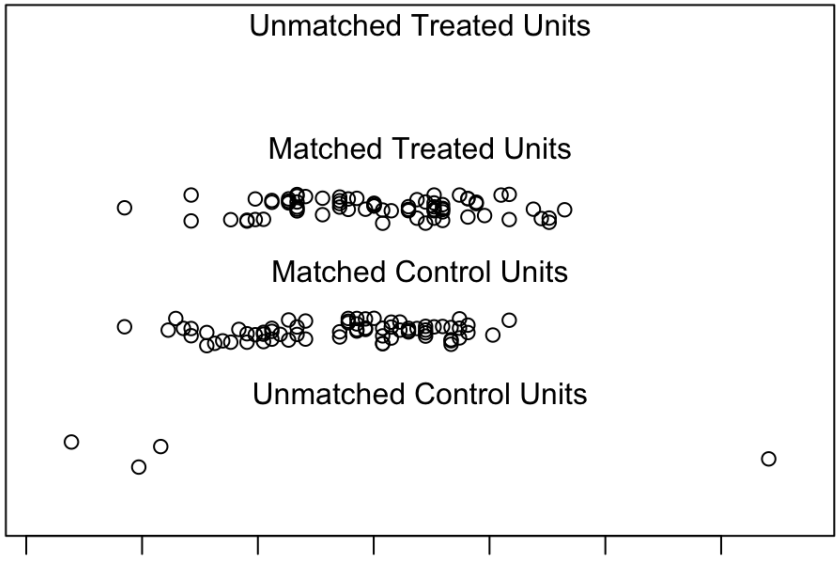

Figure 4: Stripchart of best fit pair-matching for age among male participants. Unmatched individuals were excluded from the study

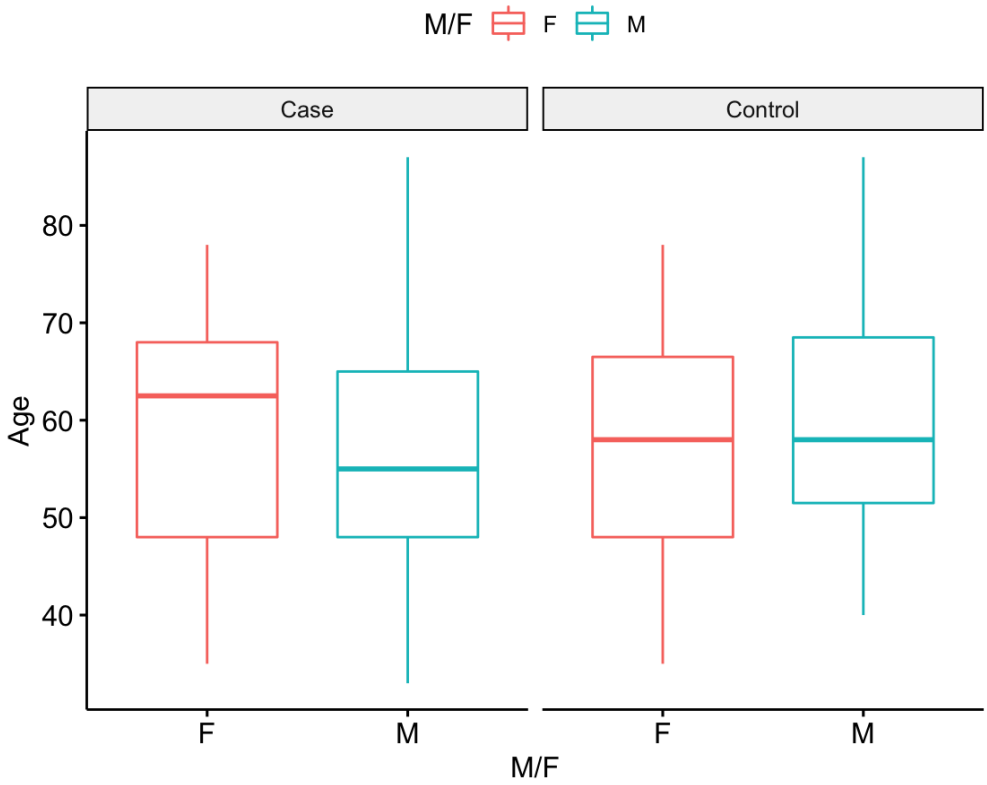

Figure 5: Boxplot contrasting the age difference of males and females in both, the case group as well as the control group. Shown is the median age, the interquartile range, as well as maximum and minimum age of individuals.

Evaluation of Study Groups

The age and sex distribution among the study population was found as displayed in tables 5 and 6. A Shapiro-Wilk test was performed and showed that the age distribution was not normally distributed (p-value = 0.023). Further comparison was therefore performed by means of the Wilcoxon rank sum test (Mann-Whitney test). The resulting p-value of 0.24 demonstrated no significant difference between the case group and the control group, visualized in figure 6.

The Fligner-Killeen test is one of the many tests for homogeneity of variances which is most robust against departures from normality [72] and was used in this setting to show

equality between the case group and the Control group. The p-value was found to be 0.3985, and therefore greater than the significance level of 0.05. We can conclude that there is no significant difference between the age variances of the two groups.

Table 5: Count of participating individuals after matching was completed, sorted by sex.

	Case	**Control**
Male	71	71
Female	42	42
Total	113	113

Table 6: Included participants in each study group´s mean age, median age, standard deviation (SD), and interquartile range (IQR) after completed matching.

	count	**Mean age**	**Median age**	**SD**	**IQR**
Case	113	56.53	58	11.83	17
Control	113	58.71	58	11.33	16

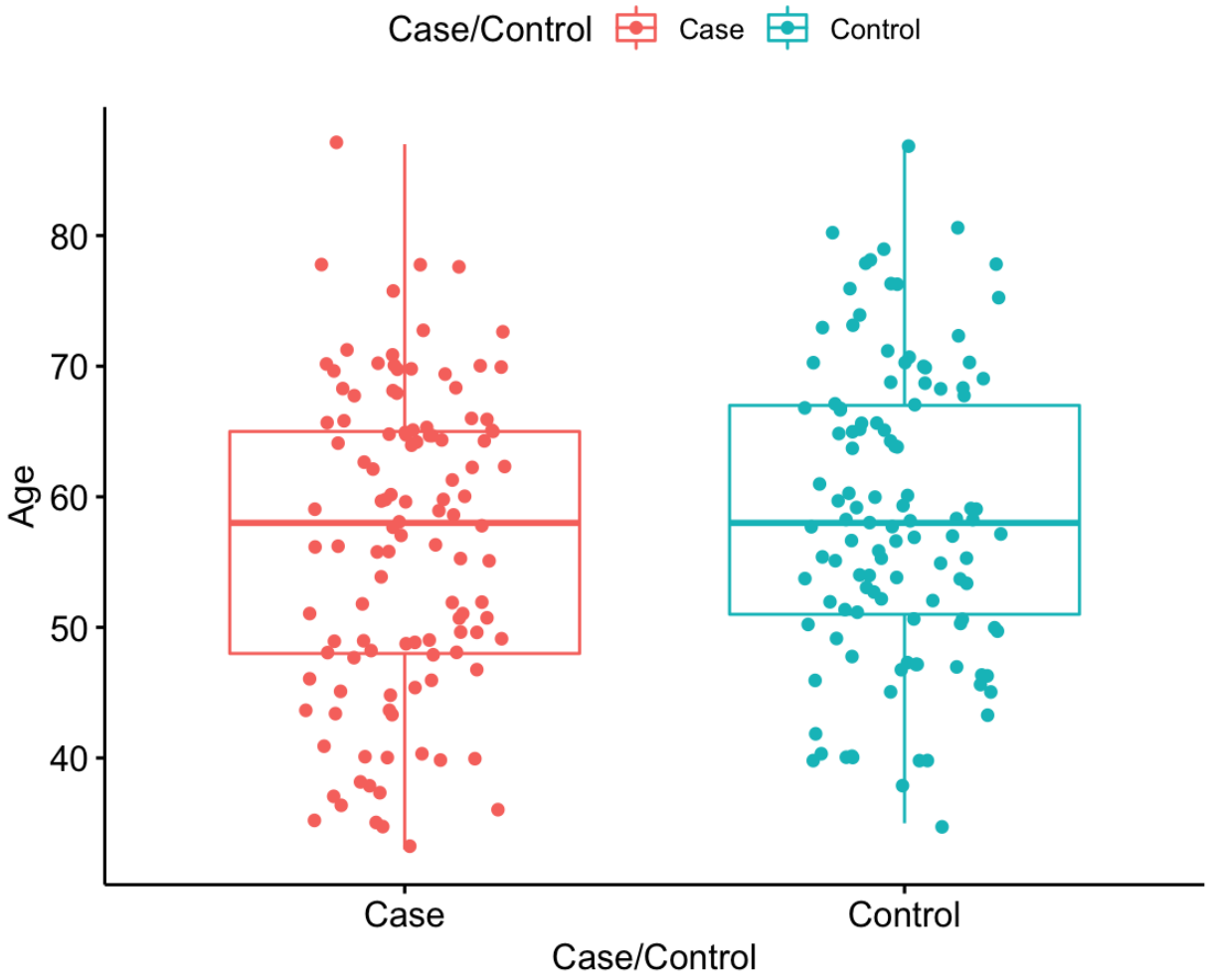

Figure 6: Boxplot contrasting the age difference in both the case group as well as the control group. Shown is the median age, the interquartile range, as well as maximum and minimum age.

Socioeconomic Status of the Study Groups

The average income among the study population was found as displayed in table 7. A Shapiro-Wilk test was performed and showed that income was not normally distributed (p-value < 2.2e-16). Further comparison was therefore performed using the Wilcoxon rank sum test. The resulting p-value of 0.49 demonstrated no significant difference between the case group and the control group, visualized in figure 7.

The Fligner-Killeen was also used to show equality between the case group and the control group. The p-value was found to be 0.6278, and therefore greater than the

significance level of 0.05. We can conclude that there is no significant difference between the income of the two groups.

Table 7: Average income of the study groups in Malawian-Kwacha (MWK)

	Mean Income	Median Income	SD	IQR
Case	49163	27500	55402	45000
Control	76740	25000	202106	30000

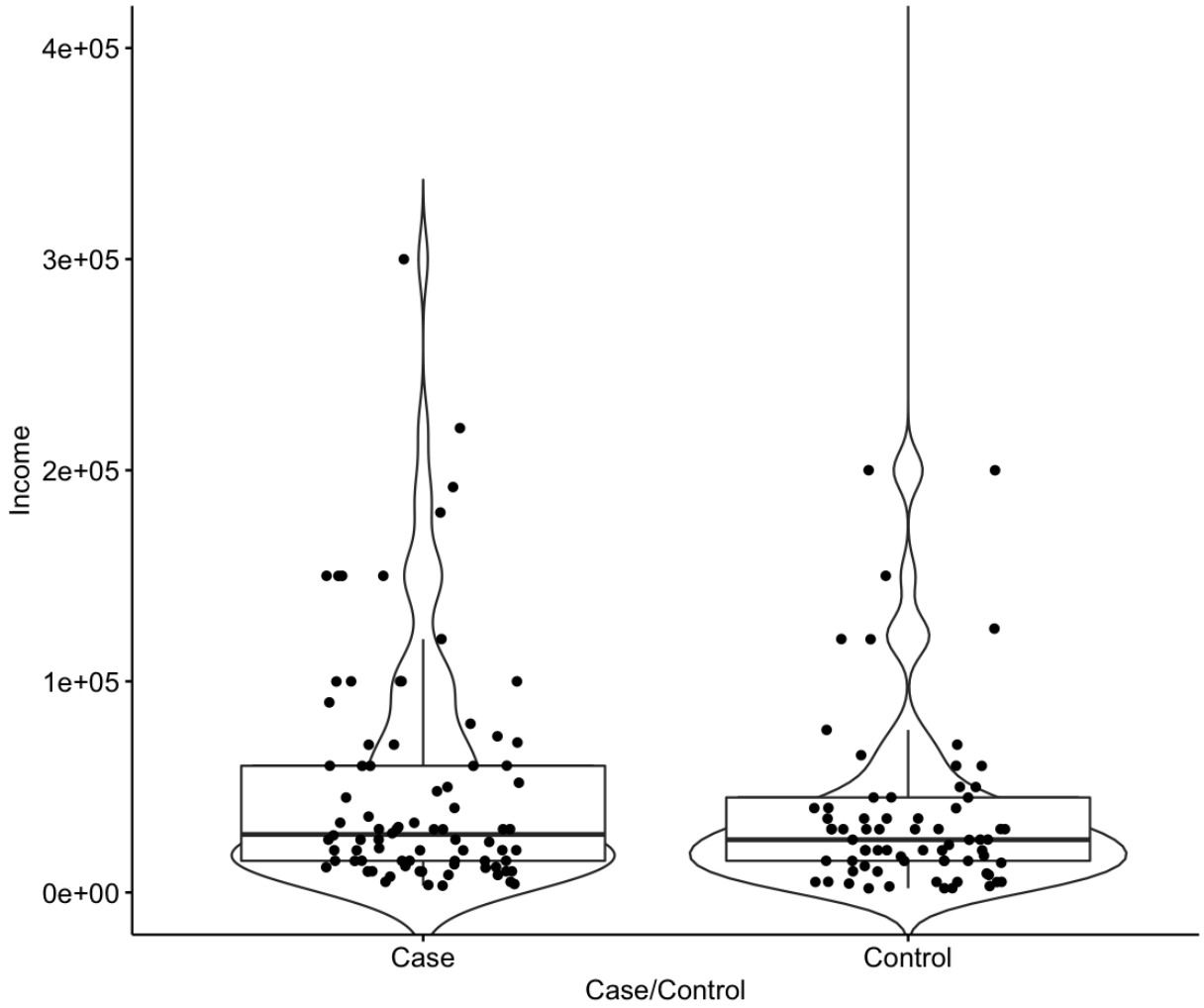

Figure 7: Average income of the two groups in Malawi-Kwacha (MKW). Three values exceeding 0.7Mil MWK were removed from the control group to maintain visibility of the plot. Furthermore a median and interquartile range are shown

Analysis of Evaluated Risk Factors

Familial Predisposition

An odds ratio (OR) measure was employed to evaluate the association between the likelihood of an individual diagnosed with cancer having a consanguineous relative also suffering from ESCC, and the general population, as outlined in table 8. The present analysis aimed to determine whether a familiar predisposition could serve as a risk factor for the onset of ESCC, and to compare the magnitude of this risk.

The OR was determined to be 2.163 (95%CI 0.838 : 5.582)

Table 8: Contingency table of individuals that have at least one direct family member suffering from esophagus cancer.

	Yes	**No**	**n**
Case	15	98	113
Control	7	106	113

Since the 95% CI of 0.838 to 5.582 intersects 1.0, the increased odds of ESCC patients with relatives suffering also from esophageal cancer does not reach statistical significance.

Geographic Accumulation

An odds ratio measure was employed to examine the relationship between the probability of a cancer patient having an individual residing in close proximity also afflicted with ESCC, and the general population, as indicated in table 9. This analysis primarily aimed to ascertain if geographic location could potentially act as a risk factor for the onset of ESCC and to compare the strength of association with the general population.

The OR was determined to be 0.525 (95%CI 0.237 : 1.162)

Table 9: Contingency table of individuals that have at least one person living in their neighborhood suffering from esophageal cancer.

	Yes	No	n
Case	26	87	113
Control	30	83	113

Since the 95% CI of 0.237 to 1.162 intersects 1.0, the increased odds of ESCC patients with relatives suffering also from esophageal cancer do not reach statistical significance.

Influence of Long-Term Medication

An odds ratio measure was used to test for an association between the likelihood of acquire ESCC for an individual taking long-term medication and the general population as shown in table 10. The objective of the analysis was primarily to determine whether persistent pharmacological treatment could influence the risk for the onset of ESCC and compare the relative magnitude of risk between individuals undergoing long-term medication and the general population.

The OR was determined to be 0.663 (95%CI 0.356 : 1.236)

Table 10: Contingency table of individuals that are under long-term pharmacological therapy.

	Yes	No	n
Case	29	84	113
Control	32	81	113

Since the 95% CI of 0.356 to 1.236 intersects 1.0, the increased odds of ESCC patients with relatives suffering also from esophageal cancer do not reach statistical significance.

Influence of Smoking

An odds ratio measure was utilized to investigate the relationship between the likelihood of an individual acquiring ESCC due to tobacco smoking, and the occurrence of ESCC in the general population, as presented in table 11. It was determined whether tobacco smoking would influence the risk for the acquisition of ESCC, and to compare the magnitude of this risk.

Never smokers were defined as individuals who have never smoked, while smokers were defined as all active smokers and those who quit smoking less than three months ago. Individuals that have stopped smoking earlier than three months ago were considered to have stopped smoking. Individuals who had not commented on smoking in the questionnaire were assumed to be never-smokers (*The terms are defined in accordance with the guidelines provided by the Center for Disease Control and Prevention. For further details on this definition, please refer to https://www.cdc.gov/nchs/nhis/tobacco/tobacco_glossary.htm*).

In the calculation of the odds ratio, smokers and former smokers were compared with the group of never smokers.

The OR was determined to be 2.569 (95% CI 1.356 : 4.868)

Table 11: Contingency table of the history of smoking among the case and control groups. Smokers are considered to have stopped smoking if they had stopped smoking for at least a three-month period.

	Smokers		**Never-Smokers**	**n**
	Yes	**Former**	**never**	
Case	24	13	76	113
Control	11	7	95	113

Since the 95% CI of 1.356 to 4.868 does not intersect 1.0, the increased odds of ESCC patients being smokers (or former smokers) is highly significant.

Influence of Alcohol

An odds ratio measure was used to test for an association between the likelihood of acquiring ESCC for an individual who is, or who has been, a regular drinker and a never-drinker in the general population as shown in table 12. It was determined whether regular consumption of alcohol would influence the risk for the acquisition of ESCC, and to compare the magnitude of this risk.

A never-drinker was defined as an individual who has never consumed more alcohol than a casual amount, while drinkers were defined as all individuals who consider themselves heavy drinkers and those who quit drinking less than three months ago. Individuals that have stopped drinking earlier than three months ago were considered to have stopped drinking. Individuals who had not commented on alcohol consumption in the questionnaire were assumed to be never-drinkers.

The OR was determined to be 1.309 (95% CI 0.687 : 2.500)

Table 12: Contingency table of the history of drinking among the case and control groups. Drinkers were considered to have stopped drinking if they had stopped drinking for at least a three-month period.

	Drinkers		**Never-Drinkers**	**n**
	Yes	**Former**	**Never**	
Case	16	10	87	113
Control	9	12	92	113

Since the 95% CI of 0.687 to 2.500 intersects 1.0, the increased odds of ESCC patients who consider themselves regular drinkers do not reach statistical significance.

Influence of the Means of Cooking on ESCC

An odds ratio measure was employed to examine the potential association between the likelihood of an individual acquiring ESCC and their preference for using charcoal, wood, or electricity to cook meals in comparison to the general population, as presented in table 13. The use of more than one fuel is common, so more than one choice was allowed. The analysis aimed to establish whether the choice of cooking method could act as a risk factor for the onset of ESCC and to compare the relative magnitude of this risk.

The OR_{wood} was determined to be 0.830 (95% CI 0.355 : 1.939)

The OR_{coal} was determined to be 1.204 (95% CI 0.662 : 2.190)

The $OR_{Electric}$ was determined to be 0.661 (95% CI 0.108 : 4.031)

Table 13: Contingency tables of the preferences in the means of cooking among the case and control groups. Each fuel is evaluated independently including individuals using more than one kind of fuel.

	Use of charcoal	**No use of charcoal**	**n**
Case	31	82	113
Control	27	86	113

	Use of wood	**No use of wood**	**n**
Case	100	13	113
Control	102	11	113

	Use of electricity	**No use of electricity**	**n**
Case	2	111	113
Control	3	110	113

Since the 95% CI_{wood} of 0.355 to 1.939 intersects 1.0, the increased odds of ESCC patients who use wood to cook do not reach statistical significance.

Since the 95% CI_{coal} of 0.662 to 2.190 intersects 1.0, the increased odds of ESCC patients who use charcoal to cook do not reach statistical significance.

The marginal use of electric energy to cook was not sufficient to display usable results for the sample size drawn.

Individuals Exposition to Kitchen Smoke

To examine the relationship between the acquisition of esophageal squamous cell carcinoma (ESCC) and cooking practices, we employed an odds ratio measure. Specifically, we investigated whether individuals who cook indoors are more likely to develop ESCC compared to those who cook outdoors. Additionally, we assessed the extent to which individuals reported being exposed to kitchen smoke, as detailed in table 14. We determined whether the location of the kitchen and associated smoke exposure influenced the risk of developing ESCC and compared the level of risk between those individuals with differing levels of smoke exposure.

The $OR_{indoor\ kitchen}$ was determined to be 0.884 (95% CI 0.504 : 1.551)

The $OR_{exposed}$ was determined to be 3.095 (95% CI 1.798 : 5.329)

Table 14: Contingency table of the location of the kitchen and the exposition of smoke during cooking. Category totals do not add up to n=113 because of missing data.

	Outdoors	**Indoors**	**n**	**Exposed**
Case	34	76	110	69
Control	31	79	110	38

Since the 95% $CI_{indoor\ kitchen}$ from 0,504 to 1.551 does intersect 1.0, the increased odds (OR 0.884) of ESCC patients who use an indoor kitchen do not reach statistical significance.

Since the 95% $CI_{exposed}$ from 1.798 to 5.329 does not intersect 1.0, the increased odds (OR 3.095) of ESCC patients who consider themselves exposed to kitchen smoke is highly significant.

Influence of Trash Burning and the Exposition of Smoke

We utilized the odds ratio measure to show an association between the likelihood of acquiring ESCC for an individual practicing trash burning and those who dispose of their trash otherwise. Additionally, we surveyed participants to determine their perceived exposure to smoke resulting from trash burning (table 15).
Our objective was to investigate whether the practice of trash burning poses a risk for ESCC acquisition due to smoke exposure and to quantify the magnitude of this risk.

The $OR_{trash\ burning}$ was determined to be 1.354 (95% CI 0.759 : 2.415)

The $OR_{exposed}$ was determined to be 2.711 (95% CI 1.798 : 5.329)

Table 15: Contingency table of the practice of burning trash and the exposition of smoke during the burning.

	Yes	**No**	**n**	**Exposed**
Case	84	29	113	40
Control	77	36	113	19

Since the 95% $CI_{trash\ burning}$ 0.759 to 2.415 does intersect 1.0, the increased odds of ESCC patients who practice trash burning do not reach statistical significance.

Since the 95% $CI_{exposed}$ of 1.798 to 5.329 does not intersect 1.0, the increased odds of ESCC patients who consider themselves exposed to trash smoke is significant.

Risk for Individuals Exposed to Smoke From Cooking and Trash Burning

We conducted an analysis using the odds ratio measure to investigate the potential association between the incidence of ESCC in individuals who are exposed to smoke resulting from both kitchen and trash burning, as compared to those who are not exposed to such smoke; see table 16. Our objective was to determine the impact the combined types of smoke exposures on the risk of ESCC acquisition and to quantify of the magnitude of this risk.

The $OR_{both:none}$ was determined to be 3.74 (95% CI 1.83: 7.80)

The $OR_{both:one/none}$ was determined to be 1.950 (95% CI 1.001 : 3.797)

Table 16: Contingency table of the exposition to smoke from cooking and trash burning.

	Exposed to none	Exposed to only one	Exposed to both	n
Case	33	51	29	113
Control	73	23	17	113

Since the 95% $CI_{both:non}$ of 1.83 to 7.80 does not intersect 1.0, the increased odds of ESCC patients who consider themselves exposed to both types of smoke is significant in comparison to those who are not exposed.

Since the 95% $CI_{both:one/none}$ of 1.00 to 3.79 lies on the 1.0 mark, the increased odds of ESCC patients who are exposed to smoke of both sources of combustion in comparison to only one or none, does not reach statistical significance.

Influence of the Consumption of Different Types of Maize

An odds ratio measure was used to examine the association between the consumption of processed and unprocessed maize and the likelihood of acquiring ESCC; see table 17. The objective was to investigate the impact of highly processed white maize and minimally

processed m'gaiwa on the risk of ESCC acquisition and to quantify the magnitude of this risk.

The OR_{white} was determined to be 1.05 (95% CI 0.57 : 1.94)

The $OR_{m´gaiwa}$ was determined to be 0.36 (95% CI 0.19 : 0.66)

The OR_{both} was determined to be 0.49 (95% CI 0.29 : 0.83)

Table 17: Contingency table of the exposition of different types of maize. The category total in the case group does not add up to 113 because of missing data.

	only White	only m´gaiwa	Both	n
Case	40	25	47	112
Control	19	27	67	113

Since the 95% CI_{white} of 0.57 to 1.94 does intersect 1.0, the increased odds of ESCC patients who consume mainly white flour do not reach statistical significance.

Since the 95% $CI_{m´gaiwa}$ 0.19 to 0.66 does not intersect the 1.0 mark, the decreased odds of ESCC patients who prefer the consumption of m´gaiwa, does reach high statistical significance.

Since the 95% $CI_{m´gaiwa}$ 0.29 to 0.83 does not intersect the 1.0 mark, the decreased odds of ESCC patients who consume white maize and m´gaiwa equally, does reach high statistical significance.

Influence of Home Grown Maize

An odds ratio measure was used to investigate the association between ESCC and home-grown maize, as shown in table 18. It was determined whether the consumption of home-grown maize would influence the risk for the acquisition of ESCC and to determine the magnitude of this risk. Individuals who did not answer with “Yes” to this question were assumed to consume commercially available maize. Individuals who grow their own maize

and also consume commercially available maize to some extent were induced in the home-growing group due to the present exposure to home-grown maize.

The OR was determined to be 0.71 (95% CI 0.34 : 1.47)

Table 18: Contingency table of individuals that grow their own maize. If at least parts of the maize were home-grown, the individual was included in the "Yes" category.

	Yes	**No**	**n**
Case	93	20	113
Control	98	15	113

Since the 95% CI of 0.34 to 1.47 intersects 1.0, the decreased odds of ESCC patients who grow their own maize do not reach statistical significance.

Influence of Hot Food

Ordinal logistic regression was used to show the association between ESCC and the consumption of hot food, as shown in table 19 and figure 8. It was determined whether the consumption of hot foods would influence the risk for acquisition of ESCC, and to determine the magnitude of this risk. Ordinal logistic regression was used to determine an odds ratio and to establish confidentiality in a trend relationship.

The proportional OR was determined to be 2.36 (95% CI 1.40 : 4.01)

Table 19: Preferred proximate cooling time that will be given before hot foods are consumed. The category total in the case group does not add up to 113 because of missing data.

	Hot of the fire	5 min to cool	10 min to cool	Cold	n
Case	74	28	9	0	111
Control	50	50	9	4	113

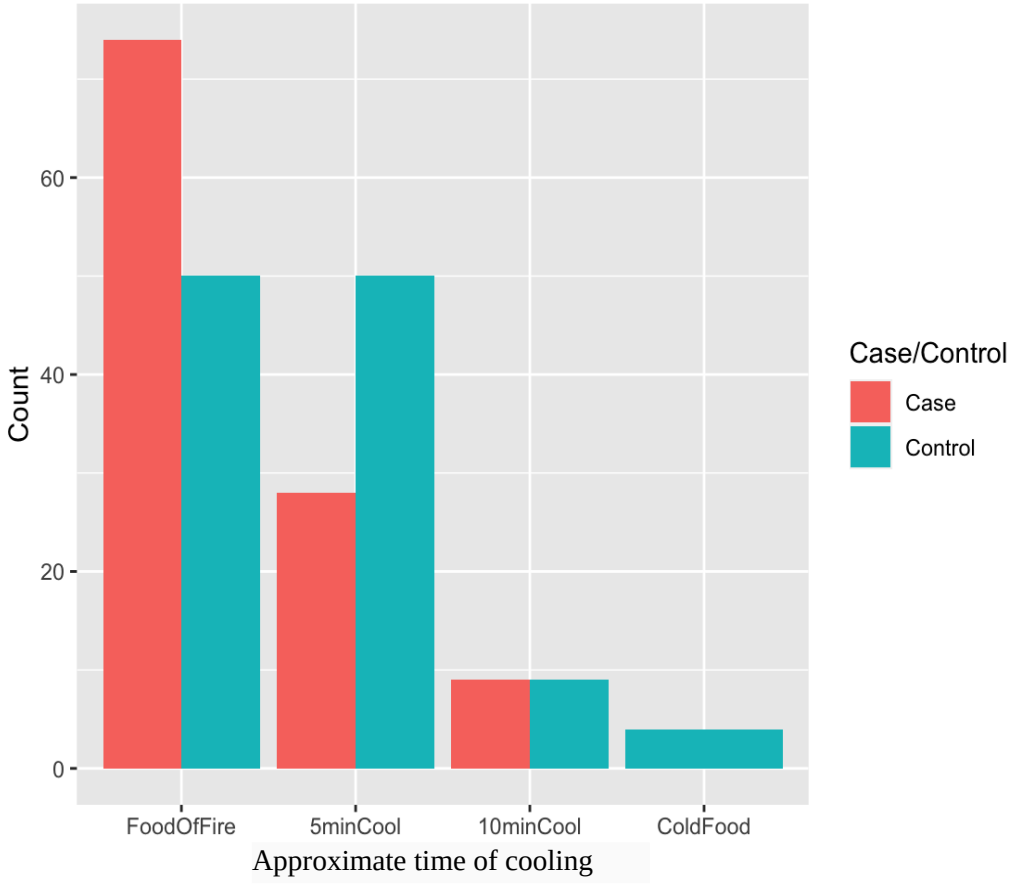

Figure 8: Distribution of the cooling-down time that individuals will give their food bore consumption.

Since the 95% CI of 1.40 to 4.01 does not intersect 1.0, the increased odds of ESCC patients with increasing consumption of hot foods do reach statistical significance.

Influence of Hot Beverages

Ordinal logistic regression was used to measure the association between ESCC and the consumption of hot beverages, as shown in table 20 and figure 9. It was determined whether the consumption of hot drinks will influence the risk for the acquisition of ESCC, and to determine the magnitude of this risk. Ordinal logistic regression was used to determine an odds ratio and to establish confidentiality in a trend relationship.

The proportional OR was determined to be 2.19 (95% CI 1.35 : 3.57)

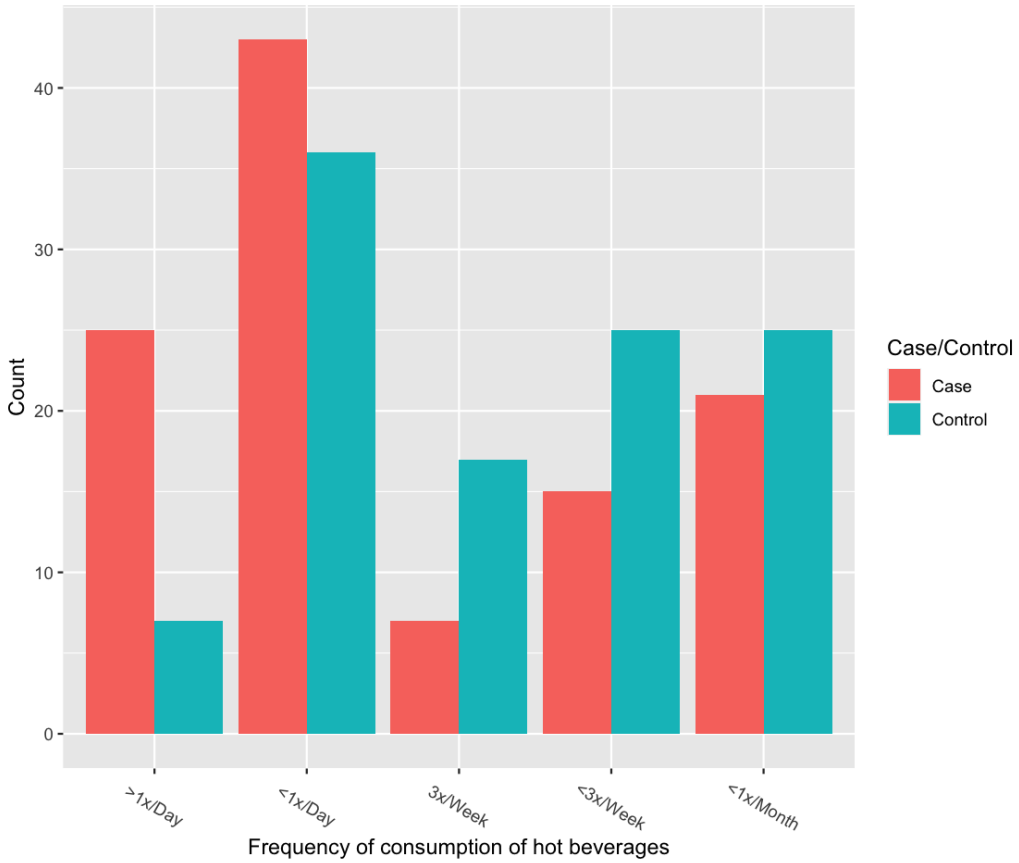

Figure 9: Distribution of the consumption frequency of hot beverages between the two groups

Table 20: Frequency of consumption of hot beverages among the study groups. Numbers do not add up to the total investigated due to missing data.

	> 1x/Day	<1x/Day	>1x/Week	<1x/Week	<1x/Month	n
Case	25	43	7	15	21	111
Control	7	36	17	25	25	110

Since the 95% CI of 1.35 to 3.57 does not intersect 1.0, the increased odds of ESCC patients with increasing consumption of hot foods do reach high statistical significance.

Influence of Spicy Foods

Ordinal logistic regression was used to examine the association between ESCC and the consumption of spicy foods, as shown in table 21 and figure 10. It was determined whether the consumption of spicy food would influence the risk for the acquisition of ESCC, and to determine the magnitude of this risk. Ordinal logistic regression was used to determine an odds ratio and to establish confidentiality in a trend relationship.

The OR was determined to be 0.93 (95% CI 0.54 : 1.60)

Table 21: Consumption frequency of spicy food. Numbers do not add up to the total investigated due to missing data.

	Daily	<3x/Wees	<3x/Week	<1x/Week	Never	n
Case	16	7	5	8	70	106
Control	8	15	15	4	70	112

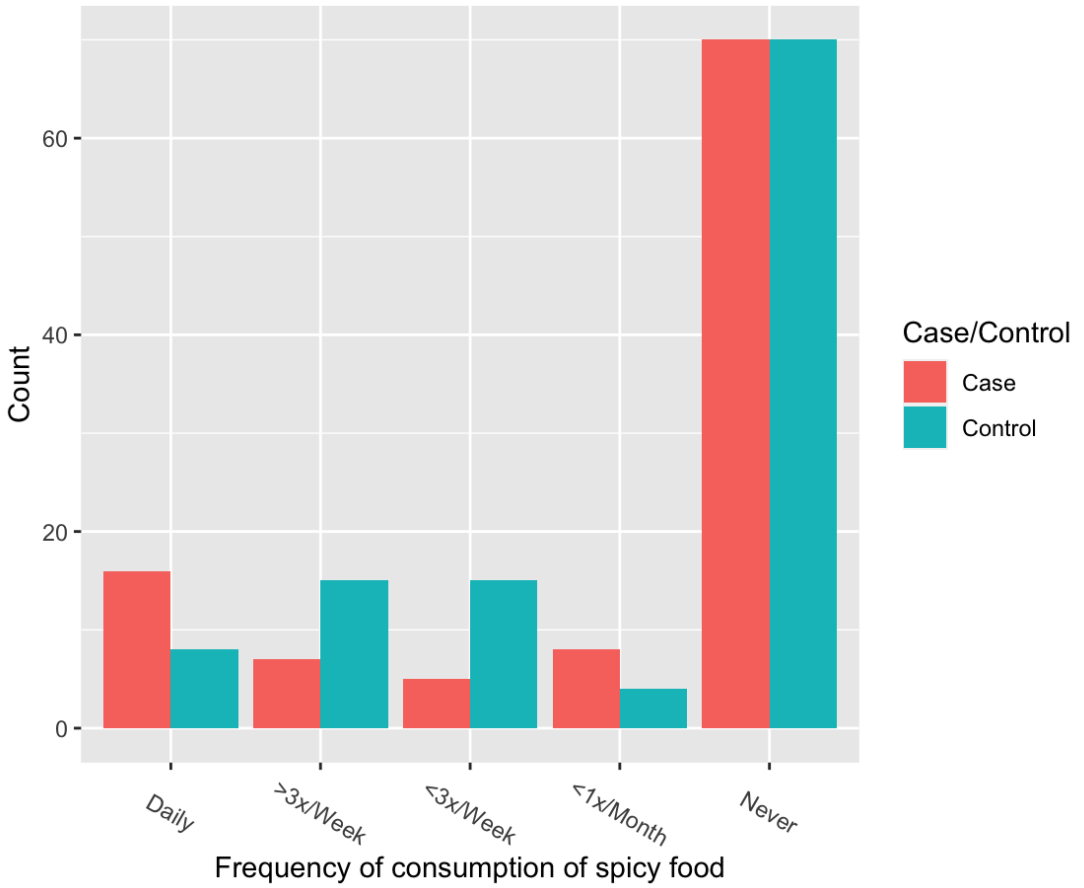

Figure 10: Trend of consumption frequency among the research groups.

Since the 95% CI of 0.54 to 1.60 intersects 1.0, the increased odds of ESCC patients with increasing consumption of hot foods do not reach statistical significance.

Summary of Results

Age and sex were matched between patients and controls in this study. Our findings indicated that patients were more likely than controls to smoke, to be exposed to smoke resulting from cooking and burning trash, and to consume hot beverages and hot foods. While not statistically significant, a familiar predisposition may be considered clinically relevant. Additionally, the consumption of m'gaiwa instead of white maize flour was found to have a protective effect, as demonstrated in figure 11.

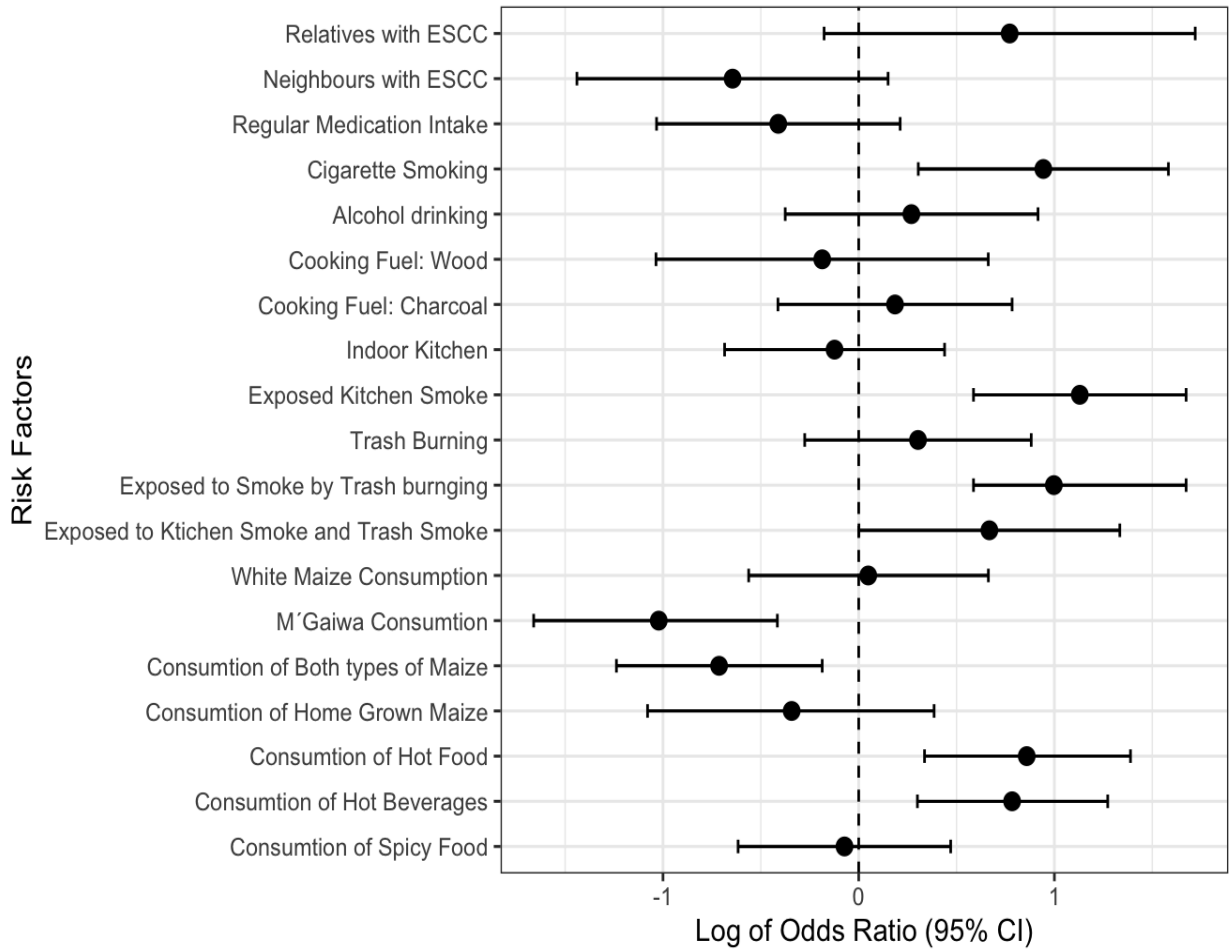

Figure 11: Forest plot summarising the investigated risk factors and their specific Log of odds ratio

Discussion

Esophageal carcinoma is a prevalent type of cancer worldwide, with squamous cell carcinoma being the most common histologic form. The highest incidence of this cancer is observed in regions such as Africa and the Middle East, with Malawi being a country that has been particularly affected [2]. In this study, we identified several risk factors associated with the development of esophageal carcinoma, including tobacco abuse, exposure to smoke resulting from cooking and burning trash, and consumption of hot foods and beverages. Our findings suggest that opting for unprocessed maize flour, such as m'gaiwa, instead of white flour, may have a protective effect against the development of this cancer. However, no significant effect was noted for the use of alcohol, consumption of spicy food, or the consumption of home-grown maize. These results suggest that the high incidence of esophageal carcinoma in countries like Malawi may be due to a multifactorial genesis, involving a combination of various environmental and lifestyle factors.

Our study revealed a 2.6-fold increase in the risk of developing ESCC among cigarette smokers. This strong association was anticipated and has been reported in previous publications [1; 11; 15]. Interestingly, the data collected in this study showed that 67% of the cases reported being never-smokers. Among the smoking cases, the average consumption was 8.8 pack-years, and an average of 7.7 cigarettes per day were smoked. Notably, smoking rates among Malawians were found to be lower than those in industrialized countries such as Germany, where smoking rates are reported to be 24% total, 27% in males, and 21% in females [73]. In data collected by Kirigia et. al. [67] in 2015, 26% of Malawian males and 4% of females were said to be smokers, compared to a global smoking population of 36% in male and 8% in female. While nicotine abuse is unarguably a leading cause of preventable ESCC, it is unlikely to be the sole cause of the increased case count in Malawi.

Not only tobacco smoking but also exposure to smoke from burning trash as well as from the kitchen stoves were shown to be strongly associated with ESCC and increased the risk about three times compared to those who are not exposed. Individuals who are exposed to both types of smoke have increased odds of ESCC of 3.7 compared to those who claim not to be exposed to either, suggesting a dose-ratio relationship. Open fires cause the

emission of combustion particles such as polycyclic aromatic hydrocarbons (PAHs), benzene, nitrosamines, and 1,3-butadiene, as well as other compounds [33][34][74] which are known risk factors for ESCC [15; 35][36]. A clinical relevance was therefore also confirmed in our study.

Roughly two-thirds of Malawians have their kitchen located inside of the building which often is poorly ventilated [32]. While it could be postulated that a kitchen located inside of the house may lead to greater indoor air pollution, the location inside or outside the living area did not show to be statistically significant in this study. The reasons for the failure to show a relationship may lie in the nature of exposition. Not all individuals with indoor kitchens are necessarily exposed to smoke, as they may not be the ones doing the cooking. Similarly, this same reasoning can be applied to the practice of trash burning, as exposure to smoke from burning trash may depend on whether an individual disposes of their trash in this manner or not.

The exposure of combustion particles such as PAH, benzene, and BaP, is also influenced by the means of cooking. Burning wood causes higher levels of particulate matter than charcoal burning or the use of electric stoves [34]. Health effects such as respiratory symptoms have been shown to be increased among wood stove users in other studies [37]. In this study, however, no significant risk for ESCC was demonstrated between wood-cooking and charcoal-cooking. Other studies are coherent with our findings, indicating an increased risk of laryngeal cancers and head and neck cancers also among those who always used coal [38]. While this study does not depict explicitly the risk of ESCC, it nevertheless demonstrates the carcinogenic potential of charcoal just as much as that of wood burning. In summary with our findings of the exposition of smoke, it may be safe to presume a potential risk for the use of both types of organic fuels.

The use of clean fuels such as electric stoves was negligible in our analysis. Only two individuals in the cases and three individuals in the control group claimed the use of electric energy for cooking. A comparison to dirty fuels was therefore not possible.

Furthermore, the temperature of ingested drinks and foods is postulated to influence the risk of ESCC. High-temperature cooking, broiling, and boiling, were associated with esophageal carcinoma in prior studies [15; 42; 43; 75]. Yet, other influencing factors have

to be taken into account by investigating the risk of hot foods and beverages. Not thermal damage alone, but also chemical constituents, which are generated by high temperatures, are said to induce malignancies [15; 43] . This is the case of cooking food by direct heat or in the production of tea during the drying process [15; 43] .

In our study, only the temperature at which foods and the frequency at which hot beverages were consumed, were considered. Both hot food and hot beverages were found to more than double the risk of ESCC. The temperature of hot food was indirectly measured by evaluating the time at which consumption takes place after heating. Questioning was performed in this manner for practical reasons. The exact numeric temperature at which an increased risk arises could therefore not be evaluated. Nevertheless, the findings suggest a higher risk of cancer due to thermal damage since chemical constituents are not expected to change during the cooling process. It is worth noting that we did not explore the impact of food preparation methods on cancer risk; however, previous literature suggests that such differences may exist and warrant further investigation [15] .

In the case of hot beverages, it was found that the risk of contracting cancer roughly doubles for individuals who consume hot beverages daily (Figure 9). Similar findings have recently been presented, where time of cooling and speed of consumption were also considered and found to increase the risk of ESCC significantly [76] .

The consumption of spicy food on the other hand was not shown to be an underlying risk factor in our setting. This was expected since spicy food was not observed to be a fundamental part of the average Malawian diet. 66% of the cases and 62% of the controls claimed to never eat spicy food. However, this shall not exclude spicy food as a risk factor in other settings where it may be consumed in a higher dosage or more frequently. A cause-relationship is still controversially discussed. A recent meta-analysis identified spicy food as a risk factor for cancer, yet not for cancer of the esophagus explicitly, due to limited numbers of such studies [53] .

Other dietary factors may play a greater role. Maize and maize-based products are a staple food in sub-saharan Africa, as it is observed in Malawi [7] , where n`sima, a maize porridge, is consumed daily. Carcinogenic mycotoxins are suspected to raise the ESCC

risk in the population due to cytotoxicity and alterations in cytokine expression [59; 63] . The extent of this contamination is dependent on agricultural location, local climate, susceptibility of the plants to fungal invasion, and crop stress [64], as well as during steps of processing of the maize to flour and other maize based products [59] .

In our investigation, we were able to show a three times lower risk of falling ill of ESCC when predominantly m´gaiwa, an unprocessed maize flour, was consumed instead of white (woyera), highly processed, flour. The risk was also significantly lower if m´gaiwa was included in the diet apart from white flour. An increased risk for the consumption of white flour could not be determined due to a too small study population for the widely practiced consumption. 77% of individuals in the study group, and 76% of the control group claimed to consume white flour.

M´gaiwa is solely milled. Woyera, however, is dehulled and winnowed. Then soaked, washed, and either wet milled and dried, or dried and then milled. Previous studies have provided evidence that during the refining process, a significant reduction of mycotoxin could be achieved [77; 78] . Provided this information, an increased risk of ESCC was expected by the consumption of unprocessed maize flour. Other studies, however, were in coherence with our findings, showing that white maize flour has a stronger association with ESCC than m'gaiwa [10] . This strongly suggests another mechanism of risk increase after processing maize flour. It has been hypothesized that the lower fiber content in the white maize flour compared to m'gaiwa may be associated with an increased ESCC risk [10], based on previous studies, showing that intake of fiber from whole grains is associated with risk reduction for cancers of the aerodigestive tract[79] , as well as to bind heavy metals and other toxicants like heterocyclic amines [80]. Yet, other factors of risk modification are possible. Factors such as secondary infection of fungi in white flour due to a potentially better growing condition after refining or unsuitable flour storing conditions. Furthermore, the effect on health due to crop contamination by toxic metals or acrylamide, as it was shown to affect health in other studies [81], is not well investigated under the given setting in Malawi.

Growing one's own maize, on the other hand, was not associated with esophageal cancer. This was contradicting previous findings, where a moderate risk increase was determined for home-grown maize [10] . However, the here collected data does not show enough

power to exclude the possibility of correlation. Most individuals of both the study group (82%), as well as the control group (87%) claimed to grow at least parts of their own maize. A larger study population would be required to exclude or show a clear relationship between home-grown maize and the development of ESCC.

Consumption of highly concentrated alcohol, or the consumption in high amounts is a well-known risk factor for ESCC development [12-16] . This finding could not be replicated by our study. Our study group did not report a regular consumption of high amounts of alcohol. A possible explanation may be that alcohol may simply not play the leading role in the high carcinoma incidence in the setting in Malawi. Nevertheless, reporting bias could also exist if individuals underreport their consumption when filling out the questionnaire in the presence of family members. A closer follow-up on alcohol consumption in a longitudinal study would be needed to determine a more precise risk in Malawi. Particularly to examine the suspected risk increase by illegally distilled alcohol such as kachasu, where no controls and regulations take place. Especially for individuals who lack the efficient elimination of acetaldehyde, the carcinogenic metabolite of ethanol.

Genetics determine the polymorphism of aldehyde dehydrogenase 2 (ALDH2) and therefore the development of cancer even in low alcohol blood levels [24] . Data on risk modulating genetic polymorphisms is scarce, particularly for squamous cell carcinoma in African settings. Yet, studies performed elsewhere demonstrate a relationship between environmental factors and cancer genes in the susceptibility to quire ESCC [82]. In order to investigate the possibility of a familial predisposition in our setting, the participants were questioned about the knowledge of relatives suffering from esophageal cancer. Here, a significant familial accumulation could not be determined but clinical relevance remains possible.

Furthermore, individuals were questioned about cases of esophagus carcinoma in their neighborhood or village to get an idea about local accumulation. However, location did not significantly contribute to risk modification. The ESCC cases have not been aware of more cases in their neighborhood than controls. Also in the distribution around the catchment area of the hospital, a suspicious geographic accumulation of ESCC cases could not be observed. To this day no cancer register for ESCC has been established for the whole of Malawi which includes an investigation of geographic accumulation. Even though many

cases are suspected to remain unreported to hospitals or governmental officials, the establishment of a cancer registrar may be able to contribute to locating groups of higher prevalence in the long run.

Some other, previously suspected risk factors for ESCC, such as viral infections, were not included in our investigation. While viral infections such as HIV and HPV have been named to potentially modify the risk of cancer, existing data have not been able to establish a significant cause-relation [10; 82-85] . Our data displays no difference in long-term medication intake between the case and control population. The long-term mediation that was taken in both groups consisted almost exclusively of antiretroviral treatment (ART). While HIV testing was not included in our study, the observed ART intake in both groups indicate no influence of HIV infection on ESCC. In the synopsis of our observations, the available data in other settings, and the cost and ethical considerations of serological testing, it was decided to exclude HIV and HPV infection from further investigation.

A clear advantage of our study in comparison to previously performed studies in Malawi, lies in the effort in selecting a control group that closely fits the study population. A control population was obtained by selecting patients from orthopedic wards who had not shown symptoms of esophagus carcinoma, lived in the same catchment area, and were typically of equal socioeconomic status to the case population. The consultation of these patients due to accidents and fractures was assumed to be random. Controls were matched by age, sex, and socioeconomic status by study design, as these factors were suspected possible confounders that do not contribute to preventable risk. The success of this match was then statistically tested. We achieved an equal proportion of both sexes in the study group and control group. Age was matched within the sex category to achieve a close age match of women and men individually. Statistical analysis showed homogeneity of the groups in terms of age. Income is a suitable indicator for socioeconomic status, which affects life-health [86] and should therefore be controlled for. Income did not show a significant deviation.

A notable limitation of this study is the missing standardization of the questionnaire due to missing resources and logistical factors. Even though a short trail of comprehensibility of the questionnaire and a subsequent adjustment of the questions was performed, proper

standardization of the questionnaire may have improved data quality. Nevertheless, questions were held simple to ensure the yield of useful results.

Another limitation included endoscopic equipment that was suboptimal. Many forceps and wires were unable to obtain proper samples due to excessive use and signs of damage. The cost of acquiring new equipment is costly and presents a financial burden that often results in funds being allocated for other equipment and departments. Many times, the facility used antiquated endoscopes, including colonoscopes, that were either donated or borrowed from other facilities making tissue difficult to visualize and biopsies difficult to obtain. Without proper equipment, the visualization of abnormal esophageal mucosa and tissue collection was a challenge and sometimes impossible.

Not all cases could be histologically confirmed. In some cases patients rejected the offer of histologic confirmation, in others, the sample amount was insufficient. In the case of insufficient sample product or loss of sample, re-sampling was not possible due to anonymization of data and loss to follow-up in most cases. In these instances, only endoscopic visualization of the tumor, barium swallow, and clinical picture were used for diagnosis. While this method is generally accepted for diagnosis, there remains a possibility of overlooking patients with esophageal adenocarcinoma. However, given the comparative rarity of adenocarcinoma in contrast to ESCC, and the high rate of successful histological confirmations, any potential distortion of results is likely to be minimal, if present at all.

Performing research in low-developed countries in Africa can also present additional limitations. These include limited access to funding, technology, and resources which have impacted the quality and completeness of histologic confirmation of ESCC. Moreover, cultural and language barriers have presented difficulties in communicating the goals and benefits of this study to patients and staff. Despite these challenges, it is important to recognize the value of research in low developed countries in Africa and to work towards overcoming these barriers in order to improve local healthcare, economic growth, and other areas.

Due to time and resources only a selection of the most relevant risk factors could be considered. In particular, the intake of fresh fruit and vegetables presents an interesting influencing factor for ESCC in literature elsewhere, worth more attention.

Since this investigation is designed to be an explorative Case-Control Study, which is based on observations, causality cannot be determined unequivocally. The results should be interpreted as potential clinically relevant life habits in the Malawian population that may raise the country´s burden of ESCC.

Conclusion

Socioeconomic factors leave Malawi vulnerable to raging esophageal squamous cell carcinoma with one of the highest incidences worldwide. Despite approaches to improve diagnosis, prevention, and treatment, no detention has been achieved in recent years. Greater effort is desirable to comprehensively understand the major risk factors for further developments to alleviate this burden.

The here presented data supports the hypothesis of a multifactorial genesis of preventable risk factors, including environmental exposures. Our study suggests that particularly high smoke exposition of wood- and coal-based cooking, trash burning, and tobacco smoking contributes to a risk increase of ESCC in Malawi. The implementation of governmental regulations, a proper trash disposal organization, as well as advancements in a comprehensive electrical energy supply could help decrease case numbers.

Furthermore, the consumption of maize flour seems to influence ESCC genesis. The preference for m´gaiwa instead of white maize flower to prepare n´sima was shown to decrease the risk of illness. The driving reasons behind this effect are not sufficiently investigated. High emphasis should be given to further investigations of a correlation with possible mycotoxin contamination. Yet, further contamination of toxins, such as toxic metals or acrylamide may also play a role.

Further investigations, including meta-analysis and longitudinal experimental trials will be critical to confirm relationships of environmental factors and indicate their absolute risk contribution. Such information could facilitate the development of tailored intervention strategies for individuals with elevated predisposition to ESCC.

Appendix A - Declaration of Interests

The author declares that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix B – Funding Source

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Appendix C – Ethics Committee

The study was approved by the University of Oldenburg Ethics Committee (2019-051), Germany, and the National Health Science Research Committee, Malawi (#20/07/2620).

Appendix D – Affidavit Declaration

The dissertation was produced independently and without unauthorized external assistance, that is, it was created without the use of any other than the specified aids and any thoughts taken from external sources, directly or indirectly, were identified as such.

The content of the dissertation has not already been predominantly used for a previous bachelor's, master's, diploma, or similar examination performance.

The regulations for good scientific practice at the Carl von Ossietzky University Oldenburg were followed.

In connection with the doctoral project, no mediation or advisory services (doctoral consultation) were used.

Appendix E – Preprint

A Preprint of an earlier version was presented under
https://papers.ssrn.com/sol3/papers.cfm?abstract_id=4181699

Appendix F – Acknowledgements

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