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DOUTORAMENTO
CIÊNCIAS BIOMÉDICAS

Esophageal cancer in Mozambique. Disease characterization for the definition of a proficient action program.

Jotamo Come

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INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR

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UNIVERSIDADE DO PORTO

JOTAMO COME

Esophageal cancer in Mozambique. Disease characterization for the definition of a proficient action program

Tese de Candidatura ao grau de Doutor em Ciências Médicas submetida ao Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto

Orientador:
Professora Carla Carrilho
Professora Catedrática da Faculdade de Medicina da Universidade Eduardo Mondlane

Coorientadores:
Doutor Lúcio Lara Santos
Professor Afiliado com Agregação do Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto

Doutor Jorge Nunes dos Santos
Professor Associado do Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto

Porto, 30 de Outubro de 2020

As opiniões expressas no presente trabalho de investigação são da exclusiva responsabilidade do seu autor.

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O que não vale a pena, descartamos.
O que for verdadeiro, abraçamos.
A árvore começa a dar frutos.
O nada torna-se tudo.
E depois de nos sentirmos tristes, sós e cabisbaixos, passamos a entender que, "é no fundo do poço que o tesouro se encontra raso"

Autor desconhecido

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LIST OF ABBREVIATIONS AND ACRONYMS

ADC	Adenocarcinoma
ALDH2	Aldehyde dehydrogenase 2 family genes
AECC	African Esophageal Cancer Corridor
AfrECC	African Esophageal Cancer Consortium
AIDS	Acquired Immuno-Deficiency Syndrome
AJCC	American Joint Committee on Cancer
ASIR	Age-Standardized Incidence Rate
BAM	Binary Alignment Map
BE	Barret´s Esophagus
CA19-9	Carbohydrate antigen 19-9
COSMIC	Catalogue of Somatic Mutations in Cancer
CT	Computed Tomography
DNA	Desoxirribonucleic Acid
DNTs	Doenças Não Transmissíveis
EC	Esophageal Cancer
EEU	Endoscopic Ultrasound
EGJ	Esophagogastric Junction
ESCC	Esophageal Squamous Cells Carcinoma
FFPE	Formalin-fixed paraffin-embedded
GERD	Gastroesophageal Reflux Disease
GLUT-1	Glucose transporter-1
GWAS	Genome wide association studies
HIF-1	Hypoxia inducible factor-1
HLA 2	Human leukocyte antigen Class II
HPV	Human Papilloma Virus
IARC	International Agency for Research on Cancer
IPO	Instituto Português de Oncologia
ISP	Ion Sphere Particles

LOX	Lysyl oxidase
LOXL4	Lysyl oxidase-like 4
MCH	Maputo Central Hospital
MRI	Magnetic Resonance Imaging
NCCP	Nacional Cancer Control Program
NGS	Next generation sequencing
NCDs	Non Communicable Diseases
NCI	National Cancer Institute
OMS	Organização Mundial da Saúde
PCR	Polymerase chain reaction
PET	Positron Emission Tomography
PAH	Polycyclic aromatic hydrocarbon
QC	Quality Control
SCC	Squamous Cell Carcinoma
SNPs	Single nucleotide polymorphisms
SNVs	Single Nucleotide Variants
TVC	Torrent Variant caller
SAM	Sequence Alignment Map
WES	Whole exome sequencing
WHO	World Health Organization
VCF	Variant Call Format

*The abreviations included in the originals research have their references in the respectives articles

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RESUMO

Introdução e Objetivos: O cancro do esófago é uma doença heterogénea e complexa, sendo atualmente classificada como um dos cancros mais mortíferos, com um prognóstico desfavorável particularmente na região da África Austral onde Moçambique se localiza. É o sexto cancro mais frequente e a oitava causa de mortalidade por cancro em todo o mundo. Dados limitados do Hospital de Referência Nacional em Moçambique, o Hospital Central de Maputo (HCM), mostram que o cancro do esófago pode ser o quarto tumor mais frequente em ambos os géneros e o mais frequente tumor maligno do tubo digestivo. Segundo estudos publicados, os principais fatores de risco são o consumo excessivo de álcool e de tabaco; fatores ambientais foram também identificados como desempenhando um papel importante na patogénese do Cancro do Esófago e na distribuição da sua incidência nas diferentes regiões do mundo.

O propósito principal desta pesquisa é resumido em quatro objetivos específicos, nomeadamente: 1) Identificar os fatores de risco relacionados com a frequência da doença no país; 2) Descrever as características demográficas e clínicas dos pacientes com o cancro do esófago; 3) Identificar os tipos histológicos e alterações moleculares mais comuns do cancro do esófago em Moçambique; 4) Prover informações relevantes que possam contribuir para a definição de ações de prevenção da doença, diagnóstico precoce e a melhoria da prestação de serviços de cuidados oncológicos no país.

Metodologia: Para responder ao primeiro objetivo, realizamos três estudos: a) Cancro do esófago em Moçambique: As micotoxinas devem constituir preocupação?; b) Microbioma do tracto digestivo superior e o carcinoma de células escamosas do esófago: Epidemiologia, patogénese e implicações clínicas em África; c) Fatores de risco do carcinoma de células escamosas do esófago em Moçambique. Para a descrição das características demográficas e clínicas dos pacientes com cancro do esófago, foi realizado um estudo

designado “Perfil clínico e patológico do cancro do esófago em Moçambique: Estudo de pacientes admitidos consecutivamente no Hospital Central de Maputo”. Para o alcance do terceiro objetivo que era tentar conhecer o perfil molecular do carcinoma de células escamosas do cancro do esófago em Moçambique, foram estudadas 27 amostras de tecido tumoral colhidas dos pacientes da mesma série tratados no HCM. No último objetivo pretendia-se recolher e fornecer informação relevante que contribua para definir ações preventivas do cancro do esófago. Para este objetivo, analisámos cuidadosamente as ferramentas académicas de saúde já existentes, tais como o Programa Nacional de Controlo do Cancro de Moçambique, as Directrizes da Organização Mundial da Saúde (OMS) sobre a prevenção do Cancro e o Plano Estratégico Nacional para a Prevenção e Controlo das Doenças não Transmissíveis (DNTs) em Moçambique. Também participámos em co-autoria no recém-aprovado Curriculum Nacional de Formação em Oncologia em Moçambique, instrumento fundamental para o treino de pessoal de saúde em diferentes áreas e níveis de oncologia no País.

Resultados: Os resultados dos estudos realizados demonstraram a premente necessidade de uma melhor compreensão do papel dos diferentes fatores de risco que podem ser encontrados em Moçambique. Além do tabaco e do álcool, o papel de outros fatores de risco como os relacionados com a alimentação ou estilo de vida, como micotoxinas frequentes no milho, o fumo da cozinha de lenha ou no carvão devem merecer particular atenção em futuros estudos. O carcinoma de células escamosas do esófago foi o tipo histológico mais comum encontrado na amostra.

Recomendações para a prevenção e a deteção precoces desta neoplasia maligna foram descritas na presente investigação.

Conclusões: O Cancro do Esófago é um dos cancros mais comuns em Moçambique, e prevê-se que o número de casos seja mais do que o dobro como resultado da transição demográfica e epidemiológica. O seu diagnóstico tardio deve ser considerado como o principal motivo de muitas perdas de vidas. Os fatores de risco modificáveis associados a este cancro já identificados em populações africanas, também estão presentes no país. Um programa de prevenção e deteção precoce deve ser urgentemente ativado.

ABSTRACT

Introduction and Objectives: Esophageal cancer is a heterogenic and complex disease, currently being ranked as one of the deadliest cancers, with a dismal prognosis particularly in Southern Africa region were Mozambique is located. It is the sixth most incident cancer in the world and the eighth leading cause of mortality related to cancer worldwide. A limited data from the National Reference Hospital in Mozambique, Maputo Central Hospital (MCH), shows that esophageal cancer may be the fourth most common tumour in both genders and the most frequent tumour of the digestive tube. Pursuant academic studies, the main and well established risk factors are tobacco and alcohol abuse but environmental factors have also been identified playing important role on the esophageal cancer pathogenesis and their incidence worldwide distribution.

The main purpose of this research is summarized in four objectives: 1) Identify the risk factors potentially related to the frequency of disease in the country; 2) Describe the demographic and clinical characteristics of patients with esophageal cancer; 3) Identify the most common histological types and molecular changes in the studied tumours; 4) Provide relevant information that can contribute to primary prevention actions, the promotion of an early diagnosis and improving the oncologic care services in the country.

Methodology: To provide a core response to the first objective, we carried out three studies: a) Esophageal cancer in Mozambique: should mycotoxins be a concern? b) The upper digestive tract microbiome and esophageal squamous cell carcinoma: Epidemiology, pathogenesis and clinical implications in Africa. c) Risk factors for esophageal squamous cell carcinoma in Mozambique. Aiming at describing the demographic and clinical characteristic of esophageal cancer patients, a study entitled “Clinical and pathologic profiles of esophageal cancer in Mozambique: a study of consecutive patients admitted to Maputo Central Hospital” was carried out. For the third objective we intended to describe the molecular profile of squamous cell carcinoma of esophagus in

Mozambique, so we studied 27 samples of tumor tissue colleted from patients of our series admitted and treated consecutively at MCH. The last objective was to collectand provide relevant information that can be used to define preventive actions of esophageal cancer. To achieve this goal we carefully reviewed existent health academic tools such the Mozambican National Cancer Control Program, World Health Organization (WHO) guidelines on cancer prevention, the National Strategic Plan for the prevention and control of Noncommunicable Diseases (NCDs) in Mozambique.

We also have participated, as co-author of the recently approved National Curriculum to Advance Surgical Oncology in Mozambique, fundamental instrument for personnel training at different areas and level of oncology.

Results: the results of our studies demonstrated the sound need of a better understanding of the role of various risk factors that can be found in Mozambique. Out of the tobacco and alcohol, the role of others esophageal cancer risk factors related with food or lifestyle such as microbiome, mycotoxins found in maize and cooking smoke from wood or coal need to be clarified in future studies. Esophageal squamous cells carcinoma was the most common characterizing histologic type found in the sample. Recommendations for prevention and earlier detection are also described.

Conclusions: Esophageal cancer is one of the most common cancers in Mozambique, and the number of cases is predicted to be more than double simply as a result of demographic changes. Its late diagnosis is taken as the main reason of many lives loss. Modifiable risk factors associated with this cancer and identified in African populations are also present in the country. A prevention and early detection program must urgently be set up.

PUBLISHED/SUBMITTED ARTICLES IN RELATION TO THE RESEARCH OF THE DOCTORAL CYCLE

SCIENTIFIC PUBLICATIONS AS FIRST AUTHOR

1. **Come J,** Cambaza E, Ferreira R, da Costa JMC, Carrilho C, Santos LL. **Esophageal cancer in Mozambique: should mycotoxins be a concern?** Pan Afr Med J. 2019;Jul 11;33:187. doi:10.11604/pamj.2019.33.187.18295; PMID: 31565147

2. **Come J**, Pereira JB, Pinto R, Carrilho C, Pereira L, Santos LL. **The upper digestive tract microbiome and oesophageal squamous cell carcinoma: Epidemiology pathogenesis and clinical implications in Africa.** Pathobiology DOI: 10.1159/000511422 . In Press.

3. **Come J,** Castro C, Morais A, Cossa M, Modcoicar P, Tulsidâs S, Cunha L, Lobo V, Morais AG, Cotton S, Lunet N, Carrilho C, Santos LL. **Clinical and Pathologic Profiles of Esophageal Cancer in Mozambique: A Study of Consecutive Patients Admitted to Maputo Central Hospital.** J Glob Oncol. 2018; Nov 4:1-9. doi: 10.1200/JGO.18.00147. PMID: 30398947.

SCIENTIFIC PUBLICATIONS AS CO-AUTHOR

1. Cunha L, Fontes F, **Come J**, Lobo V, Santos LL, Lunet N, Carrilho C. **Risk factors for esophageal squamous cell carcinoma in Mozambique**. Submitted to Eur J Cancer Prev

2. Morais A, **Come J**, Selemane C, Pires G, Tivane A, Cossa M, Tulsidás S, Antunes L, Costa MJ, Sidat M, Martins MDR, Carrilho C, Santos LL. **Understanding the bricks to build better surgical oncology unit at Maputo Central Hospital: prevalent surgical cancers and residents knowledge.** Pan Afr Med J. 2019; Feb 18;32:83. doi:10.11604/pamj.2019.32.83.18126. PMID: 31223374.

3. Morais A, Simão M, Cossa M, **Come J**, Selemane C, Tivane A, Tulsidás S, Lorenzoni C, Rodrigues J, Antunes L, Brito D, Costa MJ, Sidat M, Martins MDRO, Santos LL. **Designing a National Curriculum to Advance Surgical Oncology in Mozambique: A Delphi Consensus Study.** J Surg Educ. 2020; Jul 6:S1931-7204(20)30223-3. doi: 10.1016/j.jsurg.2020.06.030. Epub ahead of print. PMID: 32646814.

4. Antero do Vale Fernandes, Daniel Moreira-Gonçalves, **Jotamo Come**, Nilton Caetano Rosa, Victor Costa, Lygia Vieira Lopes, Paulo Matos da Costa, Lúcio Lara Santos **Prehabilitation program for African sub-Saharan surgical patients is an unmet need** Pan Afr Med J. 2020; Jun doi: 10.11604/pamj.2020.36.62.21203. PMID: 32754289

Note: The data referring to chapter V cannot be cited or published since they are part of an article that will be submitted.

CHAPTER I - INTRODUCTION

1.1 Cancer general concepts and epidemiology

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. It is a heterogenic disease frequently caused by certain changes in the genes that control the way cells function, especially how they grow and divide.The most frequent genes implicated in the carcinogenesis are the growth promoting proto-oncogenes (wich transform into oncogenes when activated), tumor suppressor genes, genes that regulate the apoptosis and genes involved in desoxirribonucleic acid (DNA) repair [1]. Mutations that activate protooncogenes generally cause an important increase in normal functions of the gene product, or sometimes lead to a new function on the mutated gene product. Mutations involving tumor suppressor genes cause a loss-of-function of the affected genes and generally need mutations in both alleles before transformation can occur. One of the most frequent tumor suppressor gene inactivated in this process is P53. Mutations in the apoptosis-regulating genes include gain-of-function mutations in genes whose products suppress apoptosis and loss-of-function mutations in genes whose products promote cell death [1]. Mutations in DNA repair genes lead to a function inhibition of the affected gene and thus contributing to carcinogenesis by impairing the ability of the cell to recognize and repair genetic damage in other genes.

Almost 95% of cancers are acquired and related to the environment or lifestyle, such as tobacco and alcohol consumption, infectious agents, inadequate diet, obesity, reproductive history and other environmental carcinogens [1]. Because that, most of cancers are potencially preventable. Only a very small percentage of cases are related with inherited genetic mutations. Althought most of cancers are of clonal origin, there is important heterogeneity in the tumour cells populations, related with both genetic and epigenetic events. During the tumor

progression in a multistep process, additional mutations are acquired, lead to the production of variant clonal populations with specific group of additional characteristics. It appears that all cancers display eight fundamental changes in cell physiology, which are considered the hallmarks of cancer: (1) self-sufficiency in growth signals; (2) insensitivity to growth-inhibitory signals; (3) evasion of programmed cell death (apoptosis); (4) limitless replicative potential (immortality); (5) sustained angiogenesis; (6) hability to invade and metastasize; (7) altered energy metabolism and (8) hability to evade host immune destruction [1,2].

Cancer is expected to rank as the leading cause of death and the single most important barrier to increasing life expectancy in every country of the world in the 21st century [3]. According to estimates from the World Health Organization (WHO) in 2015, cancer is the first or second leading cause of death before age 70 years in 91 of 172 countries, and it ranks third or fourth in an additional 22 countries [3].The incidence and mortality of cancer in the world are rapidly growing and the reasons are complex. Those can reflect both aging and growth of the population, as well as changes in the prevalence and distribution of the main risk factors for cancer, which are associated with socioeconomic development [3].

Estimative of cancer incidence and mortality produced by the International Agency for Research on Cancer (IARC), indicated that in 2018, there was an estimated 18.1 million new cancer cases and 9.6 million cancer deaths worldwide. The lung (11.6%), female breast (11.6%), prostate (7.1%) and colorectal (6.1%) cancers are the leading cause for incidence, and lung (18%), colorectal (9.2%), stomach (8.2%), and liver (8.2%) cancers for mortality, respectively [3].

Table1 below shows the estimated new cases and deaths for 36 cancers and all cancers combined in 2018 worldwide.

It is noteworthy that high-quality cancer registry data, the basis for planning and implementing evidence-based cancer control programs, are not available in most low- and middle income countries [3].

Table 1. *New Cases and Deaths for 36 Cancers and All Cancers Combined in 2018*

CANCER SITE	NO. OF NEW CASES (% OF ALL SITES)	NO. OF DEATHS (% OF ALL SITES)
Lung	2,093,876 (11.6)	1,761,007 (18.4)
Breast	2,088,849 (11.6)	626,679 (6.6)
Prostate	1,276,106 (7.1)	358,989 (3.8)
Colon	1,096,601 (6.1)	551,269 (5.8)
Nonmelanoma of skin	1,042,056 (5.8)	65,155 (0.7)
Stomach	1,033,701 (5.7)	782,685 (8.2)
Liver	841,080 (4.7)	781,631 (8.2)
Rectum	704,376 (3.9)	310,394 (3.2)
Esophagus	572,034 (3.2)	508,585 (5.3)
Cervix uteri	569,847 (3.2)	311,365 (3.3)
Thyroid	567,233 (3.1)	41,071 (0.4)
Bladder	549,393 (3.0)	199,922 (2.1)
Non-Hodgkin lymphoma	509,590 (2.8)	248,724 (2.6)
Pancreas	458,918 (2.5)	432,242 (4.5)
Leukemia	437,033 (2.4)	309,006 (3.2)
Kidney	403,262 (2.2)	175,098 (1.8)
Corpus uteri	382,069 (2.1)	89,929 (0.9)
Lip, oral cavity	354,864 (2.0)	177,384 (1.9)
Brain, nervous system	296,851 (1.6)	241,037 (2.5)
Ovary	295,414 (1.6)	184,799 (1.9)
Melanoma of skin	287,723 (1.6)	60,712 (0.6)
Gallbladder	219,420 (1.2)	165,087 (1.7)
Larynx	177,422 (1.0)	94,771 (1.0)
Multiple myeloma	159,985 (0.9)	106,105 (1.1)
Nasopharynx	129,079 (0.7)	72,987 (0.8)
Oropharynx	92,887 (0.5)	51,005 (0.5)
Hypopharynx	80,608 (0.4)	34,984 (0.4)
Hodgkin lymphoma	79,990 (0.4)	26,167 (0.3)
Testis	71,105 (0.4)	9,507 (0.1)

Salivary glands	52,799 (0.3)	22,176 (0.2)
Anus	48,541 (0.3)	19,129 (0.2)
Vulva	44,235 (0.2)	15,222 (0.2)
Kaposi sarcoma	41,799 (0.2)	19,902 (0.2)
Penis	34,475 (0.2)	15,138 (0.2%)
Mesothelioma	30,443 (0.2)	25,576 (0.3)
Vagina	17,600 (0.1)	8,062 (0.1)
All sites excluding skin	17,036,901	9,489,872
All sites	**18,078,957**	**9,555,027**

Adapted from Bray et al, 2018 [3]

1.2 Esophageal cancer in the world with emphasis in sub-Saharan Africa

Esophageal cancer (EC) ranks seventh in terms of incidence and sixth in mortality of all cancers. In 2018 it was estimated the occurrence of 572.000 new cases of EC (corresponding to 3.2% of all cancer in both sexes) and 509.000 deaths (5.4% of all cancer deaths) in the world.The distribution and frequency vary greatly in different regions of the world, with 80% of cases occurring in less developed countries [3]. Remarkably high rates of incidence are found in Southern and Eastern of Africa and Eastern Asia, where the age-standardized incidence rates (ASIR) were 3.1 to 4.1 and above per 100.000 inhabitants in 2018 (*Figure 1*) [3,4]. A third area with higher incidence was described around Uruguay in South America [5,6]. Highest incidence rates in the world are observed in the Asian EC belt, stretching from China and Mongolia (ASIR of 13.9 and 18.5, respectively) to Iran (ASIR of 6.0). In contrast, North America, most of the regions of Europe, Oceania, North Africa and Western Asia have lower incidence rates (less than 3.8 and 1.7 new cases per 100,000 in men and women, respectively) [3]. Approximately 70% of cases occur in men, and there is a 2-fold to 3-fold difference in incidence and mortality rates between the sexes worldwide [3] .

In Africa, it was estimated 28.494 new cases and 27.703 deaths of EC occurring in 2018. An African esophageal squamous cell carcinoma (ESCC) corridor was also described, stretching from South Sudan (ASIR of 7.6) to the

Eastern Cape Province of South Africa (ASIR of 7.8) [4]. EC is common in several Eastern and Southern African countries, acting as the leading cause of cancer mortality in Kenyan men (2390 deaths), whereas Malawi exhibits the highest incidence rates globally in both sexes (18.7 new cases per 100.000 inhabitants).

Currently, esophageal cancer is detected in very advanced stages with poor prognosis and higher mortality, particularly in countries with low economic income mostly in Africa and Asia. The age standardized mortality rate in this regions were estimated to be 3.9 and above for all ages and sexes per 100.000 inhabitants in 2018 *(Figure 2)* [4].

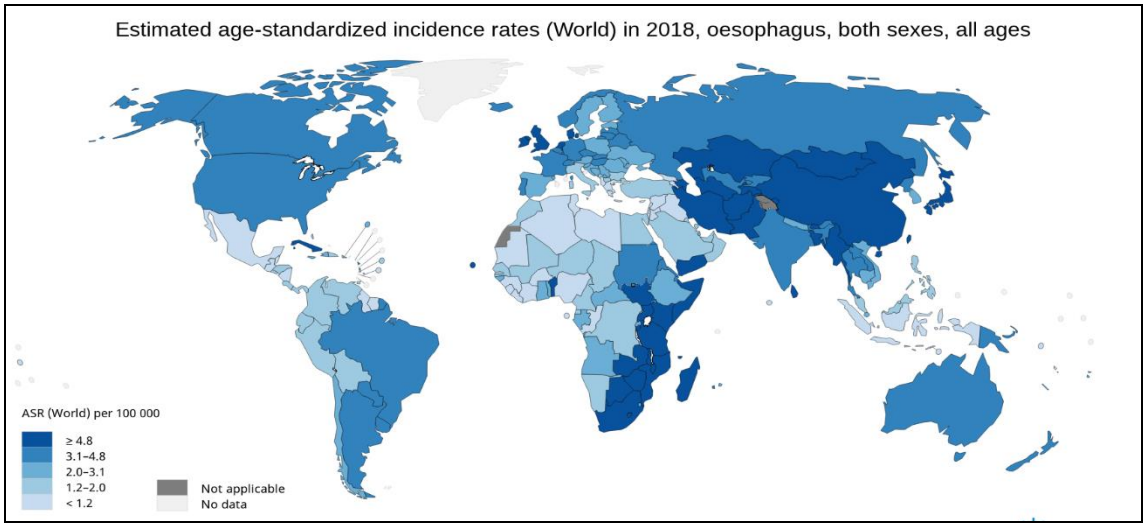

Figure 1. Estimated age-standardized incidence rates (world) in 2018, oesophagus, both sexes, all ages [4]

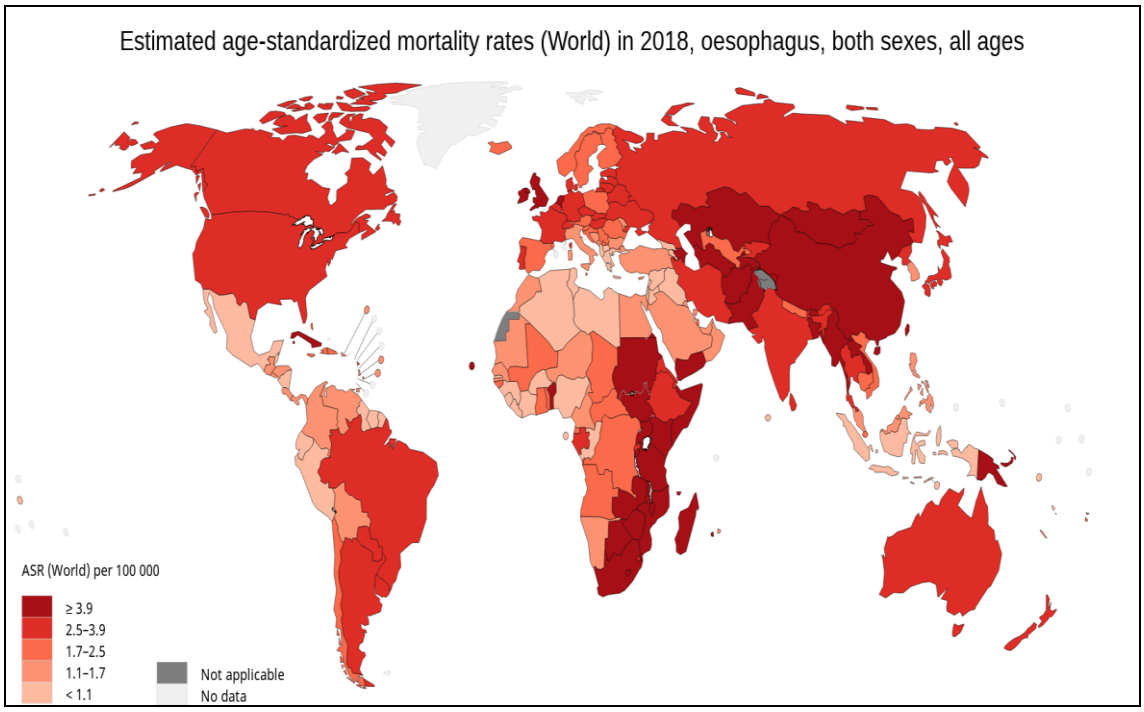

Figure 2. Estimated age-standardized mortality rates (world) in 2018, oesophagus, both sexe, all ages [4]

The high incidence rates of esophageal cancer, particularly the squamous cell carcinoma subtype, in Central, Southern, and East Africa, together with diagnosis at advanced stages, lead to high mortality rates [7,8,9].The reasons underlying the high frequency of this malignancy in Sub-Saharan Africa remain largely unknown, and investigations to evaluate potential etiologic effects of dietary, lifestyle, environmental, and other factors affecting incidence in this region, including genetics, are needed [10,11]. Liu et al (2016) demonstrated distinct subtypes of esophageal squamous cell carcinoma in sub-Saharan Africa and suggested that the endemic nature of this disease reflects exposure to other carcinogen than tobacco or oncogenic viruses [12].

1.3 Esophageal cancer in Mozambique

Mozambique is a country located in Southeast Africa bordered by the Indian Ocean to the east, Tanzania to the north, Malawi and Zambia to the Northwest, Zimbabwe to the west, and Swaziland and South Africa to the southwest *(Figure 4).*

Figure 3. Republic of Mozambique map

The country's population of around 30 million is composed mostly by Bantu people.The gender ratio (male to female) was 0.95. The population density in Mozambique is 39 per Km^2. Near 37.8% of the population is urban and the median age in Mozambique is 17.2 years [13]. Mozambique is one of the poorest and most underdeveloped countries in the world [14]. However, this country has reduced poverty in all forms measured in the last 15 years. Recent poverty assessments all show a decline in monetary poverty rates from 60.3% to 48.4%, reduction in multi-dimensional poverty rates from 92.8% to 71% and a reduction in poverty using the international poverty line from 78.5% to 62.9% [15]. According to the World Bank, Mozambique lives an economic update characterized by less poverty, but more inequality. The poverty rate has declined on average by 1% per year, but rural areas continue to lag urban areas. The Gini coefficient, which measures inequality, increased from 0.47 to 0.56 between 2008 and 2014. Mozambique's Gini coefficient has consistently remained above 0.4 even in rural areas, a high level of inequality per regional standard [16].Therefore, rural Mozambique still poor, with poor housing, a factor described in Kenya as an independent predictor of EC [17].

According to the Globocan, it was estimated the occurrence of 25631 new cancer cases and 17813 deaths related to cancer in Mozambique in 2018 (Table 2).

Table 2. Mozambique Cancer statistics 2018, according to Globocan 2018

	Males	**Females**
Number of new cancer cases	11 227	14 404
Age-standardized incidence rate (World)	127.5	135.0
Number of cancer deaths	7 704	10 109
Age-standardized mortality rate (World)	93.2	102.0
Most frequent cancers except non-melanoma skin cancer (% in relation to all cancers)	Kaposi sarcoma (36.6%) Prostate (14.4%) Liver (6.0%) Non-Hodgkin lymphoma (5.1%) Esophagus (4.1%)	Cervix uteri (29.8%) Kaposi sarcoma (15.4%) Breast (9.5%) Esophagus (4.1%) Liver (3.6%)

Adapted from Globocan 2018 [4].

Esophageal cancer was of the fourth most frequent cancer and fifth cause of cancer-related deaths in the country, responsible for 597 and 459 new cases, respectively in women and men (Figure 4) and 877 deaths in 2018 [3].

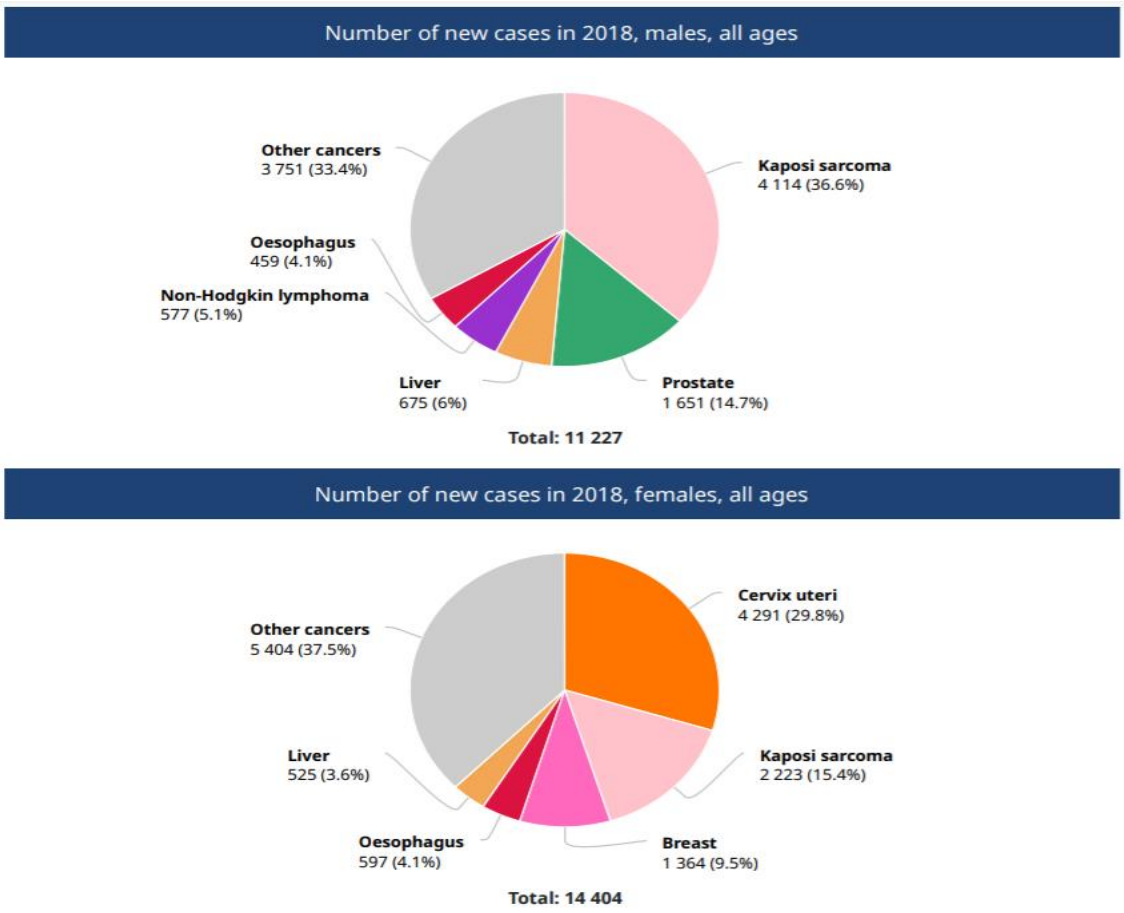

Figure 4. Number of new cancer cases in Mozambique, by sexes, all ages, in 2018 (Adapted from Globocan 2018).

Data from Maputo Central Hospital (MCH) indicated an increasing number of esophageal cancer cases between 2015-2016 in Maputo city. In this period, esophageal cancer was de fifth most commom cancer in both sexes *(Figure 5)*. Regarding the variation in ASIR between 1956-1961 and 2015-2016 in Maputo city, esophageal cancer increased from 4.4 to 7.0 among men and from 0.0 to 9.4 among women [18].

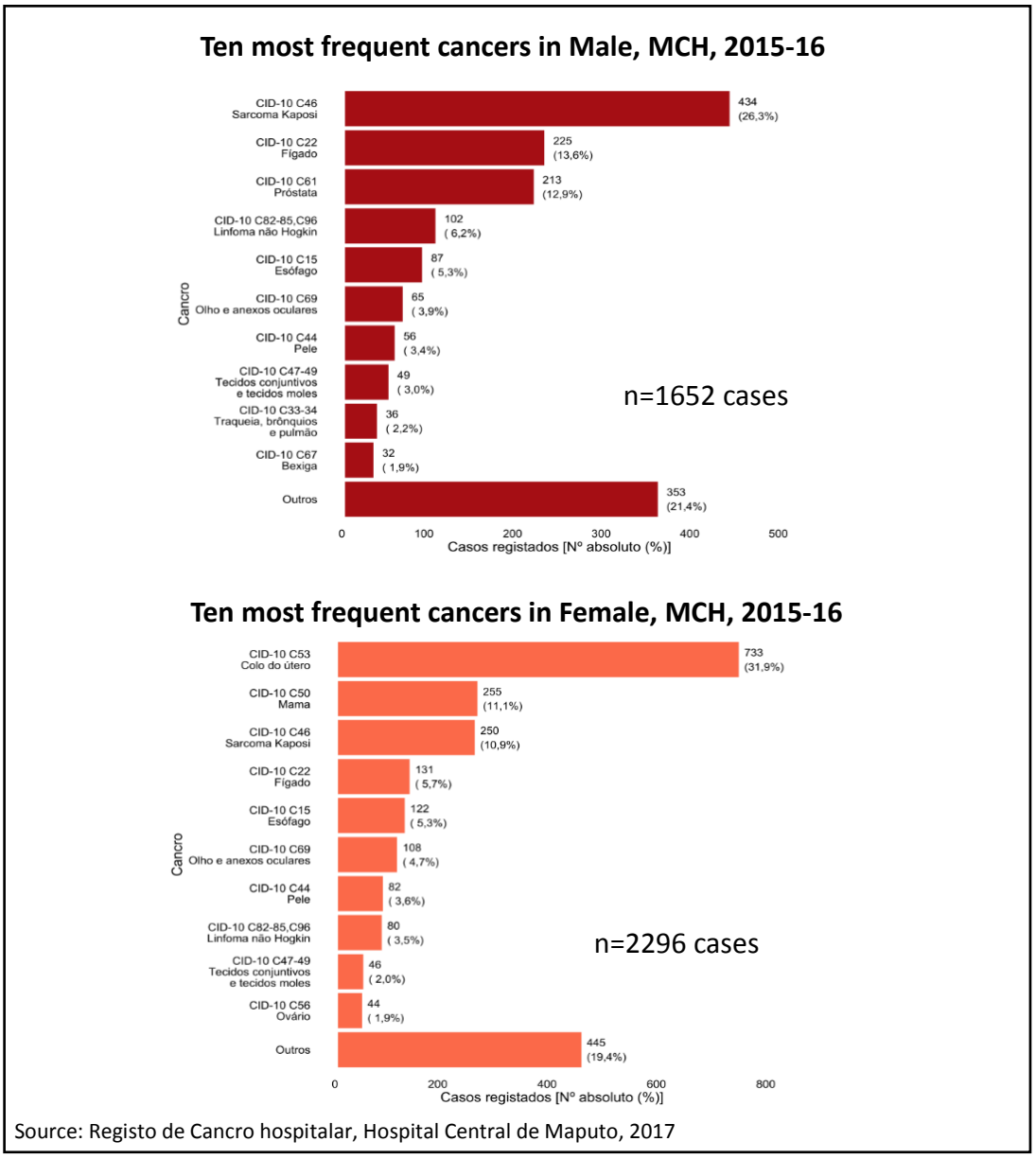

Figure 5. Ten most frequent cancers by sexes, MCH, 2015-2016 [19]

Data from population-based registry from two Mozambican cities, namely, Maputo and Beira cities in 2015-2017, demonstrated higher cumulative risk for esophageal cancer comparing with north and west African regions and US black populations.The cumulative risk of EC was 0,94 in Beira and 1,0 in Maputo (Figure 6) [20].

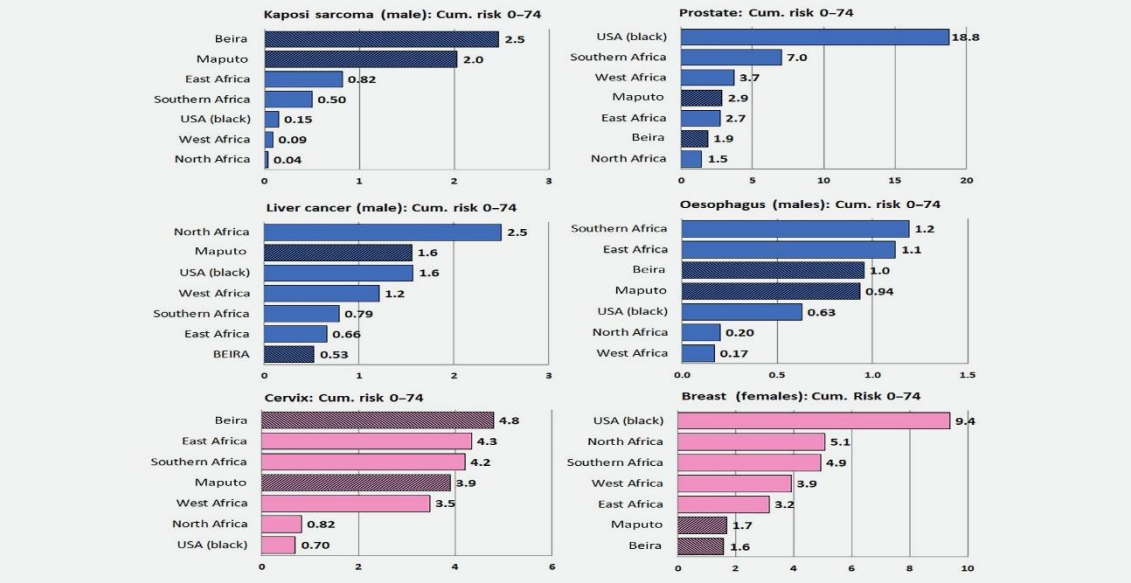

Figure 6. Cumulative risk of some of the common cancers in males and females, in Maputo (2015–2017) and Beira (2015-2017) compared to the other regions. Adapted from Lorenzoni et al, 2020 [20].

1.4 Etiology, Pathogenesis and Pathology

There are two dominant histologic types of EC: esophageal squamous cell carcinoma (ESCC), accounting to 90% of all EC, and esophageal adenocarcinoma. The etiology of ESCC is multi-factorial and is population dependent. It has been well-established that alcohol is a Group 1 carcinogen and a recognized cause of ESCC [21]. In low and medium incidence populations, ESCC is largely attributable to alcohol and tobacco consumption and low consumption of fruit and vegetables [22]. Alcohol consumption was also found to be significantly associated with EC in some regions of Africa [23]. For example, in some regions of South Africa, excessive consumption of alcohol and tobacco are related to about 90% of cases of ESCC [10].

In addition to tobacco and alcohol, many other risk factors were identified. Evidence has been presented for the apparent association between the occurrence of cancer of esophagus in Africa and the use of maize as an ingredient of beer [10,24,25]. Dietary carcinogens and insufficiencies of micronutrients were also found to be important risk factors of ESCC [26]. Consumption of beverages and food at high temperatures, which causes

thermal damage to the esophageal epithelium, has been documented to increase the risk of ESCC, mainly in China, Brazil, Iran and Tanzania [27,28,29]. Mate, an herbal tea consumed hot is very popular in South America and has been associated with an increased risk of ESCC [6]. Exposure to Polycyclic aromatic hydrocarbon (PAH) produced during incomplete combustion of organic material, including tobacco, coal, and wood, seem to be another apparent risk factor for ESCC [6]. In poor-income countries, just like Mozambique, other environmental factors such as micronutrient deficiencies (p.e. selenium), poor oral hygiene, dental fluorosis and/or mycotoxins may explain the epidemiologic pattern observed [30,31]. The role of HPV in the development of esophageal carcinoma is still controversial. An association between HPV infection and ESCC has been described in high-risk areas such as Iran, where a frequency of 23.6% was found, with the HPV16 subtypes accounting for 2/3 of HPV positive cases [32]. In other studies and a target-analysis review, no evidence of this relationship was found [33, 34]. Genetics also plays a role in the etiology of ESCC. Persons with specific variants of aldehyde dehydrogenase 2 family genes (ALDH2), have a higher risk of ESCC if alcohol assumption is added and this polymorphism is found mainly in Asian and European population [22].

The incidence of ESCC has decreased in North America and Europe, largely due to concomitant decreases in tobacco and alcohol consumption [35, 36], whereas esophageal adenocarcinoma (ADC) is increasing, which is attributed to the dramatic increase in risk factors in recent decades, namely overweight and obesity [37]. In these cases, there is evidence that the increased incidence of ADC results in an increase in tumours located in the esophagogastric junction (EGJ) [38].

The development of esophageal carcinoma is a progressive multi-stage process that goes from basal cell hyperplasia of the lining epithelium, to dysplasia, carcinoma in situ and invasive carcinoma [39,40]. The risk for development of EC is strongly associated with increasing grades of dysplasia (2.9 for mild dysplasia, 9.8 for moderate dysplasia, 28.3 for severe dysplasia and 34.4 for carcinoma in situ [39]. At the cellular level, this process is associated with changes in the process of controlling cell proliferation and differentiation and

apoptosis. Genetic changes induced by carcinogenic substances in tumor suppressor genes (P53, P27, P16, P21) and in oncogenes (mdm2, cyclin D1 and c-myc transcription factors) have been, in more recent researches, correlated with development and/or the progression of esophageal cancer [41].It is described that about 50% of EC are associated with mutations of the P53 oncossupressor gene [41].

However, Hendricks *et al* (2002) in a study on esophageal carcinoma in Africa reports that South African individuals have a low incidence of mutations in the p53 gene, a condition described as common by other authors [42]. More recent studies attempt to identify possible potential markers of disease aggressiveness that can be used in clinical practice. One example is the expression of the p16 protein, which seems to be associated with a more favorable prognosis [43].

As previously refered, the two main histological types of esophageal carcinoma include squamous cell carcinoma (SCC) and adenocarcinoma [44]. The primary SCC lesions are subtle and may not be detected in a digestive endoscopy performed for other purposes. In an endemic area of China (Linxian), in a series of 31 samples from biopsies obtained in areas of the mucosa considered friable, erythema, erosion, plaques or nodules, 25 showed moderate dysplasia or cancer [45]. The most advanced EC are characterized by infiltrative or ulcerated masses that may be circumferential involving the diameter of the esophageal lumen. The SCC invades submucosa in the initial phase and extends along the muscular wall of the organ, usually in the cephalic direction [46] with the establishment of stenosis. Local lymphatic invasion occurs early due to the location of lymphatics in the sheet itself. Distant metastasis most often affects the liver, bone and lungs [47].

Adenocarcinoma of the esophagus occurs in the distal esophagus approximately three-fourths of the time [48] and has a distinct link to gastroesophageal reflux disease (GERD). Untreated GERD can progress to Barrett's esophagus (BE), where the stratified squamous epithelium that normally lines the esophagus is replaced by a columnar epithelium.The chronic reflux of gastric acid and bile at the gastroesophageal junction and the subsequent damage to the esophagus has been implicated in the pathogenesis of Barrett metaplasia [49]. Most esophageal adenocarcinomas (ADC) are located close to the gastroesophageal junction and are associated with

endoscopic evidence of BE. Esophageal ADC with origin in BE may appear as an ulcer, nodule, alteration of the mucosa or an abnormality not visible on endoscopy [50]. Early adenocarcinoma not associated with BE grows from an ulcer, plaque or nodule near the esophagogastric junction (EGJ). The involvement of the celiac and peri-hepatic lymphatic chains is more common in adenocarcinoma due to the location of the tumor nearby or in the EGJ [50]. Another risk factor for esophageal ADC is obesity, specifically in those individuals with predominantely abdominal centered fat distribution. Hypertrophied adipocytes and inflammatory cells within fat deposits create an environment of low-grade inflammation and promote tumor development through the release of adipokines and cytokines [51]. Adipocytes in the tumor microenvironment supply energy production and support tumor growth and progression [52].

1.5 Clinical presentation

Esophageal carcinoma in general has a similar clinical presentation whether it is an ADC or SCC, except for localization. ADC is preferentially located in the distal portion of the esophagus or in the EGJ. Usually, patients show in an advanced stage of the disease with progressive signs of obstruction of the esophageal lumen causing dysphagia and weight loss [53]. Obstructive signs usually appear when the diameter of the esophagus is less than 13 mm, which indicates an advanced stage of the disease [54]. Dietary changes conditioned by dysphagia, as well as anorexia related to the presence of a tumor, determine progressive weight loss. Early symptoms are subtle and often imperceptible, and can only be reduced to discomfort or a burning sensation in the back. Regurgitation of food or saliva without evidence of gastric contamination occurs in patients with advanced disease. Hoarseness suggests invasion of the recurrent laryngeal nerve and regional lymph nodes. Chronic blood loss from the tumor results in iron deficiency and anemia. The presence of a tracheal bronchial fistula is a very late complication of the disease. Currently, tumors in early stages can be detected during disease screening by endoscopy in patients with BE, most of them are asymptomatic (intramucosal tumors) [45].

1.6 Early detection and Diagnosis

The disease diagnosis is made based on the clinical presentation, radiologic esophagus study, gastric esophagic endoscopy and on the tumor sample histology obtained by biopsy collected during the endoscopy [45]. The recognition that ESCC arises from precursor lesions (squamous dysplasia), gives the possibility of intervention to early detect and manage this lesions in order to prevent tumor development. Endoscopic screening with Lugol´s iodine, in adults without symptoms has been described in China [40], in order to detect esophageal squamous dysplasia. Although this technique may be not possible to be applied as mass screening, especially in low resource settings, it could be used in clinical practice, in a case-finding manner, approaching groups at risk for EC [55].The use of a less invasive method of screening such as sponge cytology, to detect dysplasia and carcinoma among asymptomatic individuals was previously described, and can be considered a relevant tool for screening specially in areas of high risk for EC [22, 56].

1.7 Staging, Treatment, and Prognosis

Aspects of staging assessed with the TNM system developed by the American Joint Committee on Cancer (AJCC) (Table 3) [57] is essential in determining stage-specific protocols. A chest and abdominal computed tomography scan (CT) improves the disease staging and the tumor evaluation. Other more accurate techniques include magnetic Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET) scan and endoscopic ultrasound (EEU). Treatment generally follows criteria defined for each disease stage and includes surgery, chemotherapy and radiotherapy (Table 4). ESCC early detection (or their precancerous lesions) is highly desirable, since surgical and endoscopic resection offers the only possible cure for esophageal cancer [58].

Table 3. TNM system, specifically referring to depth of invasion in T staging [57]

Category	Description
Tis	Carcinoma *in situ*
T1	Tumors invade lamina propria or submucosa
T2	Tumors invade muscularis propria
T3	Tumors invade adventitia
T4	Tumors invade adjacent structures
N0	No regional lymph node metastases
N1	Regional lymph node metastases
M0	No distant metastasis
M1a, M1b	Distant metastasis

Table 4. Treatment of oesophageal cancer at different stages*

Stage	Tumor	Node	Metastasis	Therapeutic options
0	Tis	N0	M0	Local ablative therapy
I	T1	N0	M0	Surgery
IIA	T2	N0	M0	Surgery
	T3	N0	M0	
IIB	T1	N1	M0	Neoadjuvant therapy with or without surgery
	T2	N1	M0	
III	T3	N1	M0	Neoadjuvant therapy with or without surgery
	T4	Any N	M0	
IVA	Any T	Any N	M1a	Chemotherapy or radiation therapy with or without surgery
IVB	Any T	Any N	M1b	Palliative treatment

**Adapted from National Cancer Institute (NCI) protocols [59]*

The disease prognosis is directly related to the stage at the time of diagnosis and the capacity for its early detection. The role of neoadjuvant chemotherapy for the prognosis is unclear [60]. In general, the prognosis and survival of most of patients are poor, which is related to the later detection and advanced stage

at time of diagnosis [60]. The overall 5-year survival rates for esophageal cancer vary from 21% in China to less than 5% in low resource settings [8]. As in other types of cancers, a multidisciplinary approach is also recommended in the esophageal cancer treatment [61]. Early surgical recession improves the survival patients as long as the tumor is detected early before local invasion occurs [58]. Metastases are the main cause of the high mortality [62]. Despite the significant advanced and innovative diagnosis techniques, the esophageal cancer prognosis remains poor [63]. The early detection, before tumor spread is the only way that will condut to the efficient tumor recession and improve patient's survival and quality of life [52]. The advent of endoscopic treatment, such as radiofrequency ablation, cryotherapy, endoscopic mucosal resection and submucosal dissection, for high-grade esophageal squamous dysplasia or early ESCC can be promisor for early detection and cancer prevention in regions of high incidence of EC [64].

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CHAPTER II - AIMS AND SCOPE

Esophageal cancer is a heterogenic and complex disease. This neoplasm is one of the deadliest cancers, with a dismal prognosis. It can be subdivided into two distinct groups: squamous cell carcinoma and adenocarcinoma, based on histological appearance.

The aim of this thesis is to identify the global dimension of esophageal cancer in Mozambique. We intend to identify the associated risk factors. Its clinical, pathological, and molecular profile in order to mitigate its incidence, improve the treatment opportunities, best practices, survival rates and quality of life.

Specific aims are:

1. Identify the risk factors potentially related to the frequency of esophageal cancer in the country;
2. Describe the demographic, clinical and pathological characteristics of patients with esophageal cancer;
3. Identify the most common molecular changes in the studied tumors;
4. Provide information that can contribute to promote prevention actions, early diagnosis and to improve the oncologic care services at MCH and in Mozambique as a whole.

CHAPTER III – RISK FACTORS FOR ESOPHAGEAL CANCER

Risk is the possibility that an event will happen. A cancer risk factor is anything that increases a person's chance of getting cancer. The most common risk factors for cancer include aging, tobacco, sun exposure, radiation exposure, chemicals and other substances, some viruses and bacteria, certain hormones, family history of cancer, alcohol, poor diet, lack of physical activity, or being overweight.

It is important to know the risk factors and talk about them with populations. It will help people make better lifestyle choices to improve health and develop risk mitigation programs.

This chapter focuses on risk factors for esophageal cancer, mainly for ESCC, and is based on three papers that are part of the thesis.

3.1 Esophageal cancer in Mozambique: should mycotoxins be a concern?

Fumonisin B1 (FB1) is a mycotoxin frequently found in agricultural commodities. The toxin poses a considerable risk for human and animal health. FB1 is among several mycotoxins produced by Fusarium spp. contaminating virtually any cereal and other Poaceae. Their intracellular action includes the promotion of oxidative stress through the generation of reactive oxygen species (ROS) that damage biomolecules such as DNA. These toxic effects were observed in vivo and in vitro. However, the association between esophageal lesions and oxidative stress induced by FB1 remain debatable. Studies in China, Iran and South Africa showed higher exposure to fumonisins in areas with higher risk of esophageal cancer (EC). Exposure to mycotoxins may be inevitable in Mozambique. How mycotoxins, particularly fumonisins from the contaminated

food, can be associated with the emergence of EC in Mozambique? This issue is discussed in the following article.

PanAfrican Medical Journal

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Essay

Esophageal cancer in Mozambique: should mycotoxins be a concern?

Jotamo Come[1], Edgar Cambaza[2], Rita Ferreira[3], José Manuel Correia da Costa[4], Carla Carrilho[5], Lúcio Lara Santos[6,7,&]

[1]Department of Surgery, Maputo Central Hospital, Maputo, Mozambique, [2]Department of Biological Sciences, Faculty of Sciences, Eduardo Mondlane University, Maputo, Mozambique, [3]QOPNA-Química Orgânica, Produtos Naturais e Agroalimentares, Departamento de Química, Aveiro University, Aveiro, Portugal, [4]Center for the Study of Animal Science, ICETA, University of Porto and INSA-National Health Institute Dr. Ricardo Jorge, Porto, Portugal, [5]Department of Pathology, Faculty of Medicine, Eduardo Mondlane University, Maputo Central Hospital, Maputo, Mozambique, [6]Experimental Pathology and Therapeutics Research Group, Surgical Oncology Department, Portuguese Oncology Institute, Porto, Portugal, [7]ONCOCIR, Education and Care in Oncology, Lusophone Africa

[&]Corresponding author: Lúcio Lara Santos, Experimental Pathology and Therapeutics Research Group, Surgical Oncology Department, Portuguese Oncology Institute, Porto, Portugal, ONCOCIR, Education and Care in Oncology, Lusophone Africa

Key words: Mycotoxyns, esophageal cancer, Mozambique

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Abstract

Fumonisin B1 (FB1) is a mycotoxin frequently found in agricultural commodities. The toxin poses a considerable risk for human and animal health. FB1 is among several mycotoxins produced by Fusarium spp. contaminating virtually any cereal and other Poaceae. Their intracellular action includes the promotion of oxidative stress through the generation of reactive oxygen species (ROS) that damage biomolecules such as DNA. These toxic effects were observed in vivo and in vitro. However, the association between esophageal lesions and oxidative stress induced by FB1. Studies in China, Iran and South Africa showed higher exposure to fumonisins in areas with higher risk of esophageal cancer (EC). Exposure to mycotoxins may be inevitable in Mozambique. How mycotoxins, particularly fumonisins from the contaminated food, can be associated with the emergence of EC in Mozambique? Herein, we revise the literature and present some pieces of evidence in order to highlight the burden of mycotoxins and to provide evidence-based considerations for the stakeholders involved in the management of the EC agenda in Mozambique. The information presented herein supports the need to implement novel and/or to revisit the existent detoxification methods to reduce the global burden of mycotoxins and its outcomes in health management.

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Essay

Cancer of esophagus is a serious health problem in sub-Saharan Africa and it is associated with high lethality [1]. Carcinogenesis of esophageal cancer (EC) is still largely unknown in sub-Saharan Africa. Several individual factors have been considered but the main cause is most likely multifactorial [2]. EC, mainly squamous cell carcinoma, is highly prevalent in Western Kenya, especially among members of the Kalenjin community, who reside in the northern and southern areas of the rift valley [3]. According to Kigen *et al.* (2017), the most plausible causes of the high incidence of EC among the Kalenjin community are mycotoxins, particularly fumonisins from the food chain resulting from poor handling of cereals [4]. Aflatoxins and fumonisins are mycotoxins contaminating a large fraction of the world's food, including maize, cereals, groundnuts and tree nuts [5]. Contamination is due to high-level chronic exposure [6]. This is particularly true in subsistence farming communities where regulations to control exposure are either non-existent or practically unenforceable [7].

Esophageal cancer in Mozambique

Mozambique has a high rate of EC. According to previous studies, EC is the 4th most incident malignant tumor in Maputo in both genders [8]. A study of 522 consecutive cases of EC diagnosed and treated at the Maputo Central Hospital revealed that most patients were female (n=291, 55.7%), and born in the southern region of the country (n=418, 80.1%) where the consumption of maize in food and in fermented beverages is high [9]. The prognosis is highly undesirable as the median survival time was 3.5 months for all patients [9]. Therefore, it is necessary to implement a nationwide esophageal cancer program in Mozambique encompassing the detection of risk factors and the implementation of early diagnosis programs. Can mycotoxins play a role in the malignant transformation process of the esophagus in Mozambique? Kigen *et al.* (2017) suggest that mycotoxins, particularly fumonisins, combined with traditional alcohol, dietary deficiencies and viral infections acting synergistically are risk factors for EC in the Western Kenya [4]. So, one can suspect of a similar effect in Mozambique once food habits are similar.

Fusarium verticillioides and carcinogenesis

Fusarium verticillioides, a mold that grows mostly on maize, has the ability to produce Fumonisin B1 (FB1). FB1 is a toxic secondary metabolite linked to EC and neural tube defects in humans and lung edema in swine and leukoencephalomalacia in equines [10]. Their intracellular action, favouring oxidative stress and the generation of reactive oxygen species (ROS), sustain their toxic effects observed in vivo [11,12] and in vitro [13,14]. Mycotoxins have a strong tendency and ability to penetrate the human and animal cells and reach the cellular genome where it causes a major mutagenic change in the nucleotide sequence, which leads to strong and permanent defects in the genome (adduct formation targeting guanine bases, which induces G→T transversions at codon 249 in TP53) [15,16]. FB1 might also disrupt sphingolipid metabolism therefore impairing the balance between apoptosis and mitosis [17]. These defects will eventually be transcribed, translated and lead to the development of cancer (Figure 1). FB1 is a known animal carcinogen and has been shown to cause tumors of the liver and kidney in mice and rats [18]. Likewise, chronic dietary exposure to FB1 (≥50 ppm) is carcinogenic to rodents: hepatocarcinogenic in male BD IX rats and female B6C3F1 mice and nephrocarcinogenic in male F344 rats [19,20]. The weight of evidence indicates that the mechanism of carcinogenesis is epigenetic and related to compensatory cell proliferation accompanying apoptosis [21]. Fumonisin was categorized as a Group 2B carcinogen by the International Agency for Research on Cancer (CIRC) [22].

Fumonisins and esophageal cancer

So far, evidence for human carcinogenicity of fumonisins is circumstantial and limited. Yet, studies in China, Iran, and South Africa showed higher exposure to fumonisins in areas with higher risk of EC [23]. Consumption of contaminated maize has been associated with an elevated risk of EC in the Transkei region in South Africa and China [24,25]. Maize consumption by different age groups in these communities was measured in Mbizana (formerly known as Bizana) and Centane magisterial areas of the former Transkei region of the eastern cape province of South Africa, an area of high EC incidence [25]. Mean fumonisin exposures in all age groups were above the provisional maximum tolerable daily intake according FAO/WHO Expert Committee on Food Additives. Mwalwayo and Thole (2016) observed that populations in the rural areas of Malawi, where the incidence of EC is also high, may be at a high risk of exposure to unacceptably high levels of aflatoxins and fumonisins, according to the *Codex Alimentarius*. This seems more preoccupant in the Chikhwawa and Machinga districts from the southern part of the country where relatively high levels of both aflatoxins and fumonisins were observed [26]. This region is bordered by Mozambique. However, the only case-control study on fumonisin exposure in

relation to EC risk was conducted in Linxian, China, and no association between exposure and risk was found [27]. Urinary fumonisin B1 (UFB1) was the exposure biomarker assayed once it offers an integrated estimation of exposure from all sources for either aflatoxin or fumonisin [5]; however, the results were inconclusive. UFB1 has been measured in human samples in regions with known high exposure to dietary fumonisins [28]. In general, statistically significant relationships between UFB1 and either estimated or measured FB1 intakes were reported; however, the data indicate that urinary measure was only moderately reflective of the intake level [29]. According to Liu *et al.* (2016), mutation analysis revealed common signatures across esophageal cancer samples from Malawi patients associated with aging, cytidine deaminase activity, and a third signature of unknown origin. Signatures of mycotoxins were notably absent [30]. Therefore, epidemiologic studies are needed to establish or refute any association between fumonisins and EC.

Mycotoxin in Mozambique

Aflatoxins B1 (AFB1) and G1 (AFG1) have been found in Mozambican commodities, especially groundnuts and maize [31]. Casadei found aflatoxins in food samples from different areas of Mozambique, though it was almost 40 years ago [32]. Yet, more recent evidences reported by Sineque *et al.* (2017) and Zuza *et al.* (2018) demonstrate that aflatoxin exposure is still a major issue in Mozambique [33,34]. According to Cambaza *et al.* (2018a,2018b), the highest prevalence of aflatoxins contamination was found in Nacala, followed by Maputo city. Inhambane and Nampula also had high aflatoxin levels in their foods [31,32]. Warth *et al.* (2012) studied mycotoxins in food and feed from Burkina Faso and Mozambique and observed that FB1 concentration in maize were higher in Mozambique (92% incidence, median = 869 µg/kg) than in Burkina Faso (81% incidence, median = 269 µg/kg). Their samples were purchased in markets from Nampula city [35]. New strategies for fighting food contamination by mycotoxins are urgently needed [36].

The working group from IARC regarding food contamination with fumonisin B1 performed the present evaluation: there is inadequate evidences in humans for the carcinogenicity of fumonisins. However, there is sufficient evidence from experimental animals for the carcinogenicity of FB1. Thus, FB1 is possibly carcinogenic to humans (Group 2B). These subjects are usually exposed to other risk factors among which are indoor air pollution caused by cooking with charcoal. So, the combination of these risk factors may be at the genesis of the high esophageal cancer rates in Mozambique. We believe that it is necessary to study these issues, to educate the population, to move beyond ecological evidence and to promote food security with cost-effective measures.

Competing interests

The authors declare no competing interests.

Authors' contributions

LLS was responsible for primary conception and design of the article with input from co-authors. Initial drafts of the article were prepared by LLS, JC, EC. Additions, modifications and revisions critical for important intellectual content of the article were performed by JC, EC, RF, JMC, CC, LLS, including final approval of the version to be published.

Figure

Figure 1: overview of the putative molecular pathways involved in EC induced by FB1. (C-Me - DNA hypermethylation; CYP - cytochrome P450; dG-8-oxo - 8-Oxo-2'-deoxyguanosine; FB1- fumonisin B1; UFB1- urinary fumonisin B1)

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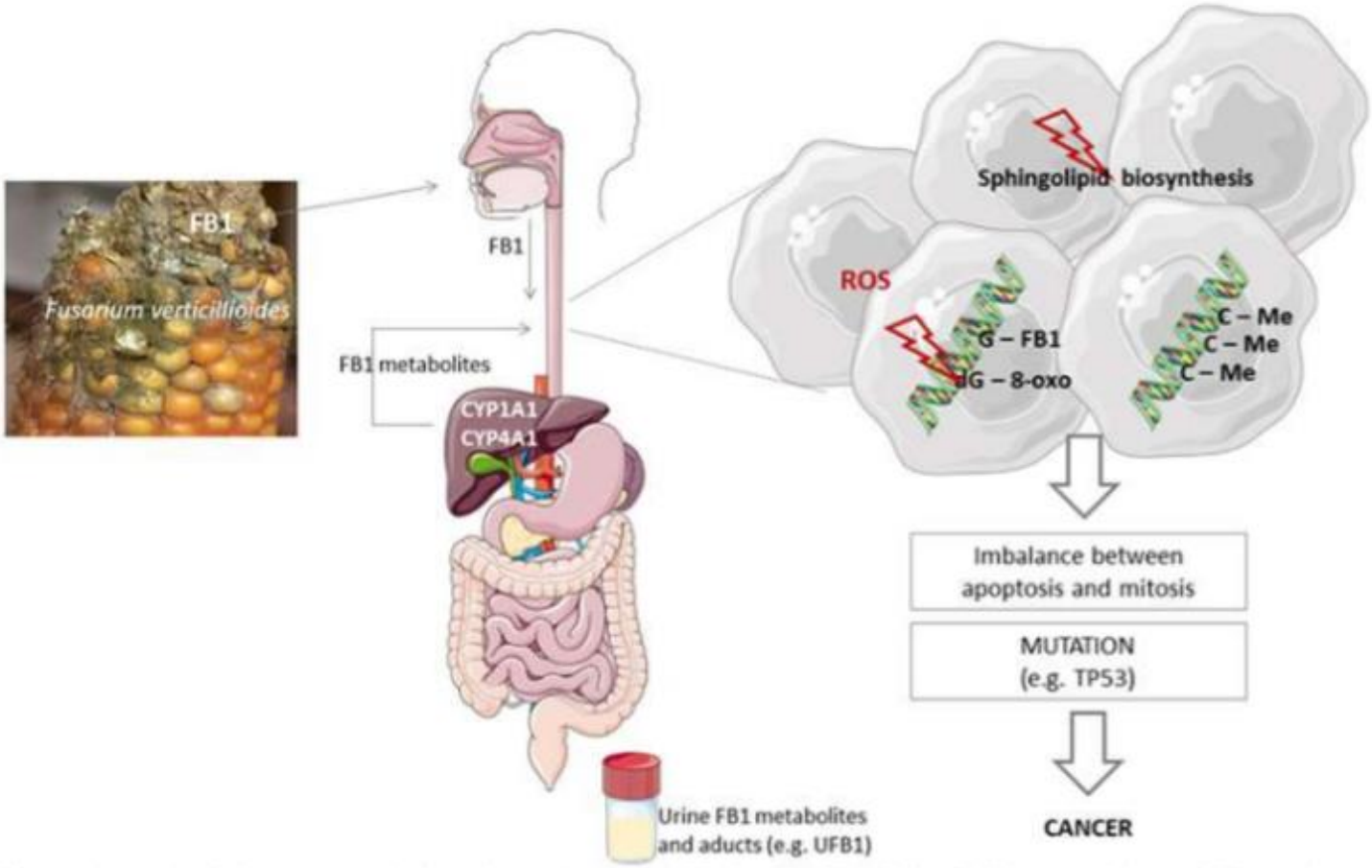

Figure 1: overview of the putative molecular pathways involved in EC induced by FB1. (C-Me - DNA hypermethylation; CYP - cytochrome P450; dG-8-oxo - 8-Oxo-2'-deoxyguanosine; FB1- fumonisin B1; UFB1- urinary fumonisin B1)

3.2 The upper digestive tract microbiome and Esophageal squamous cell carcinoma: Epidemiology pathogenesis and clinical implications in Africa

The study of the microbiome has significantly contributed to our understanding of complex diseases including cancer, with a profound influence of the microbiota on clinical prognosis and efficacy of cancer treatments. Esophageal cancer (EC) is positioned amongst the most aggressive malignant diseases, resulting from a complex interaction between anthropometric, genetic, immune response and environmental factors. Esophageal squamous cell carcinoma (ESCC) is the most common type of esophageal cancer and is a serious burden in eastern Africa, in the area known as the African esophageal cancer corridor (AECC). ESCC is often diagnosed at a late stage, with patients already suffering from severe malnutrition and dehydration due to swallowing difficulties, conducting to high mortality rates. So far, etiologic factors have been individually analysed with an inappropriate contextualisation. The upper digestive tract microbiome has been proposed to contribute to the onset and progression of ESCC but with limited understanding of the mechanisms behind this interaction. Data on African populations is limited, and the aetiology of AECC is still poorly understood. This review discusses the current knowledge on the aetiology of ESCC in Africa, with special focus on the probable influence of the upper digestive tract microbiota. (*According to the version of the English language indicated by the journal the word esophagus, "American English") or associated words were written in the article according to the British version of the English language namely oesophagus "British English"*).

The Upper Digestive Tract Microbiome and Oesophageal Squamous Cell Carcinoma: Epidemiology, Pathogenesis, and Clinical Implications in Africa

Jotamo Come[a] Joana Barbosa Pereira[b, c] Ricardo Pinto[b, c] Carla Carrilho[d, e]
Luisa Pereira[b, c] Lúcio Lara Santos[f, g]

[a]Departamento de Cirurgia, Hospital Central de Maputo, Maputo, Mozambique; [b]i3S, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal; [c]IPATIMUP, Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Porto, Portugal; [d]Departamento de Patologia, Faculdade de Medicina, Universidade Eduardo Mondlane, Maputo, Mozambique; [e]Departamento de Patologia, Hospital Central de Maputo, Maputo, Mozambique; [f]Grupo de Patologia e Terapêutica Experimental e Departamento de Oncologia do Instituto Português de Oncologia do Porto, Porto, Portugal; [g]ONCOCIR – Education and Care in Oncology, PALOP – Lusophone Africa, Porto, Portugal

Keywords
Oesophageal squamous cell carcinoma · Microbiota · Microbiome · African oesophageal cancer corridor · Epidemiology

Abstract
The study of the microbiome has significantly contributed to our understanding of complex diseases including cancer, with a profound influence of the microbiota on clinical prognosis and the efficacy of cancer treatments. Oesophageal cancer is positioned amongst the most aggressive malignant diseases, resulting from a complex interaction between anthropometric, genetic, immune response, and environmental factors. Oesophageal squamous cell carcinoma (OSCC) is the most common type of oesophageal cancer and is a serious burden in Eastern Africa, in the area known as the African oesophageal cancer corridor (AOCC). OSCC is often diagnosed at a late stage, with patients already suffering from severe malnutrition and dehydration due to swallowing difficulties, leading to high mortality rates. So far, aetiological factors have been individually analysed with an inappropriate contextualisation. The upper digestive tract microbiome has been proposed to contribute to the onset and progression of OSCC but with limited understanding of the mechanisms behind this interaction. Data on African populations are limited, and the aetiology of AOCC is still poorly understood. This review discusses the current knowledge of the aetiology of OSCC in Africa, with special focus on the probable influence of the upper digestive tract microbiota.

Introduction

Oesophageal cancer is extremely aggressive, ranking eighth in of most diagnosed cancers worldwide and being the sixth most mortal one [1]. The 2 main histological types – oesophageal adenocarcinoma (OAC) and oesophageal squamous cell carcinoma (OSCC) – are easily distinguished in terms of epidemiology, pathogenesis, and tumour biology. OAC develops in the lower portion of the oesophagus and predominates in high-income settings, mainly affecting middle-aged and elderly peo-

J.C. and J.B.P. contributed equally to this work.

karger@karger.com
www.karger.com/pat

Lúcio Lara Santos
Department of Surgical Oncology and Experimental Pathology and Therapeutics Group
Instituto Português de Oncologia do Porto Francisco Gentil, EPE
Rua Dr. António Bernardino de Almeida 865, PT–4200-072 Porto (Portugal)
llarasantos@gmail.com

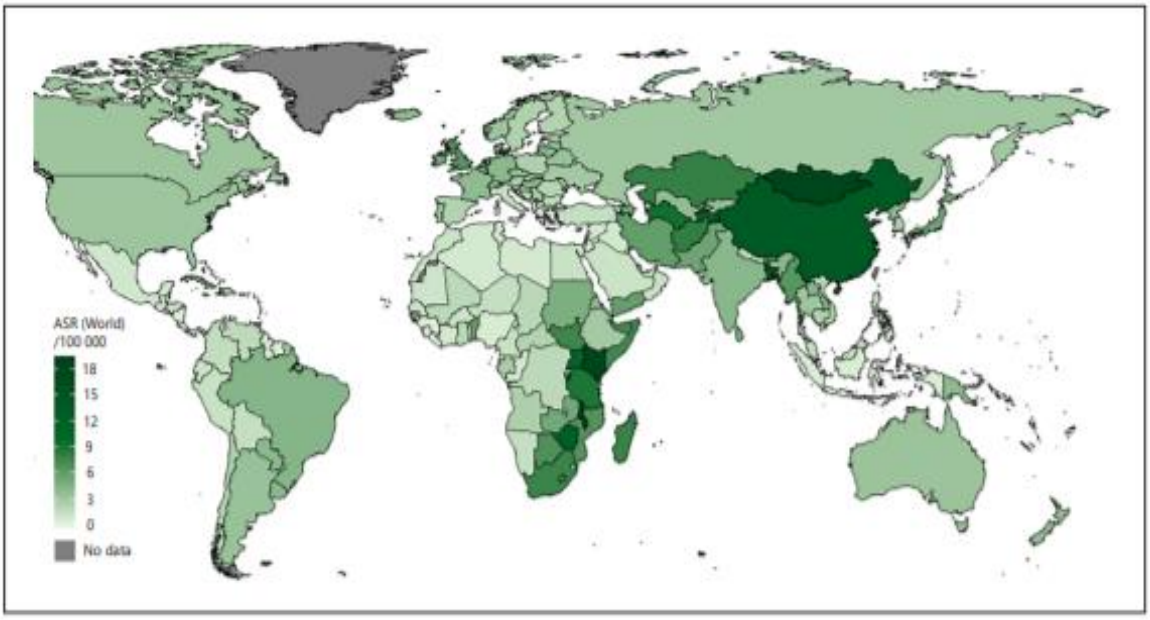

Fig. 1. Oesophageal cancer hotspot regions. Age-standardised incidence rates (ASR) per 100,000 individuals of oesophageal cancer worldwide, according to GLOBOCAN 2018. The incidence of OSCC is higher in the so-called AOCC, the north-south corridor in easterly lying African countries extending from Ethiopia and Kenya to South Africa, and the AOCB, which extends from western/northern China and across Mongolia, Kazakhstan, Turkmenistan, Uzbekistan, Tajikistan, the Middle-East, and eastern Turkey.

ple. OSCC occurs in all parts of the oesophagus and accounts for over 3 quarters of all oesophageal cancers in the world, yet is much less understood than the former type. In fact, in high-income settings, the main OAC risk behaviours include smoking, tobacco, and a diet poor in fruits and vegetables. In low-income regions, these factors hardly explain the high burden of the disease, especially among young people, and other factors such as micronutrient deficiencies, poor oral hygiene, dental fluorosis (a defect in the tooth enamel characterised by hypomineralisation), mycotoxins, as well as genetic factors, may explain the pattern observed [2]. The existence of 2 main OSCC endemic regions in the world (Fig. 1) is striking: the so-called African oesophageal cancer corridor (AOCC) in Eastern and Southern Africa, and the Asian oesophageal cancer belt (AOCB), which extends from western/northern China through Mongolia, Kazakhstan, Turkmenistan, Uzbekistan, Tajikistan, the Middle-East, and eastern Turkey [3, 4]. The 2 regions present a 20-fold higher incidence than any other region, with variations in sex ratio that most likely reflect aetiological factors [2]. OSCC is more common in men than in women, but the ratio varies greatly between countries, ranging from 4:1 in high-incidence areas such as China to 1:1 in other hotspot countries such as Mozambique [5]. Yet there is an overall male excess in OSCC incidence across Africa, especially in individuals aged 30–39 years old [6]. Factors driving the high prevalence in such marked geographical regions and the higher incidence in younger patients is nevertheless still poorly understood.

OSCC is usually preceded by a premalignant lesion called oesophageal squamous dysplasia (OSD) [7]. This asymptomatic precursor lesion was found to be common even among asymptomatic residents of southwestern Kenya, especially in those aged over 50 years [8]. Nevertheless, OSCC is usually detected at late stages of the disease in low-income settings, carrying a poor prognosis since the disease presents too late for a therapeutic intervention. Only 13% of patients in sub-Saharan Africa are potential surgical candidates, and many more do not even have access to healthcare providers [2]. Despite the technological advancements in surgical therapies and chemoradiotherapy, the prognosis remains poor even in patients who have undergone complete resection. The 5-year survival rate in western countries does not exceed 15–25% frequency amongst African patients. This situation is aggravated as many African patients already suffer

from severe malnutrition and dehydration due to swallowing difficulties at the time of diagnosis [7, 9]. The poor prognosis leads to high mortality rates, being the fourth and second cause of death in Mozambique and Malawi, respectively [1].

This review will sum up evidence for the multifactorial profile of OSCC, addressing not only relevant environmental and genetic risk factors for the disease development, but also mainly the oral, oesophageal, and gastric microbiomes. The correct evaluation of multiple possible factors may challenge the burden of oesophageal cancer in Africa, empowering medical staff, researchers, and politicians to understand the disease and the importance of implementing prevention strategies to improve overall quality of life in high-prevalence settings such as AOCC.

Table 1. Association between epidemiologic risk factors and OSCC

Risk factor	Effect	References
Tobacco and alcohol		
Smoking	↑↑↑	11, 13, 17
Alcohol	↑↑	14
Nutritional status		
Selenium	↓↓	3, 24
Zinc	↓↓	3, 24
Iron	↓↓	3, 24
Thermal damage		
Hot coffee	↑↑	31
Hot tea	↑↑	31
Hot/very hot beverages	↓↓	34

↑ indicates an increased risk for OSCC development; ↓ indicates a diminished risk of OSCC. The magnitude of the effects was categorised as follows: ↓, odds ratio (OR) ≤1.70; ↑↑, 1.70< OR <5; ↑↑↑, OR ≥5.

Review Methodology

The bibliographic search consisted of a generic approach that provided analysis of recent and up-to-date literature on a wide range of topics related with oesophageal cancer in Africa (1990–2020). Terms such as "squamous cell oesophageal cancer," "Africa oesophageal cancer corridor," "epidemiologic risk factor," "alcohol and tobacco," "micronutrients deficiencies," "thermal injury," "mycotoxins," "microbiome," "oesophageal microbiota," "oral microbiota," and "gastric microbiota" were used in PubMed searches, with Boolean operators to broaden or narrow down the output results.

Epidemiological Risk Factors

The unusual existence of high OSCC incidence areas strongly suggests a population-dependent role of environmental risk factors. The list of potential carcinogens is long and ranges from thermal damage, cooking fire and inhaled smokes, to micronutrient deficiencies and dietary habits [2].

Tobacco and Alcohol

The evidence on the influence of tobacco on OSCC susceptibility is still uncertain (Table 1), especially in low-income countries [2]. In China, a country lying within the AOCB, smoking does not seem to significantly affect the risk of disease [10], but in eastern Turkey it was indicated as a major risk factor [11]. Besides susceptibility variation due to distinct smoking habits, different ethnicities with similar smoking behaviours show different OSCC risk. African Americans show incidence rates greater than twice as high as Hispanics, Asians, and Native Americans [12]. The risk of OSCC increases with both exposure intensity and duration, with long-standing tobacco smokers presenting a higher risk [13].

The link between alcoholic beverage consumption and OSCC seems to be well established although data must be taken with caution due to improper multifactorial evaluation (Table 1). In Asia there is an OSCC increased risk of 1.6- to 5.3-fold and about 3-fold in Africa and South America associated with alcohol consumption. This increased susceptibility is even greater in low incidence areas, with about a 6-fold increase in Europe and 9-fold in America [5]. In Africa, the traditional home-made alcoholic beverages often contain carcinogenic substances, such as a greater ethanol percentage or acetaldehyde, due to preparation methods which represent an additional risk factor. A case-control study in endoscopy-confirmed OSCC Kenyan patients indicated that alcohol consumption, particularly of busaa and chang'aa, may contribute to half of the OSCC cases in the region, with a linear increased risk with number of drinks [14]. The identification of the carcinogenic compounds in drinks and their damaging effects is, nevertheless, a challenge due to the great heterogenicity of beverages and lack of systematic evidence [15, 16].

The combined consumption of tobacco and alcohol nearly doubles the risk of OSCC than either one alone [17]. The underlying mechanism is not clear but most likely includes DNA damage, increased epithelial permeability to carcinogenic compounds, and/or altered

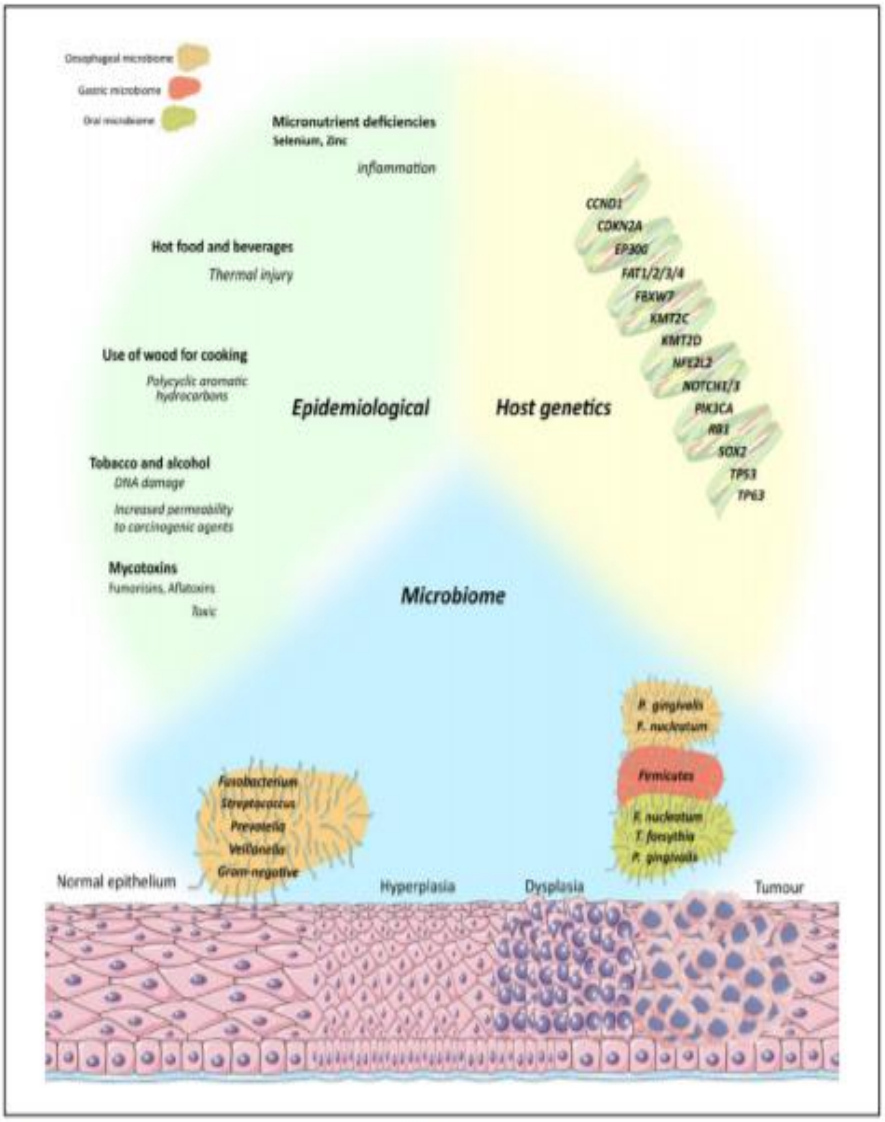

Fig. 2. OSCC multiple risk factors. Risk factors of different origins that can influence the risk of OSCC development: epidemiological (micronutrient deficiencies, hot food and beverages, use of wood for cooking, tobacco and alcohol, and mycotoxins); oesophageal, oral and gastric microbiomes; and genetic. Altogether, different risk factors can increase the risk of OSCC through multiple mechanisms (described in detail in the text) such as enhancement of inflammation, DNA damage, and changes in microbiota diversity.

upper digestive tract microbiota (Fig. 2) [2]. Nevertheless, in Africa other dominant factors besides alcohol and tobacco consumption must explain the burden of the disease, particularly among young adults and women. Okello et al. [18] showed that only 13% of Uganda's OSCC cases were linked to smoking and alcohol, supporting the main influence of other factors in AOCC carcinogenesis.

Nutritional Status

Micronutrient deficiencies have been implicated in OSCC predisposition by making the oesophageal epithelium more prone to inflammation (Fig. 2), which may lead to tumour development and progression [2]. The association between unbalanced intake of minerals and oesophageal cancer risk is still debateable (Table 1), but evidence points to the protective effect of selenium and zinc

[19–23]. Selenium deficiency seems to accelerate oxidative stress and DNA damage, and selenium supplementation trials in nearly 30,000 participants from Linxian, an area of north central China with some of the world's highest rates of oesophageal cancer and a population with a chronically low intake of several nutrients, showed promising results [24], with beneficial effects on mortality still evident up to 10 years after ending supplementation [25]. Zinc deficiency affects cell homeostasis and may induce the overexpression of proinflammatory mediators. Nevertheless, contrary to the selenium trials, the Linxian trials showed no significant results for multivitamin supplementation containing zinc [24]. Other potential OSCC protective micronutrients are vitamins A, C, and E, riboflavin, carotenoids, or folate, though prospective data are missing. In contrast, diets with high levels of saturated fats and cholesterol seem to increase the risk of OSCC development [2].

Nutrient deficiency, mainly zinc, selenium, and iodine, in the AOCC is particularly severe, such as the region falling within the Rift Valley, where soils are greatly exposed to degradation causing micronutrient imbalance, perpetuated by a diet mostly or exclusively relying on local agriculture [3, 26]. Despite the striking co-location, there is an acute lack of studies to access this possible correlation. Schaafsma et al. [3] examined gender-specific oesophageal cancer incidence rates in relation to dietary nutrient supplies. The authors showed that AOCC countries appear to have lower estimated dietary supplies of selenium and zinc in similarity to studies performed outside Africa, supporting the importance of dietary nutrient deficiencies in OSCC. Several studies in Malawi, Tanzania, Kenya, and South Africa showed crop uptake of selenium varies greatly due to the geochemical variation of soils [3]. In Malawi, the most affected country by OSCC, selenium intake was lower than the average adult requirements for over 80% of the population [27]. Kenya and Mozambique were the other countries with the lowest mean dietary selenium and zinc supplies, whereas other nutrients such as magnesium or iron tended to have adequate supplies [3, 28]. In contrast to what was expected, a study that evaluated the association between higher serum selenium concentration and OSD found a positive association between the 2 parameters, implying that further studies are needed to clarify the role of selenium in OSCC carcinogenesis [29]. Nevertheless, simple nutritional prevention measures might have a great impact in decreasing the burden of OSCC in Africa, such as implementation of fortified/enriched food or drinking water, supplements, fortified fertilizers or other agronomic strategies, and the promotion of nutritional diversity [28]. A study was performed in a Chinese population exploring the efficacy of selenium-enriched rice in improving human selenium status to evaluate and potentially prevent the progression of oesophageal cancer and dysplasia [30]. This simple approach might be tailored to AOCC crops and has great potential in the region, where simple but effective measures are urgent.

Thermal Damage and Cooking Habits

Consumption of hot food and beverages has been associated with an increased risk of OSCC (Table 1), especially in high incidence areas, by causing thermal injury and the production of inflammatory heat shock proteins, cytokines, and chemokines that promote carcinogenesis (Fig. 2). Thermal damage can also induce nitrosamines formation, which are carcinogenic compounds. The epithelium injury may also allow the permeation of carcinogens [2]. Hot beverage consumption of hot tea, hot coffee, and hot maté shows increased OSCC risk, with the latter having been classified by the International Agency for Research on Cancer (IARC) as probably carcinogenic to humans [31]. In African countries like Tanzania, Malawi, and Kenya, hot tea consumption, especially milk tea with a high fat content that retains heat, is quite frequent and the contribution of this custom to OSCC needs to be evaluated in the AOCC, together with other risk factors acting synergistically in this malignancy. Studies in the region described hot beverage consumption could reach over 71 °C [32, 33]. Middleton et al. [34] conducted a case-control study in Kenya observing that "very hot" and "hot" beverage drinkers had a 3.7- and 1.4-fold OSCC risk, respectively, when compared to "warmer" drinkers. Yet, the potential carcinogenic mechanisms involved are unclear.

African populations are greatly exposed to polycyclic aromatic hydrocarbons (PAH) that are a large class of noxious and carcinogenic compounds formed by incomplete burning of several organic substances. In low-income settings, populations are more prone to use charcoal and wood for cooking, being more exposed to inhaled smoke with high amounts of PAH, predisposing to respiratory infections as well as respiratory tract cancers [2, 35]. Nevertheless, the heterogenicity of epidemiological evidence presents the need for more data to clarify thermal damage and PAH in OSCC pathogenesis.

Mycotoxins

The cereal-based diet adopted by most people in Africa expose them to maize contamination by fumonisins,

human health carcinogenic mycotoxins produced by the fungus *Fusarium* spp. [36]. Fumonisins are categorised as possibly carcinogenic to humans (Group 2B) by the International Agency for Research on Cancer [36]. *F. verticillioides*, a fungus that is virtually present in almost all maize samples, produces the mycotoxin fumonisin B1 (FB1), a toxic compound linked to oesophageal cancer (Fig. 2) and neural tube defects in humans [36]. The presence of the fungus does not necessarily implicate FB1 production since most strains do not produce the toxin. Nevertheless, its presence in low-income countries, especially Africa, represents a public health problem. FB1 contaminates a large fraction of the crops, including maize, cereals, groundnuts, and tree nuts, in high OSCC incidence areas [36]. Besides crop contamination, mycotoxins are also found in traditional alcoholic beverages due to customary preparation methods [2]. Mycotoxin exposure is more likely to occur where poor food handling and storage conditions are common, and poverty and malnutrition prevent the disposal of contaminated food. In Malawi it was found that rural populations were exposed to extremely high levels of aflatoxins and fumonisins [37], and in Mozambique high levels of aflatoxins were also found in groundnuts and maize, demonstrating that aflatoxin exposure is still a major problem [36]. Few food safety regulations protect the exposed populations.

Fumonisins contamination is associated with an increased risk of hepatocellular and renal cell carcinoma, but its implication in OSCC is still unclear [2, 36, 38]. Kigen et al. [39] suggested that mycotoxins, particularly fumonisins, combined with home-made alcoholic beverages, dietary deficiencies, and viral infections, contribute to the development of oesophageal cancer in Kenyan communities along the Rift Valley. However, Shephard et al. [40] showed that fumonisin exposure in all age groups in 2 regions with high and low oesophageal cancer incidence was above the short-term maximum tolerable daily intake according to the FAO/WHO Expert Committee on Food Additives. In rodents, FB1 was shown to cause tumours of the liver and kidney [41–43]. The carcinogenic mechanism triggered remains unclear but most likely implicates oxidative damage, production of reactive oxygen species, and apoptosis [39, 44–46]. Mycotoxins also have a strong mutagenic effect causing permanent defects in the genome and disrupting sphingolipid metabolism [36]. A recent study showed fumonisins inhibit sphingolipid metabolism, suggesting that disruption of the de novo pathway of sphingolipid biosynthesis may be a crucial event in diseases associated with the consumption of fumonisins [47].

A few epidemiological studies also support the association of fumonisins and OSCC. The substitution of sorghum (a plant that does not enable *Fusarium* fungi growth) for maize in South Africa and Italy led to an increase of oesophageal cancer incidence [48, 49]. Conversely, the prevalence of sorghum in Nigeria shows very low rates of OSCC prevalence [50]. The only case-control study on fumonisin exposure and oesophageal cancer risk was conducted in China, where 98 OSCC cases were randomly selected from the Linxian trial cohort and compared to 185 controls. The study evaluated concentrations of serum sphingolipids as these molecules are biomarkers of fumonisin exposure. For each of the 3 sphingolipid measures no significant relationship was found, with odds ratios of 0.69 (95% CI 0.33 ± 1.4) for sphingosine, 0.63 (95% CI 0.29 ± 1.4) for sphinganine, and 0.98 (sphinganine 0.50 ± 2.0) for the sphinganine/sphingosine ratio, adjusted for covariates such as age, sex, smoking, and drinking [51]. However, given the heterogenicity of OSCC risk factors across the world and the sharp delineation of high incidence areas, the extensive available results for AOCB may not reflect Africa's analogous high incidence area.

Genetics

The research community has made a great effort to perform molecular analysis on the most common malignancies, but OSCC is still relatively understudied. The Cancer Genome Atlas (TCGA) consortium, a leading cancer genomics program, has a single publication on oesophageal cancer [52]. Besides, most published data refer to western countries, where OSCC has been in decline for decades, leaving this type of cancer poorly understood in terms of molecular studies. An inherited genetic component to OSCC ethology has been indicated in Chinese populations [53, 54], but family history involves a series of habits and inherited genes that most likely influence disease susceptibility. In China and Japan, OSCC molecular studies described frequent mutations in genes associated with other squamous cell carcinomas (Table 2) as well as other cancer-associated and histone modifier genes, such as *TP53*, *RB1*, *CDKN2A*, *PIK3CA*, *NOTCH1*, and *NFE2L2* (Fig. 2) [55–58]. In sub-Saharan Africa, a few molecular studies have addressed OSCC, mostly targeting single-nucleotide polymorphisms (SNPs) and indels in specific genes and chromosome regions [59–67]. The findings point to population-specific differences, most likely reflecting differences across ethnic groups.

Liu et al. [68] performed one of the few whole-exome DNA sequencing studies in Africa, characterising 59 untreated OSCCs and matched normal pairs from Malawian patients, reporting similar genetic alterations as shown in North American and Asian cohorts in *TP53*, *CDKN2A*, *NFE2L2*, *CHEK2*, *NOTCH1*, *FAT1* and *FBXW7* genes (Table 2). Another study observed a significant enrichment of inactivating or dominant-negative events in known tumour suppressor genes such as *TP53*, *CDKN2A*, *NOTCH1/3*, *FAT1/2/3/4*, and *FBXW7*, as well as known activating events of *PIK3CA* and *NFE2L2* (Table 2). The authors also reported an enrichment of mutations in genes encoding the chromatin-modifying enzymes KMT2D (MLL2), KMT2C (MLL3), and EP300 [68].

Dental fluorosis has a striking co-location with AOCC and there are specific mutations linked to fluorosis in different populations [69]. The few epidemiological studies on the subject have shown that some ethnic groups are more likely to develop the disease, especially African American children [70]. Genetic variants in some candidate genes have already been linked to fluorosis susceptibility, such as COL1A2, CTR, ESR, COMT, GSTP1, MMP-2, PRL, VDR, and MPO [70]. Despite the high incidence of fluorosis in the AOCC, to date there have been no studies on the potential individual genetic background influence in African populations. One of the few molecular studies in OSCC studied genetic variants from 7 loci identified in Asian and European populations for association in South African populations. An SNP in CHEK2, a tumour-suppressor gene involved in cell cycle regulation and DNA repair, was significantly associated with OSCC indicating that genetic risk factors most likely change in African and non-African populations [71]. Nevertheless, there is a great urge to fill in this gap given the high incidence of the disease in AOCC and the marked differences in population ancestry that may reflect in cancer epidemiological and clinical features. Ancestry-specific predisposing genetic variants is well established in other cancer types and needs to be better addressed in the context of AOCC. For instance, prostate and breast cancers show an increased genetic susceptibility risk in Africans in comparison to other populations, even though the actual magnitude of risk is not well characterised [72, 73].

Table 2. Association between molecular risk factors and OSCC

Risk factor	Association	References
TP53	↑↑↑	55–57, 59, 66, 68
RB1	↑↑↑	55–57
CDKN2A	↑↑↑	55–57, 68
PIK3CA	↑↑↑	55–57, 68
NOTCH1	↑↑↑	55–57, 68
NFE2L2	↑↑↑	55–57, 68
D2S123	↑↑↑	60, 64
D3S659	↑↑↑	60, 64
D3S1255	↑↑↑	60, 64
DCC	↑↑↑	60
D18S34	↑↑↑	60
D18S58	↑↑↑	60
D5S346	↑↑↑	60
1p	↑↑↑	61
4p	↑↑↑	61
18q	↑↑↑	61
19p	↑↑↑	61
19q	↑↑↑	61
22q	↑↑↑	61
1q	↑↑↑	61
2q	↑↑↑	61
3q	↑↑↑	61
5p	↑↑↑	61
7p	↑↑↑	61
7q	↑↑↑	61
8q	↑↑↑	61
Xq	↑↑↑	61
COL1A2	↑↑↑	62
Bat 25	↑↑↑	64
Bat 26	↑↑↑	64
Bat 40	↑↑↑	64
FAT1/2/3/4	↑↑↑	68
FBXW7	↑↑	68
KMT2D (MLL2)	↑↑↑	68
KMT2C (MLL3)	↑↑↑	68
EP300	↑↑	68

Genes associated with OSCC. The magnitude of the association was categorised as follows: ↑, <2% mutated samples; ↑↑, 2–5% mutated samples; ↑↑↑, >5% mutated samples.

Upper Digestive Tract Microbiome

Microbial organisms interact with each other and with the host immune system, having a great influence on the development of disease [74]. Alterations in the microbiome's composition may induce significant changes in host homeostasis, and lead to inflammatory diseases and increased cancer susceptibility [75]. Homeostasis of the microbiome is essential for adequate host immune responses [76]. The most efficient way to characterise the microbiome involves the analysis of microbial genomes through metagenomics. Prokaryotic 16S ribosomal RNA (rRNA) is usually used as a marker for the taxonomic classification and phylogenetic analysis of the microbiome, affording enough information for

secure identification of strains [77]. Sequencing is currently mostly performed by next-generation methods, which process thousands of low-sized reads (approx. 100 base pairs) that are then mapped against reference panels of bacterial genomes [74]. The sequencing of thousands of reads allows us to identify and quantify many microbes that constitute the microbiome, even the ones that are less frequent.

The upper digestive tract (oral cavity and oesophagus) and gut microbiota assemble complex microbial compositions involved in diseases such as OSCC (Fig. 2) and OSD [78, 79]. Despite the numerous microorganisms harbouring in the human upper digestive system, little is known about the relationship of the oral and/or oesophageal microbiome with the host and OSCC susceptibility. Sampling of the oesophagus usually consists in endoscopy with brushing or biopsies with limited sample sizes due to the invasiveness of these procedures, impending, namely, informative microbiome studies. The available non-endoscopic procedures for oesophageal microbiome analysis, such as "Esophageal String Test" or "Cytosponge," indirectly also sample the stomach or oral microbiome, biasing the results [80, 81].

Oesophageal Microbiome

Initial evidence suggested the oesophageal microbiome was the result of momentary exposure to the oral microbiota [82], but recent studies showed that, in addition to swallowed bacteria, the oesophagus is exposed to refluxed gastric microbes, being a sum up of both oral and gastric microbiota, and having its own identity [83]. Several factors may alter the oesophageal microbiome, such as proton pump inhibitors by increasing the pH of gastric secretions, and also by directly targeting the bacterial proton pumps of certain bacteria such as *Streptococcus pneumoniae* and diet [84]. Early studies in Chinese populations showed that *Streptococcus* is the dominant taxon in the healthy oesophageal mucosa, followed by the genera *Prevotella* and *Veillonella* [85, 86]. Later, a study of a Caucasian cohort that underwent upper gastrointestinal endoscopy showed the oesophageal microbiota clusters in 3 community types with unique dominant organisms defined by the relative abundances of *Streptococcus* and *Prevotella*, and microbial richness and evenness. However, the findings in healthy oesophageal microbiota are not completely consistent and some genera abundances differ between cohorts. These differences are most likely explained by the different geographical locations of the populations, reflecting diverse lifestyles, sex proportions in the cohorts, and age that together significantly affect the microbiome. Different sampling and sequencing methods are also important variables [87].

Evidence of different oesophageal microbiome composition in healthy individuals and in patients with cancerous oesophageal mucosa or cancer-predisposing conditions is indisputable. A cross-sectional study performed on a Chinese cohort provided the first evidence of increased Gram-negative bacteria associated with adenocarcinoma and the related cancer-predisposing Barrett's oesophagus [88]. Liu et al. [89] also showed that bacterial communities differed among normal oesophagus, reflux esophagitis, and Barrett's oesophagus in Japanese patients. While the most prevalent genus in normal oesophagus and reflux esophagitis patients was *Streptococcus*, *Veillonella* was predominant in Barrett's oesophagus. Also, while *Fusobacterium* was found in patients, it was absent in the normal oesophagus [89]. Another cohort displayed dominance of *Streptococcus* and *Prevotella* and found a significant association of these genera with Barrett's oesophagus and OAC risk factors [90]. Later, Deshpande et al. [87] found that taxonomic and functionally distinct microbial compositions were associated with the OAC cascade, indicating that these functionally distinct community types should be further explored. The authors identified 3 main community types with distinct functional signatures: cluster 1, with average levels of *Streptococcus* and *Prevotella* but increased levels of *Haemophilus* (*H. parainfluenzae*) and *Rothia* (*R. mucilaginosa*), was found to be enriched for glycolysis as well as pathways related to short-chain fatty acid metabolism; cluster 2, dominated by *Streptococcus* (*S. mitis/oralis/pneumoniae*), was enriched for the pentose phosphate pathway and fructose and mannose metabolism; and cluster 3, dominated by *Prevotella* (*P. melaninogenica* and *P. pallens*) and to a lesser extent *Veillonella*, was enriched for lipopolysaccharide biosynthesis.

Far less is known about the association of oesophageal dysbiosis and OSCC as the great majority of studies refer to OAC that has a particularly higher incidence in high-income countries. These results may not be reflected in OSCC due to the epidemiologically and biologically distinct characteristics of the 2 types of tumour. The few studies addressing the association of OSCC and the oesophagus microbiota showed compelling evidence between bacterial composition and disease risk (Table 3). Yu et al. [91] showed that Chinese individuals with lower microbial richness are more prone to develop OSCC since there was an inverse association between microbial richness and the risk of OSD. However, this study exam-

Table 3. Association between the upper digestive microbiome risk factors and OSCC

Risk factor	Effect	References
Oesophageal microbiome		
Microbial richness	↓[1]	91
P. gingivalis	↑[2]	92
F. nucleatum	↑↑[2]	93
Oral microbioma		
Microbial diversity	↑	100
Gastric microbiome		
Clostridiales	↑↑↑ (% OTU)	102
Erysipelotrichales	↑ (% OTU)	102
H. pylori	↑↑	105
H. pylori (Eastern individuals)	↓	106
H. pylori (Middle East)	↓	107

↑ indicates an increased risk for OSCC development; ↓ indicates a diminished risk of OSCC. The magnitude of the effects was categorised as follows: ↑, odds ratio (OR) ≤1.70; ↑↑, 1.70 < OR < 5; ↑↑↑, OR ≥5, except where indicated. OTU, operational taxonomic unit.

[1] OSCC precursor lesion – OSD.

[2] OSCC prognosis.

ined the upper digestive tract cells collected by 2 different devices, which does not allow the distinct microbiota of the oral cavity, oesophagus, and stomach to be distinguished.

Gao et al. [92] analysed oesophageal tissues from OSCC Chinese patients, finding that the Gram-negative anaerobic bacterium *Porphyromonas gingivalis* infects the cancerous and adjacent oesophageal mucosa of the patients but not the healthy mucosa of controls. This opportunistic pathogen falls within the "red complex" of periodontal pathogens, which are the species most strongly associated with severe periodontitis – an inflammatory disease that causes oral dysbiosis. The presence of *P. gingivalis* in the unhealthy mucosa was positively correlated with cancer cell differentiation and metastasis, which may represent a good marker for poor clinical outcome. The presence of *F. nucleatum* (a bacterium that inhabits the oral cavity also causing periodontitis) in oesophageal cancer specimens of Japanese patients was also associated with a significantly shorter survival time since "cytokine-cytokine receptor interaction" pathways were significantly affected in *F. nucleatum*-positive oesophageal cancer tissues, suggesting that this bacterium contributes to a more aggressive tumour behaviour by activating chemokines such as CCL20 [93].

Oral Microbiome

Given the proximity of the oral cavity and the oesophagus, the oral microbiome has been strongly implicated in OSCC susceptibility. Disruption of the oral microbiome is stimulated through direct metabolism of chemical carcinogens such as alcohol and tobacco, systemic inflammatory effects, or poor oral hygiene [94–99]. Few studies have addressed the association between the oral microbiota and OSCC, being almost limited to the Asian corridor (Table 3). Chen et al. [100] reported that 87 OSCC Chinese patients had lower microbiome diversity than 85 healthy individuals, including an increased relative abundance of *Prevotella*, *Streptococcus*, and *Porphyromonas*. Although these genera seem to be non-pathogenic to the host, certain bacteria are associated with increased risk of disease – *F. nucleatum*, *Tannerella forsythia*, and *P. gingivalis* – while others were linked to lower risk – *Streptococcus* and *Neisseria* [80, 92, 93]. The detection of adverse bacteria in pre-cancer lesions may become a key disease biomarker and prognostic indicator [92, 101]. A recent prospective study in an American cohort of 25 OSCC cases and 50 matched controls strengthened the association of *P. gingivalis* with OSCC, indicating an association of the bacteria with OSCC lymph node metastasis and decreased survival time [79]. Peters et al. [79] found that ecological networks between bacteria may play an important role in oesophageal cancer risk since there are bacterial taxon associations unique to OAC and OSCC. The authors reported a promising new perspective on the correlation between oral microbiome and oesophageal cancer types, where specific ecological networks are associated with adenocarcinoma and OSCC, in line with the different origins of these cancers. Although the authors did not report significant associations between the oral microbiota diversity and disease risk, they strengthened the association of *P. gingivalis* with OSCC, showing an association of this bacterium with OSCC lymph node metastasis and decreased survival time. Despite the exciting evidence presented by Peters et al. [79], there is an overrepresentation of adenocarcinoma cases in relation to the number of OSCC which leaves this cancer type underexplored. Moreover, the study lacked the periodontal status of the patients to properly address the possible independent implication of periodontal pathogens in patient outcome.

Menya et al. [69] conducted one of the few studies on oral health and OSCC in western Kenya, where dental fluorosis is endemic due to early-life excessive fluoride intake. The authors concluded that 430 cases with poor oral hygiene and dental fluorosis had increased OSCC-

associated risk in comparison with 440 controls. Moderate/severe fluorosis cases, which consisted of 44% of cases, had an increased risk as high as 9.4-fold. Moreover, the occurrence of oral leucoplakia and tooth loss/decay increased with fluorosis severity and, consequently, contributed to cancer risk. Geographic differences also showed the striking co-location of areas with a high OSCC incidence and those of high groundwater fluoride levels, highlighting an urgent need for focused research into primary prevention strategies. The encouraging results of the role of the oral microbiota in OSCC strengthens the need to further explore periodontal disease and/or periodontal pathogens in OSCC carcinogenesis. The identification of oral pathogenic bacteria and/or protective specimens could lead to preventive interventions for their eradication or colonisation in susceptible individuals. This has particular importance in OSCC hotspot regions, particularly in sub-Saharan Africa, where prevention strategies are crucial to decrease the high incidence of the disease.

Gastric Microbiota

The gastric microbiota has been associated with oesophageal cancer susceptibility [102]. While in OAC the relationship with gastric microbiota may be more evident due to the biological proximity of this type of tumour to the stomach, the association with OSCC may not be so clear but nonetheless important. Observational studies disclosed an association of the gastric microbiota environment with OSCC (Table 3), implying that changes of gastric mucosa interfere with OSCC susceptibility [103, 104].

Gastric colonisation by *Helicobacter pylori*, a Gram-negative bacterium that grows in the mucus layer of the stomach, is the leading cause of gastric carcinoma. Contradictory, *H. pylori* is believed to reduce the risk of OAC, but the underlying mechanism is not understood [105]. While some studies found a protective effect of the bacterium to the risk of the disease [106], others did not find a significant association [102] or even showed a higher risk of the disease [105]. A recent meta-analysis study based on 345,886 patients addressed these inconsistent results, revealing that they are probably due to the high geographic heterogeneity, translated into multiple risk factors, such as smoking and alcohol consumption or dietary habits [107]. It is worth noting that this systematic review with 35 studies only includes one African population from Uganda, with the vast majority being Caucasian individuals from westernised countries. The results presented by Gao et al. [107] strengthen the need for well-designed prospective cohort studies with representative samples of the great heterogeneity of OSCC.

Besides *H. pylori*, other organisms of the gastric microbiota have been associated with OSCC. By comparing the gastric corpus microbiota in OSCC and oesophageal dysplasia patients with healthy individuals, Nasrollahzadeh et al. [102] found significant differences between diseased subjects and controls, with patients showing a higher abundance of Clostridiales and Erysipelotrichales, both Firmicutes phyla.

Microbiome Potential Clinical Applications

Better understanding of the dynamics between OSCC and the microbiome would contribute to clarify cancer aetiology and potentially develop new approaches for prevention and treatment. The microbiome may act as a biomarker for diagnosis and/or clinical outcome and can greatly improve the development of targeted therapies and strategies by modulating the microbiota. Microbiota can be changed by antibiotics, probiotics, prebiotics, or microbiota transplants, representing an enormous potential for cancer prevention, particularly in high-risk populations [78]. While antibiotics can remove or supress pathogenic organisms, probiotics may provide the introduction of lost beneficial microbial components and prebiotics can enhance the proliferation of microbes with beneficial functions. Moreover, treatment outcomes may be influenced by the microbiota due to microbial influence on the toxicity and efficacy of therapeutic intervention [108]. The microbiome can also influence host response to immunotherapy. Host-microbial interactions may provide an important basis for a more inclusive view of pharmacology and nutrition [78].

Several studies strongly indicate a link between commensal bacteria and cancer therapeutic efficacy by modulating responses to cancer immunotherapy [109]. The gut microbiota is the one most frequently implicated in cancer therapy response across a wide variety of treatments, such as chemotherapy, immune checkpoint blockade, and stem cell transplant, through several potential mechanisms that require deeper understanding. Iida et al. [110] conducted one of the first studies in antibiotics-treated and germ-free mice to show that tumour-infiltrating myeloid-derived cell response to immunotherapy and chemotherapy was mediated differentially by commensal bacteria, impairing the inflammatory effect and stressing the importance of manipulating the microbiome to improve treatment. Several other studies have subsequently demonstrated that differential gut microbiota environments were associated with patients who responded fa-

vourably to treatments with an enhanced systemic immunity response [111]. Preclinical and clinical studies suggested that the gut microbiome affects antitumor immunity via numerous mechanisms such as the induction of cytokine production, enhancing the therapeutic response [109]. The gut microbiota has also been shown to influence responses to a range of chemotherapies, with both beneficial and microbiome-dependent effects [109].

Increasing acknowledgment that the microbiome plays a critical role in carcinogenesis and therapeutic outcome offers a great opportunity for research, from basic to translational research in both clinical and epidemiological studies. Simple strategies such as probiotics supplementation may be easily implemented in low-income African countries to monitor and modulate disease development and outcome in order to realise the full potential of host-human dynamics.

Cancer Research, Prevention, and Health Care Resources in AOCC

Africa faces a rising burden of cancer, namely oesophageal cancer along the AOCC. Yet, cancer research is still scarce in the continent to meet this public health challenge and few people are equipped to conduct research in the area despite remarkable progress in building human research capacity over the last decade [7, 112, 113]. In Tanzania, a great part of oncology trainees would like to incorporate research into their careers, but inadequate training in research methodology and absence of longitudinal mentorship is a drawback [114].

Additionally, the lack of well-trained health professionals and differential infrastructures pose a great challenge for clinical and research oncology performed at low-income settings. In Mozambique, the knowledge in prevalent cancer domains is well characterised, as well as the prevalent cancers treated by surgery at Maputo Central Hospital (MCH), the main hospital in Mozambique, together with residents' oncological knowledge. Results obtained, besides confirming oesophageal cancer as the most prevalent malignant tumour treated by general and thoracic surgery, with advanced-stage diagnoses requiring multimodal treatment, informed the need to improve residents' oncology knowledge, supporting the necessity of a surgical oncology training tailored to suit local needs [7, 112]. The improvement of resources, such as tests and procedures to diagnose OSCC are also urgent. This problem is transversal to other African countries and personalised oncology education programs should be implemented in AOCC countries at the undergraduate level, with specific training for residents and continuing oncological education for general surgeons to improve the practice of surgical oncology [113].

The African Esophageal Cancer Consortium (AfrECC) coordinates aetiological and molecular studies of OSCC and could represent a great platform to implement multisite and multidisciplinary research programs [115]. Teams of surgeons, gastroenterologists, and pathologists are crucial to develop promotion plans, identify staff needs, establish general clinical procedures, and select risk assessment models for each region [7]. It is also important to design case studies to implement dietary diversification and the consumption of fortified/enriched food [3, 116], as well as call attention to soil degradation and the importance of fertilizer use and improved crop varieties to increase nutrient levels in harvests [26, 116]. The empowerment of people with oesophageal cancer information and decision-making skills will promote disease prevention strategies and ultimately improve the population's quality of life. Awareness of cancer risk factors is urgently needed in Africa. For instance, a study in Tanzania showed that despite high awareness of the cancer risk of tobacco or alcohol, knowledge was quite low regarding infections or food contamination by fungi. Moreover, most participants were not aware of cancer warning symptoms and many myths still endure in the community [117].

Conclusion

OSCC is a burden in impoverished countries, with a special incidence in the AOCC. Oesophageal cancer symptoms are associated with impaired quality of life, especially if help is not sought in the earliest stages of the disease. This scenario worsens in Africa due to limited clinical organisations and weak public health education that delays not only treatment but also the implementation of prevention strategies and research. It is urgent to evaluate OSCC multifactoriality in Africa, addressing relevant environmental (e.g., alcohol and tobacco, diet, mycotoxins), biological (oral microbiome and metabolome), and genetic risk factors (Fig. 2). So far, these elements have been mostly individually analysed with an inappropriate contextualisation. Easy and inexpensive solutions may decrease the OSCC burden in Africa, and these solutions must be studied with the best up-to-date technologies and robust scientific approaches. An oesophageal cancer prevention approach will be crucial not only be-

cause of the OSCC burden, but also because prevention strategies are quite deficient throughout Africa in general. The urge to change population mentalities and capacitation is demanding and only makes sense in light of new initiatives and collaborations between academic, clinical, and scientific institutions. Nevertheless, prevention strategies are pointless if we do not understand the causes for such high incidence rates in the AOCC. Understanding of the molecular oesophageal cancer pattern and microbiome risk profile along with complementary classical epidemiological studies is essential to implement oesophageal cancer prevention strategies and programmes.

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Statement of Ethics

This article is in compliance with internationally accepted standards for research practice and reporting.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

L.L.S. designed the manuscript and coordinated all contributions. All authors drafted, read, and approved the final manuscript.

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3.3 Risk factors for esophageal squamous cell carcinoma in Mozambique

The African Esophageal Cancer Consortium (AfrECC) since September 2016 have begun quality studies, including case-control studies in: Addis Ababa, Ethiopia, Eldoret, Kenya, Bomet, Kenya, Moshi, Tanzania, Dar es Salaam, Tanzania, Lilongwe, Malawi, Blantyre, Malawi, Lusaka, Zambia and Johannesburg and Cape Town, South Africa. These studies have allowed to know the most plausible risk factors to be in the genesis of esophageal cancer in sub-Saharan Africa, as well as to know the genetic risk profile. Mozambican data are scattered and fragile. So we decided to study the colligible data from the central Hospital of Maputo. Therefore, the study that follows aimed to quantifying the association between alcohol, tobacco and dietary history, and the occurrence of ESCC in Mozambique.

Risk factors for esophageal squamous cell carcinoma in Mozambique

Lina Cunha*[1], Filipa Fontes*[2,3,4], Jotamo Come[5], Vitória Lobo[6,7], Lúcio Lara Santos[8,9], Nuno Lunet[2,3], Carla Carrilho[10,11]

[1] Serviço de Gastroenterologia, Hospital Privado de Maputo, Maputo, Moçambique

[2] EPIUnit – Instituto de Saúde Pública, Universidade do Porto, Porto, Portugal

[3] Departamento de Ciências da Saúde Pública e Forenses e Educação Médica, Faculdade de Medicina da Universidade do Porto, Porto, Portugal

[4] Departamento de Imagem, Instituto Português de Oncologia do Porto, Porto, Portugal

[5] Departamento de Cirurgia, Hospital Central de Maputo, Maputo, Moçambique

[6] Departamento de Gastroenterologia, Hospital Central de Maputo, Maputo, Moçambique

[7] Departamento de Medicina, Faculdade de Medicina, Universidade Eduardo Mondlane, Maputo, Moçambique

[8] Departamento de Oncologia Cirúrgica, Instituto Português de Oncologia do Porto, Porto, Portugal

[9] Grupo de Patologia e Terapêutica Experimental, Instituto Português de Oncologia do Porto, Porto, Portugal

[10] Departamento de Patologia, Faculdade de Medicina, Universidade Eduardo Mondlane, Maputo, Moçambique

[11] Departamento de Patologia, Hospital Central de Maputo, Maputo, Moçambique

* these authors contributed equally to the manuscript

ABSTRACT

Objective: Studies evaluating risk factors for the occurrence of esophageal squamous cell carcinoma (ESCC) in high risk regions, might contribute to a better understanding of the esophageal cancer etiology and incidence variation worldwide. We aimed to quantify the association between alcohol, tobacco and dietary history, and the occurrence of ESCC in Mozambique.

Methods: A case-control study was conducted at Maputo Central Hospital. Cases (n=143) were patients with newly diagnosed esophageal cancer recruited in the Gastroenterology Service. Controls (n=212) were selected in the Orthopedic Ward among subjects with pathologies related to trauma. Crude and adjusted odds ratios (OR), and the corresponding 95% confidence intervals (CI) were computed using non-conditional logistic regression.

Results: The risk of ESCC was higher in older participants and lower in those with higher household income. Alcohol drinking (lifetime consumption ≥55.1 *vs* 0 Kg ethanol: OR=5.01; 95%CI: 2.41 – 10.42) and tobacco smoking (lifetime consumption ≥20 *vs* 0 pack/years: OR=6.38; 95%CI: 1.48 – 27.40) were associated with increased risk of ESCC. Tea (at least twice daily *vs* less than daily: OR=5.02; 95%CI: 2.49 – 10.01) and coffee consumption (at least once daily *vs* less than daily: OR=3.18; 95%CI: 1.09 – 9.29) were also associated with the occurrence of ESCC. No significant differences were observed for fruit and vegetable, smoked meat or fish consumption.

Conclusion: The occurrence of ESCC in Mozambique is strongly influenced by lifetime consumption of tobacco and alcohol, and with tea and coffee drinking. This highlights the importance of preventive measures based on the promotion of healthier lifestyles.

Keywords: Africa, Alcohol, Diet, Esophageal cancer, Mozambique; Tobacco.

INTRODUCTION

According to the most recent estimates, worldwide there were more than 570 000 new cancer cases (3% of all cancers) and around 500 000 deaths due to esophageal cancer (5% of all cancer deaths) in 2018; the highest figures for both age-standardized incidence and mortality rates are observed in Eastern Asia, and in Eastern and Southern Africa (Ferlay et al. 2018). Although esophageal cancer has two predominant histopathological subtypes, esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EA), ESCC has been estimated to account for approximately 90% of all esophageal cancer cases in many sub-Saharan African countries (Ocama et al. 2008, Somdyala et al. 2010, Arnold et al. 2015, Come et al. 2018).

In developed regions, tobacco smoking has been consistently considered the major risk factor for ESCC (Castro et al. 2018). Together with alcohol drinking and low consumption of fruits and vegetables, it is estimated to account for approximately 90% of the total number of cases in this context (Engel et al. 2003). However, the reasons explaining the high frequency of ESCC in high risk developing regions, such as Eastern and Southern Africa, remain largely unknown (Cheng et al. 2015, McCormack et al. 2017). Despite the increase of Western influences in this context, the prevalence of tobacco and alcohol consumption remains much lower than the observed in Western countries (Peacock et al. 2018). Furthermore, the strength of the association between tobacco and alcohol consumption, and the occurrence of ESCC were shown to be weaker in developing regions (Castro, Peleteiro et al. 2018). Studies from high risk areas addressing the role of the major knowing risk factors for ESCC are scarce, but the few available suggest that other factors might also contribute to the geographic variations in the occurrence ESCC worldwide (Yu et al. 1993, Okello et al. 2016, Chetwood et al. 2019).

Studies assessing the relation between plausible risk factors and the occurrence of ESCC in high risk regions, such as Eastern and Southern Africa, might contribute to a more comprehensive explanation of the esophageal cancer incidence variation worldwide. According to a recent systematic review of the epidemiology and risk factors of esophageal cancer in Africa, there are no studies assessing risk factors for ESCC in Mozambique (Asombang et al. 2019), although data from the Maputo Central

Hospital (MCH) registry highlights that it is the fourth most frequent tumor for both sexes (Carrilho et al. 2019). Therefore, this study aimed to quantify the association between alcohol, tobacco and dietary history, and the occurrence of ESCC in Mozambique.

METHODS

Study design and setting

We conducted a case-control study in MCH. The MCH is a 1500-bed hospital quaternary hospital and the national reference hospital for cancer in Mozambique. A total of 143 cases of esophageal cancer and 212 controls were identified between 2006 and 2010, and included in this study.

Participants

Cases were patients recruited in the Gastroenterology Service of the MCH, among those with newly diagnosed esophageal cancer. Those with histological confirmation of the diagnosis were considered eligible. From the initial number of cases included (N=145), the pathological diagnosis was ESCC in 143 (98.6%) and EA in 2 (1.4%) cases; the latter were excluded from the present analysis because they were in a very small number.

Controls were selected from the Orthopedic Ward of the MCH and included subjects with several pathologies related to trauma. Those without a diagnosis of tobacco or alcohol related diseases, or esophagus or stomach diseases recorded as part of the admission cause, were considered eligible.

Exposures assessment

Face-to-face interviews were conducted by trained interviewers, using a standardized questionnaire covering information on sociodemographic characteristics, coffee and tea drinking, smoking and alcohol consumption, and dietary history.

Alcohol consumption was assessed through questions exploring the usual intake for each type of beverage separately, i.e. beer, wine, spirit drinks and traditional drinks (e.g. brandy, *sura*, *mahéu*, *canho* and *caju*). Current and ex-drinkers were asked to report the

age when they started to drink and the usual number of units consumed per day, week or month, as applicable. The four types of alcoholic beverages were combined to provide an overall estimate of alcohol consumption. Ex-drinkers, defined as those who had permanently quit drinking before the interview, were asked to report at what age they stopped to drink. We estimated the lifetime cumulative quantity of alcohol consumed in Kg of ethanol for each participant; for analysis, data was further categorized using the quartiles of the distribution among controls as cut-offs (0, >0 and <6.5, ≥6.5 and <23.9, ≥23.9 and <55.1 and ≥55.1 Kg of ethanol). In addition, current drinkers of each type of beverage were categorized according the amount of drinks consumed per day.

Participants were asked if they currently were smokers of any tobacco product, and current and ex-smokers were asked to report the age when they started to smoke and the number of units consumed per day, week or month, as applicable. In addition, ex-smokers, defined as those who had permanently quit smoking before the interview, were asked to report what age they stopped to smoke. We estimated the lifetime cumulative quantity of tobacco smoked in pack/years (average number of 20-cigarette packs per day multiplied by the number of years smoking) for each participant; for analysis data was further categorized as 0, >0 and <15, ≥15 and <20 and ≥20 pack/years.

Tea and coffee consumption were assessed by asking participants if they currently were drinkers of tea or coffee, and consumers were asked to report the number of times they drink per day, week or month, as applicable, and the usual tea or coffee temperature (very hot, hot or warm). As only one participant reported the consumption of warm tea and no one reported the consumption of warm coffee, for data analysis the “warm” and “hot” categories were merged.

The consumption of fruits and vegetables were evaluated through questions on the usual intake of fruits and vegetables, and the number of times they consumed each type (fruits and vegetables) per day, week or month, as applicable. For data analysis, fruits and vegetables consumption frequency were combined and participants were categorized using the quartiles of the distribution among controls as cut-offs (< 1 time per day, ≥1 and <2 times per day and ≥ 2 times per day). In addition, participants were asked if they consumed smoked meat and/or fish.

Data analysis

Odds ratio (OR), and the corresponding 95% confidence intervals (CI), for the association between sociodemographic characteristics, alcohol, tobacco, tea and coffee consumption and dietary history, and the occurrence of ESCC, were computed using non-conditional logistic regression. Variables included in each model are described as footnotes of the respective table.

The potential interaction between smoking and alcohol consumption was assessed by including interaction terms in the regression models. For the purpose of this analysis, both ex-drinkers and current drinkers, and ex-smokers and current smokers, respectively, were combined in the same categories.

Statistical analysis was conducted STATA®, version 11.2 (StataCorp, College Station, TX, USA).

Ethical considerations

The study protocol was approved by the Mozambican National Bioethics Committee for Health. All participants provided written informed consent.

RESULTS

The association between sociodemographic characteristics and ESCC are summarized in Table 1. In the multivariate analysis, the risk of ESCC was higher in older participants (>65 *vs* <45 years: OR = 2.39; 95%CI: 1.15 – 4.95) and lower in those with higher household income (>1000 *vs* <500 MZN per capita: OR = 0.25; 95%CI: 0.13 – 0.48). Although not statistically significant, a protective independent effect was observed for male participants, for those with a secondary or higher education and for those with freezer at home.

Table 2 presents the association between alcohol and tobacco consumption and ESCC. Concerning alcohol consumption, ex-drinkers and current drinkers of more than 12 or 24g of alcohol per day experienced, respectively, a 2.4 and 6.2-fold increase of risk when compared to never drinkers. When the lifetime consumption of alcohol was considered, a significant increase of risk was observed for those with higher levels of consumption (≥55.1 *vs* 0 Kg ethanol: OR = 5.01; 95%CI: 2.41 – 10.42). In relation to the type of beverage consumed, beer was the most commonly consumed (30.4%), followed by wine (27.3%), traditional drinks (19.7%) and spirit drinks (11.6%). Those

who consumed at least one drink per day of beer and wine had a 6.7 and 5.1-fold higher ESCC risk, respectively, when compared to non current drinkers. The strength of the association remains similar after additional adjustment to the intake of other types of beverage.

Concerning smoking, no significant differences were observed when comparing current or ex-smokers with never smokers but those with the highest lifetime consumption presented a significant higher risk when compared to those who never smoked (OR = 6.38; 95%CI: 1.48 – 27.40).

No significant interaction was observed between smoking and alcohol drinking (P-value for interaction = 0.213), despite the association between alcohol consumption and the occurrence of ESCC was lower among never smokers (OR = 1.55; 95%CI: 0.89 – 2.71) than among ever smokers (OR = 4.01; 95%CI: 0.78 – 20.57).

Table 3 shows the association between dietary habits and ESCC. There is a tendency for a higher risk with the increase of the daily frequency of consumption of tea (at least once daily *vs* less than daily: OR = 4.19; 95%CI: 2.20 – 7.99; at least twice daily *vs* less than daily: OR = 5.02; 95%CI: 2.49 – 10.01) and coffee (at least once daily *vs* less than daily: OR = 3.18; 95%CI: 1.09 – 9.29). No significant differences were observed across different temperatures of tea or coffee consumption, frequencies of fruit and vegetable intake or smoked meat or fish consumption.

DISCUSSION

To our knowledge, this is the first study evaluating risk factors for esophageal cancer in Mozambique. We demonstrated that alcohol, tea and coffee drinking are associated with a higher ESCC risk, while no significant association was observed for fruit and vegetable intake or smoked meat or fish consumption. Regarding smoking, there was an increased risk for higher lifetime consumptions.

Alcohol drinking is widely accepted as a risk factor for ESCC (Castro, Peleteiro et al. 2018). Data from one systematic review on the effect of alcohol on different types of cancer showed that when comparing light drinkers, moderate drinkers and heavy drinkers with nondrinkers, the pooled RR for ESCC was 1.3, 2.2 and 5.0, respectively (Bagnardi et al. 2015). Although direct comparisons are difficult due to the heterogeneous characteristics of the studies and the use of different criteria to classify participants according to different levels of consumption, our data supported a dose-response effect in the Mozambican context. In fact, the risk increased with the increase

of the lifetime alcohol consumption and, when comparing the lowest drinkers (≤12/24 g/day) and the highest drinkers (>12/24 g/day) with never drinkers, the ORs were 1.2 and 5.3, respectively. Some studies from other Eastern African countries found similar associations (Patel et al. 2013, Sewram et al. 2016). In a case control study from South Africa, participants who consumed between 16.5 and 52.8 grams of ethanol per day and those who consumed more than 52.8 grams of ethanol per day presented an approximately 3 and 5-fold higher risk of ESCC, respectively, than never drinkers (Sewram, Sitas et al. 2016). In another case control study from Kenya, it was found that ever users presented a 2.6-fold higher ESCC risk than never users (Patel, Wakhisi et al. 2013).

In our data, the risk of ESCC increase with the quantity of drinks of wine and beer consumed per day, with ORs of 5.10 and 6.75, respectively, for the highest levels of intake (≥1 drink/day). Nevertheless, previous results suggested that the quantity of ethanol consumed was the most important factor in ESCC development rather than any individual type of beverage consumed (Sewram, Sitas et al. 2016).

Tobacco smoking has been consistently considered the major risk factor for ESCC in developed regions, accounting for 47% to 57% of the total incidence in these settings (Siemiatycki et al. 1995, Engel, Chow et al. 2003, Pandeya et al. 2013). In our study, no significant differences were observed between current and never smokers, which could be justified, in part, by the small number of cigarettes smoked per day by each smoker. This is in accordance to the first World Health Organization Stepwise Approach to Chronic Disease Risk Factor Surveillance survey, conducted in 2005 in Mozambique, that found a prevalence of current tobacco consumption of 9.6% in urban women and 31.6% in urban men and that most of the daily smokers reported to consume less than 5 cigarettes/day (Padrão et al. 2013). Despite the non-significant result, our findings suggested a tendency to a higher risk for smokers when compared with never smokers, which is in accordance with results from case-control studies from South Africa (Patel, Wakhisi et al. 2013) and Uganda (Okello, Churchill et al. 2016) that found a risk of developing ESCC in smokers of approximately 2.51 and 1.38, respectively. In addition, despite the small number of participants in this exposure category, those with the highest lifetime consumption (≥20 pack/years) presented a 6-fold higher risk when compared to never users, which supported a dose-response effect. Our results suggest that in the current stage of the tobacco epidemic in Mozambique (Padrão, Damasceno et

al. 2013), the cumulative consumption of tobacco was more important as risk factor for ESCC development than the tobacco consumption status.

The effect of some dietary aspects on ESCC risk has been previously addressed in other studies from Eastern Africa countries (Sewram et al. 2014, Asombang et al. 2016). Contrary to our findings, in a study from the Eastern Cape Province of South Africa, the authors found that males and females consuming green leafy vegetables 5-7 day/week had 38% and 50% reduced odds of developing ESCC, respectively, compared with those consuming ≤ 1 day/week; a similar reduction in odds was also observed with fruit consumption (Sewram, Sitas et al. 2014). In contrary, in a previous study from Zambia, the authors found that the consumption of fruits and vegetables did not differ significantly between esophageal cancer cases and controls (Asombang, Kayamba et al. 2016), which is in accordance to our findings.

Regarding hot beverages, it has been noted an association with ESCC, that led the International Agency for Research on Cancer (IARC) to recently classify very hot beverages (>65ºC) as "probably carcinogenic" to humans (Group 2A) based on epidemiological evidence, although it was noted insufficient evidence for coffee or tea when not consumed hot, which were therefore classified as "not classified as to its carcinogenicity to human" (Group 3) (Loomis et al. 2016, International Agency for Research on Cancer 2020). In our study, no significant differences in the ESCC risk were observed across different temperatures of tea or coffee consumption, while an increase of the risk was observed for more frequent consumers of tea or coffee, which is apparently inconsistent. We may hypothesize that this could be, at least in part, explained by the absence of participants reporting warm tea or coffee consumption, and by misclassification of some participants regarding temperature consumption (hot/very hot). Therefore, our finding of an increase of the ESCC risk with the consumption frequency is more likely to be related with a cumulative effect of the beverage temperature than an independent effect of tea or coffee.

Although this study provides new insights on risk factors for ESCC in Mozambique, some limitations need to be discussed. First, this case-control study was hospital-based and therefore more prone to selection bias. However, controls included patients with several pathologies related to trauma and not related to the exposures of interest, which may have minimized bias. The presence of information bias was minimized by using the same interviews procedures in both cases and controls, to avoid any differential misclassification. Second, data regarding tea, coffee, fruit and vegetables, and smoked

meat or fish consumption, were reported referring to the currently consumption, and may not represent changes in consumption over time. Finally, future studies may include information regarding other potential risk factors for the ESCC risk, that could be particularly important in this setting, including smokeless tobacco, mycotoxins, cooking habits, Human Papillomavirus and *Helicobacter pylori* infections (Castro, Peleteiro et al. 2018, Chetwood, Garg et al. 2019, Come et al. 2019), that were not evaluated in the present study.

In conclusion, this case-control study shows that the occurrence of ESCC is strongly influenced by lifetime consumption of tobacco and alcohol, and with tea and coffee drinking. This highlight the importance of preventive public health measures based on the promotion of healthier lifestyles.

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Table 1. Association between sociodemographic characteristics and esophageal squamous cell carcinoma.

	Controls	Cases	OR (95%CI)	
	n (%)	n (%)	Crude	Adjusted *
Sex				
Female	120 (56.6)	90 (62.9)	1 [reference]	1 [reference]
Male	92 (43.4)	53 (37.1)	0.77 (0.50 – 1.19)	0.75 (0.45 – 1.23)
Age (years)				
<45	55 (25.9)	26 (18.2)	1 [reference]	1 [reference]
45-55	68 (32.1)	38 (26.6)	1.18 (0.64 – 2.18)	1.29 (0.66 – 2.50)
55-65	51 (24.1)	39 (27.3)	1.62 (0.86 – 3.02)	1.84 (0.91 – 3.72)
>65	38 (17.9)	40 (28.0)	2.23 (1.17 – 4.24)	2.39 (1.15 – 4.95)
Education (level completed)				
None	53 (25.0)	41 (28.7)	1 [reference]	1 (ref.)
Primary	97 (45.8)	85 (59.4)	1.13 (0.69 – 1.87)	1.60 (0.90 – 2.83)
Secondary or higher	62 (29.2)	17 (11.9)	0.35 (0.18 – 0.70)	0.87 (0.39 – 1.93)
Household income (MZN per capita)				
<500	35 (16.5)	48 (33.6)	1 [reference]	1 [reference]
500-999	84 (39.6)	68 (47.6)	0.58 (0.34 – 1.01)	0.57 (0.33 – 0.99)
>1000	93 (43.9)	27 (18.9)	0.21 (0.11 – 0.39)	0.25 (0.13 – 0.48)
Freezer (at home)				
No	85 (40.1)	78 (54.5)	1 [reference]	1 [reference]
Yes	127 (59.9)	65 (45.4)	0.55 (0.36 – 0.86)	0.77 (0.48 – 1.22)

OR, Odds Ratio; 95%CI, 95% Confidence Interval

* Obtained from models including all variables presented in Table 1

Table 2. Association between alcohol and tobacco consumption and esophageal squamous cell carcinoma.

	Controls	Cases	OR (95%CI)	
	n (%)	n (%)	Crude	Adjusted *
Alcohol drinking				
Never drinker	99 (46.7)	60 (42.0)	1 [reference]	1 [reference]
Ex-drinker	27 (12.7)	24 (16.8)	1.47 (0.78 – 2.77)	2.42 (1.16 – 5.04)
Drinker (≤12g/day for females and ≤24 g/day for males)	82 (38.7)	46 (32.2)	0.93 (0.57 – 1.50)	1.22 (0.70 – 2.13)
Drinker (>12g/day for females and >24 g/day for males)	4 (1.9)	13 (9.1)	5.36 (1.67 – 17.20)	6.23 (1.79 – 21.62)
Lifetime alcohol consumption (Kg ethanol)				
0	99 (46.7)	60 (42.0)	1 [reference]	1 [reference]
>0 and <6.5	28 (13.2)	16 (11.2)	0.94 (0.47 – 1.89)	1.18 (0.56 – 2.51)
≥6.5 and <23.9	29 (13.7)	9 (6.3)	0.51 (0.23 – 1.16)	0.67 (0.28 – 1.62)
≥23.9 and <55.1	28 (13.2)	11 (7.7)	0.65 (0.30 – 1.40)	1.32 (0.55 – 3.20)
≥55.1	28 (13.2)	47 (32.9)	2.77 (1.57 – 4.88)	5.01 (2.41 – 10.42)
Current type of alcohol consumption (drinks/day)				
Beer				
0	158 (74.5)	100 (69.9)	1 [reference]	1 [reference]
>0 and <1	49 (23.1)	32 (22.4)	1.03 (0.62 – 1.72)	1.11 (0.63 – 1.97)
≥1	5 (2.4)	11 (7.7)	3.47 (1.17 – 10.30)	6.75 (1.90 – 24.03)
Wine				
0	147 (69.4)	100 (69.9)	1 [reference]	1 [reference]
>0 and <1	62	38	0.90 (0.56	1.02 (0.59

	(29.2)	(29.6)	– 1.45)	– 1.78)
≥1	3 (1.4)	5 (3.5)	2.45 (0.57 – 10.48)	5.10 (1.08 – 24.12)
Spirits				
0	188 (88.7)	126 (88.1)	1 [reference]	1 [reference]
>0 and <1	23 (10.8)	16 (11.2)	1.04 (0.53 – 2.04)	1.42 (0.65 – 3.11)
≥1	1 (0.5)	1 (0.7)	1.49 (0.09 – 24.07)	2.53 (0.14 – 44.46)
Traditional				
0	168 (79.2)	117 (81.8)	1 [reference]	1 [reference]
>0	44 (20.8)	26 (18.2)	0.85 (0.49 – 1.45)	0.84 (0.45 – 1.56)
Tobacco consumption				
Never smoking	149 (70.3)	106 (74.1)	1 [reference]	1 [reference]
Ex-smoker †	41 (19.3)	18 (12.6)	0.62 (0.34 – 1.13)	0.83 (0.41 – 1.69)
Smoker †	22 (10.4)	19 (13.3)	1.21 (0.63 – 2.35)	1.62 (0.73 – 3.57)
Lifetime tobacco consumption (pack/years)				
0	149 (70.3)	106 (74.1)	1 [reference]	1 [reference]
>0 and <15	52 (24.5)	25 (17.5)	0.68 (0.39 – 1.16)	0.86 (0.45 – 1.63)
≥15 and <20	8 (3.8)	3 (2.1)	0.53 (0.14 – 2.03)	0.81 (0.16 – 4.01)
≥20	3 (1.4)	9 (6.3)	4.22 (1.12 – 15.95)	6.38 (1.48 – 27.40)

OR, Odds Ratio; 95%CI, 95% Confidence Interval

* Obtained from models including all variables presented in Table 1.

† The median (percentile 25 – percentile 75) number of cigarettes consumed per day was 6 (3–15) among ex-smokers and 5 (3 –10) among smokers. The median (percentile 25 – percentile 75) years of tobacco consumption was 17 (9 – 26) among ex-smokers and 31 (25 – 40) among smokers

Table 3. Association between dietary history and esophageal squamous cell carcinoma.

	Controls	Cases	OR (95%CI)	
	n (%)	n (%)	Crude	Adjusted *
Tea consumption (frequency)				
Less than daily †	77 (36.3)	18 (12.6)	1 [reference]	1 [reference]
Once daily	82 (38.7)	76 (51.8)	3.86 (2.12 – 7.05)	4.19 (2.20 – 7.99)
At least twice daily	53 (25.0)	51 (35.7)	4.12 (2.17 – 7.82)	5.02 (2.49 – 10.1)
Tea consumption (temperature) ‡				
Warm/hot	165 (79.7)	117 (81.8)	1 [reference]	1 [reference]
Very hot	42 (20.3)	26 (18.2)	0.87 (0.51 – 1.50)	0.85 (0.48 – 1.54)
Coffee consumption (frequency)				
Less than daily ¥	202 (95.3)	133 (93.0)	1 [reference]	1 [reference]
At least once daily	10 (4.7)	10 (7.0)	1.52 (0.61 – 3.75)	3.18 (1.09 – 9.29)
Coffee consumption (temperature) §				
Warm/hot	148 (86.6)	44 (88.0)	1 [reference]	1 [reference]
Very hot	23 (13.4)	6 (12.0)	0.88 (0.34 – 2.29)	0.65 (0.23 – 1.85)
Fruit and vegetable consumption				
<1 times per day	97 (46.0)	68 (48.2)	1 [reference]	1 [reference]
≥1 and <2 times per day	62 (29.4)	44 (31.2)	1.01 (0.62 – 1.66)	1.24 (0.72 – 2.13)
≥2 times per day	52 (24.6)	29 (20.6)	0.80 (0.46 – 1.38)	0.93 (0.51 – 1.67)
Smoked meat or fish consumption				
No	186 (88.2)	132 (92.3)	1 [reference]	1 [reference]
Yes	25 (11.8)	11 (7.7)	0.62 (0.28 – 1.30)	0.53 (0.24 – 1.18)

OR, Odds Ratio; 95%CI, 95% Confidence Interval

* Obtained from models including all variables presented in Table 1.

† Includes four non-drinkers.

‡ Data available for 350 tea drinkers.

¥ Includes 134 non-drinkers.

§ Data available for 221 coffee drinkers.

CHAPTER IV - CLINICAL AND PATHOLOGIC PROFILES OF ESOPHAGEAL CANCER IN MOZAMBIQUE: A STUDY OF CONSECUTIVE PATIENTS ADMITTED TO MAPUTO CENTRAL HOSPITAL

Eastern Africa, as already mentioned, was described as a high-incidence geographic area for esophageal cancer. Mozambique is included in this region. Little is known about the clinical and pathological characteristics of this malignant tumor in Mozambique. The next study aimed to characterize this malignant disease at Maputo Central Hospital (MCH) in order to develop a global program for esophageal cancer management in the country.

original reports

Clinical and Pathologic Profiles of Esophageal Cancer in Mozambique: A Study of Consecutive Patients Admitted to Maputo Central Hospital

Jotamo Come
Clara Castro
Atílio Morais
Matchecane Cossa
Prassad Modcoicar
Satish Tulsidâs
Lina Cunha
Vitória Lobo
Alberto Gudo Morais
Sofia Cotton
Nuno Lunet
Carla Carrilho
Lúcio Lara Santos

Author affiliations and support information (if applicable) appear at the end of this article.

Corresponding author: Lúcio Lara Santos, MD, PhD, Instituto Português de Oncologia, Porto. Rua Antonio Bernardino de Almeida, 4200-072, Portugal; e-mail: llarasantos@gmail.com.

abstract

Purpose Eastern Africa was recently described as a high-incidence geographic area for esophageal cancer. Mozambique is included in this region. This study aimed to characterize this malignant disease at Maputo Central Hospital (MCH) to develop a global program for esophageal cancer management in Mozambique.

Methods MCH records from between 2012 and 2016 were used to assess the clinical, pathologic, and outcome profiles of esophageal tumors. A descriptive analysis of data collected was performed. Overall survival was evaluated using Kaplan-Meier curves.

Results In the study, 522 consecutive patient cases of esophageal cancer were recorded. The median patient age was 56.1 years (range, 27 to 97 years); 291 (55.7%) patients were women, and 230 (44.1%) were men. Regarding tumor site, 113 patients (21.6%) had a tumor in the lower third, 154 (29.5%) in the middle, and 50 (9.6%) in the upper third of the esophagus; in the remaining 196 (37.5%), tumor site was unknown. Squamous cell carcinoma comprised 94.4% of cases with documented histopathology (74.9% of the sample). Surgical treatment was possible in 32 patients (6.1%). Disease stage was documented only in these 32 surgical patients; 28.1%, 53.1%, and 18.8% had stage I, II, and III disease, respectively. The remaining patient cases seemed to involve clinically advanced tumors. The median follow-up time was of 1.6 months. The median survival time was of 3.5 months for all patients; for patients treated with curative intent, it was of 8.7 months.

Conclusion Esophageal carcinoma is a common malignant tumor at MCH and is diagnosed in the advanced stages resulting in poor prognosis. Therefore, implementation of an Esophageal Cancer Program in Mozambique is essential.

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INTRODUCTION

Esophageal cancer is the eighth most frequent cancer worldwide and sixth most common cause of death resulting from cancer.[1] The distribution of esophageal cancer varies geographically, with 80% of cases occurring in developing countries.[2] The highest incidences of esophageal cancer in the world have been observed in China, northeastern Iran, southeastern United States, and Southern Africa.3 Among both sexes, there are more than 20-fold differences in incidence in world regions, with rates ranging from 0.8 per 100,000 in West Africa to 17.0 per 100,000 in East Asia in men and 0.2 per 100,000 in Micronesia/Polynesia to 7.8 per 100,000 in East Africa in women.[4] Mortality rates are elevated in East Asia (14.1 per 100,000) and Southern Africa (12.8 per 100,000) in men and in East Africa (7.3 per 100,000) and Southern Africa (6.2 per 100,000) in women.[4] The high incidence rates of esophageal cancer (esophageal squamous cell carcinoma) in Central, Southern, and East Africa, together with diagnosis at advanced stages, lead to high mortality rates.[1,2,5-7] The reasons underlying the high frequency of this malignancy on that continent remain largely unknown, and investigation to evaluate potential etiologic effects of dietary, lifestyle, environmental, and other factors affecting incidence in this region, including genetics, is needed.[2,8-10] In their study, Liu et al[11] demonstrated discrete subtypes of esophageal squamous cell carcinoma in sub-Saharan Africa

Table 1. Descriptive Analysis of Demographic and Clinical Characteristics of Patients With Esophageal Cancer Registered at MCH Over Study Period (N = 522)

Characteristic	No.	%
Year of diagnosis		
2012	89	17.0
2013	95	18.2
2014	96	18.4
2015	119	22.8
2016	123	23.6
Sex		
Male	230	44.1
Female	291	55.7
Unknown	1	0.2
Race		
Black	520	99.6
Mixed	2	0.4
Region of origin		
North	7	1.3
Center	26	5.0
South	418	80.1
Other	2	0.4
Unknown	69	13.2
Age, years		
Mean	56.1	
Standard deviation	13.23	
Basis of diagnosis		
Histology	391	74.9
Death certificate	35	6.7
Clinical	82	15.7
Upper GI endoscopy	14	2.7
Tumor site		
Upper third	50	9.6
Middle third	154	29.5
Lower third	113	21.6
Esophagogastric junction	9	1.7
Unknown	196	37.5
Surgery		
Feeding gastrostomy only	199	38.1
Surgery with curative intent	32	6.1
None	291	55.8
Chemotherapy		
Yes	18	3.4
No	504	96.6
Microscopically verified cases (n = 391)		
Morphology		
Adenocarcinoma	10	2.6
Adenosquamous carcinoma	3	0.8

(Continued on following page)

and suggested that the endemic nature of this disease reflects exposure to a carcinogen other than tobacco or oncogenic viruses.

In Mozambique, data on the basis of the Maputo Central Hospital (MCH) registry reveal that esophageal cancer is the fourth most frequent tumor for both sexes, and it is the most frequent occurring in the digestive tract.[12,13] Dysphagia related to esophageal cancer has been a major cause of hospitalization. Many of these patients are admitted with severe malnutrition and dehydration resulting from difficulties in swallowing, which are related to poor prognosis.[14] With the aims of creating a global program to fight this disease, promoting good practices, and empowering disease management, MCH records were used to evaluate the burden of esophageal cancer in the hospital; assess the clinical and pathologic profiles of such tumors, treatment approach; and barriers in collection of data in this context; and define a plan of action that can contribute to the improvement of care management for these patients. The study was performed at MCH, located in Maputo, the capital of Mozambique. It is a 1,500-bed quaternary hospital and serves the Eduardo Mondlane University Medical School as a teaching hospital. MCH is the national reference hospital of the country.

METHODS

MCH records were retrospectively assessed from the Pathology and Surgery Services database to obtain the clinical and pathologic characteristics of patients with esophageal cancer admitted to and treated at MCH between January 1, 2012, and April 30, 2014. From May 2014 to December 2016, data were obtained from the MCH hospital-based cancer registry. This registry was implemented in May 2014 and includes information about patient cases of esophageal cancer identified in the departments of pathology and oncology, as well as data on cancer-related deaths from the Intrahospital Death Registration System.[13] In addition, surgery department files were also assessed to complete information on patients diagnosed on the basis of clinical data only. Demographic variables collected for each patient included sex, age, race, and origin of the patient; clinical and pathologic data included anatomic site, histologic subtype, grade, basis of diagnosis, and treatments performed for each patient (surgery and chemotherapy). For patients

Table 1. Descriptive Analysis of Demographic and Clinical Characteristics of Patients With Esophageal Cancer Registered at MCH Over Study Period (N = 522) (Continued)

Characteristic	No.	%
Squamous cell carcinoma	369	94.4
Carcinoma, no other specification	9	2.3
Histologic grade		
Well differentiated	83	21.2
Moderately differentiated	88	22.5
Poorly differentiated	21	5.4
Unknown	203	50.9

Abbreviation: MCH, Maputo Central Hospital.

undergoing surgery with curative intent, information on stage (American Joint Committee on Cancer seventh edition[15]), tumor size, resection status, and occurrence of lymphatic, vascular, muscular, perineural, adventitia, and/or lymph node invasion was also collected. A descriptive analysis of data collected was performed to describe the clinicopathologic profile of esophageal cancer at MCH. Overall survival was evaluated using Kaplan-Meier curves. The study was approved by the Mozambican National Bioethics Committee (reference No. 432/CNBS/2017).

RESULTS

Over the study period, 522 consecutive patient cases of esophageal cancer were recorded (Table 1). The mean age at diagnosis was 56.1 years (standard deviation, 13.23 years; range, 23 to 97 years); 53 patients (10.2%) were age < 40 years; 14 patients (2.6%) were age ≤ 30 years. Most patients were women (n = 291; 55.7%), black (n = 520; 99.6%), and born in southern regions of the country (n = 418; 80.1%; Fig 1). The male-to-female ratio was between 0.7 and 0.9 in all 10-year age groups (20 to 29, 30 to 39, 40 to 49, 50 to 59, 60 to 69, and ≥ 70 years). Dysphagia to solids was documented in all patients and required nutritional support. However, nutritional management with gastrostomy is unsuccessful in a large number of patients. Information on the anatomic site of the tumor was not available for 196 patients (37.5%); among the remaining patients, 154 (47.2%) had a tumor located in the middle third of the esophagus, 113 (34.7%) in the lower third, 50 (15.3%) in the upper third, and nine (2.8%) in the esophagogastric junction (Table 1). Diagnosis was histologically confirmed for 391 patient cases (74.9%; Table 1). Among those, most cases were squamous cell carcinomas (n = 369; 94.4%); 10 (2.6%) were adenocarcinomas, and three (0.8%) were adenosquamous carcinomas. Among the 192 patient cases with available information on histologic grade, 83 (43.2%) were well differentiated, 88 (45.8%) were moderately differentiated, and 21 (10.9%) were poorly differentiated. In the studied series, 55.8% of patients underwent no staging imaging. Surgical treatment with curative intent (Ivor-Lewis procedure) was only possible in 32 patients (6.1%); surgical feeding gastrostomy was performed using a urinary catheter tube in 199 patients (38.1%), and the remaining 291 patients (55.7%) received best support care. For patients undergoing surgery with curative intent, the median tumor size was 40 mm (interquartile range, 25.5 mm; range, 15 to 100 mm); 28.1%, 53.1%, and 18.8% of patients had stage I, II, and III disease, respectively; 21 patients (65.6%) had negative margins; and lymphatic, vascular, perineural, muscular propria, adventitia, and lymph node invasion were found in 14 (43.8%), 14 (43.8%), 11 (34.4%), 31 (96.9%), 20 (64.5%), and eight patients (25.0%), respectively. The neoadjuvant chemotherapy protocol used (cisplatin 75-100 mg/m^2 intravenously on day 1 plus fluorouracil 1,000 mg/m^2 continuous infusion over 24 hours on days 1 to 5. Cycled every 28 days), included four cycles before operation and two after the operation. In adjuvant/palliative context the same protocol was used during six cycles. Chemotherapy was administered to 18 patients (3.4%).Only one patient received neoadjuvant chemotherapy; for the remaining 17 patients, chemotherapy was adjuvant or palliative. Radiotherapy was not available. In survival analyses, 172 patients (32.9%) were excluded (127 patients were lost to follow-up, 26 had as date of diagnosis the date of death, and 19 had only the year of diagnosis and/or follow-up available.). The median follow-up time was of 1.6 months (1.3 *v* 2.1 months for alive and dead patients, respectively, at the date of last contact). The median survival time was of 3.5 months for all patients (Fig 2), whereas for patients treated with curative intent (either surgery alone or with neoadjuvant chemotherapy, according to stage), it was of 8.7 months.

DISCUSSION

This study reveals that esophageal cancer is a common condition at MCH. The surgery, gastroenterology, medical oncology, and pathology departments in the hospital were the sources

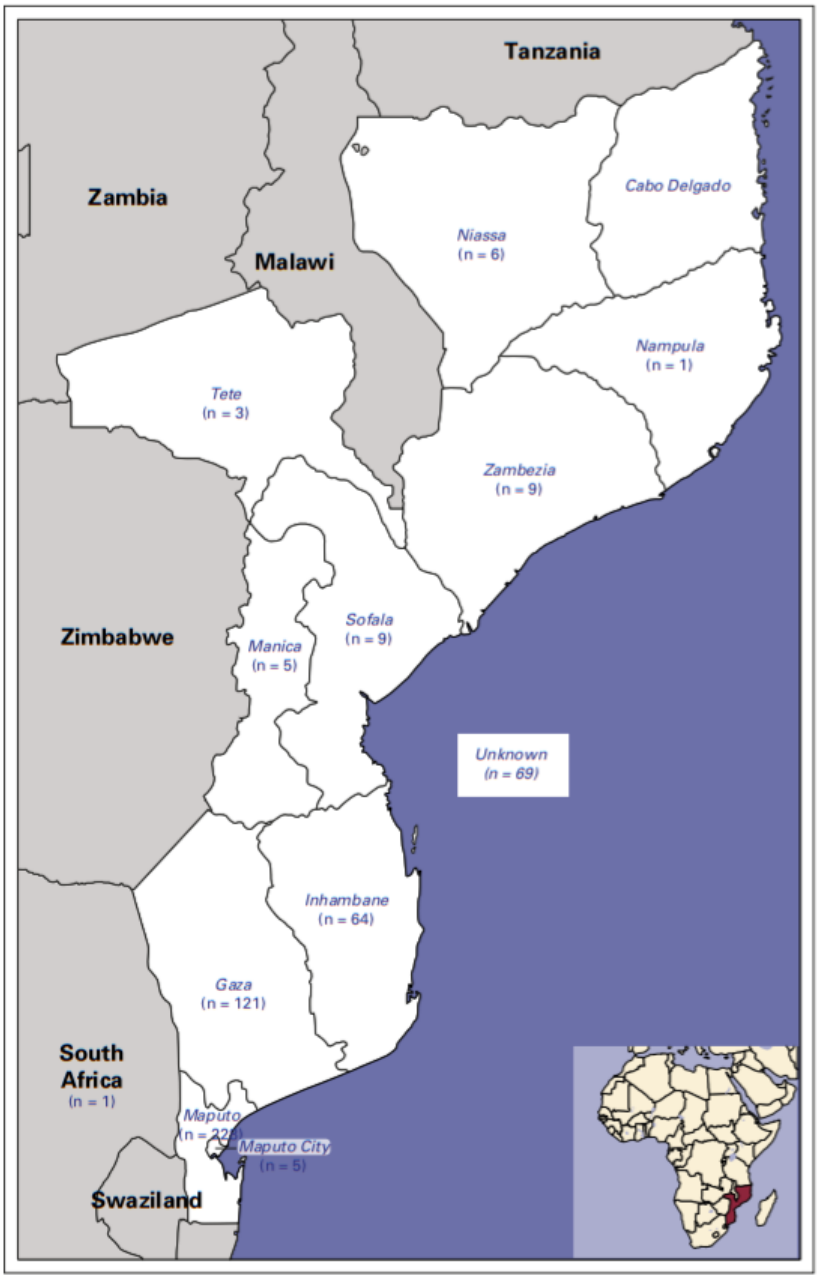

Fig 1. Geographic distribution of place of birth of patients by provinces in the country.

of information used in this study. However, a multidisciplinary approach toward treating esophageal cancer was nonexistent. In our study sample, most patients were black women. The sex ratio found in our study was different from that in other African countries; however, it was closer to values found in Ethiopia, Kenya, and Sudan; furthermore, a decrease in sex ratio to

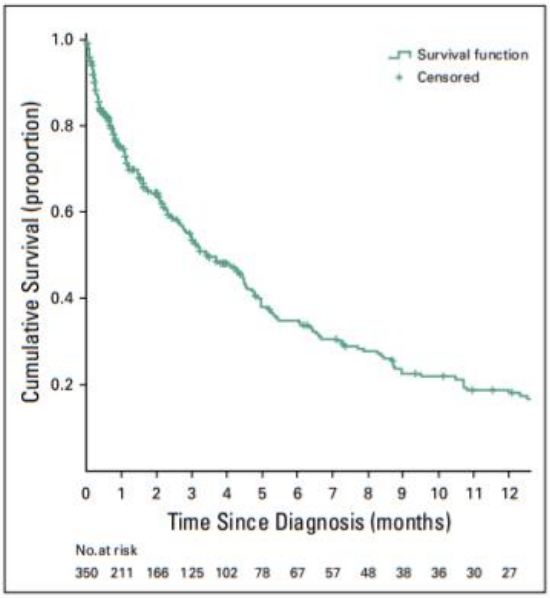

Fig 2. Overall survival of patients with esophageal cancer since date of diagnosis.

increasing incidence of women (used as an indicator of underlying incidence in the general population) was described in a recent report.[16] In the same study, a sex ratio of 1.4 was reported by another cancer registry in Mozambique (Beira Cancer Registry), but only 30 patients were included in the analyses, hindering extrapolation to the general population in that country. Although no information on migration or place of residence could be obtained for the patients included in our study, a report related to the study of migration in Mozambique reveals that there are low internal migration rates in Mozambique, so this lack of information is not expected to have greatly influenced our results.[17] Diagnosis was usually determined at an advanced stage of disease, precluding the curative approach, and nutritional status at diagnosis was usually poor according to clinical data. In a South African study involving 1,868 patients, similar stage, performance status, weight loss, and long history of dysphagia were described; furthermore, only 19.8% of those patients could be treated with curative intent, and squamous cell carcinoma was also the most frequent histologic type.[7] In our study, most patients required nutritional support, as observed in South African reports.[7,18] Gastrostomy was the most frequently performed palliative surgery, but it is often responsible

Table 2. Synopsis of Esophageal Cancer Program in Mozambique

Actions	Summary	Responsibility
Awareness	Increase public understanding about esophageal cancer and the risk factors that can lead to this devastating disease	National Department of Education and Promotion of Health at different levels in the MoH, including specific programs and community organizations/partners, national programs of cancer control
Advocacy	Help patients and their families and caregivers navigate the esophageal cancer landscape	National Department of Education and Promotion of Health at different levels in the MoH
Diagnosis and staging	Improve resources, such as the tests and procedures used to diagnose esophageal cancer and to determine the extent of disease (endoscopy, imaging, and pathology)	National Department of Medical Assistance, central hospitals, donors, and other MoH partners, national programs of cancer control
Medical education and training	Create a multidisciplinary team for esophageal cancer approaches; improve the knowledge and training of all involved in diagnosis, staging, and treatment to optimize patient outcomes	UEM School of Medicine, MCH, MoH, and partners
Treatment	Provide resources for the best treatment options at all esophageal cancer stages, including surgery, neoadjuvant chemoradiotherapy, radical radiotherapy, palliative chemotherapy, pain management, nutritional support, and best supportive care	National Departments of Medical Assistance and Pharmacy, national clinical programs, MCH, donors, and other MoH partners
Follow-up	Organize an outpatient follow-up and patient support service	MCH
Registry	Improve the quality of information in clinical records	Surgical department and oncology service of MCH; cancer registry
Research	Understand the risk factors and other epidemiologic determinants of research in Mozambique; understand the biologic profile of esophageal cancer and advance strategies for preventing the disease	Multidisciplinary team, hospitals and schools of medicine, and internal and external partnerships

Abbreviations: MCH, Maputo Central Hospital; MoH, Ministry of Health; UEM, Universidade Eduardo Mondlane.

for complex skin lesions and additional suffering.[19,20] Stent palliation represents a more efficient approach.[21] However, the retail price for the imported stent is a barrier.[22] Therefore, we have performed palliative surgical procedures, such as bypass. The clinical and pathologic profiles observed in our study are similar to those observed in other African countries in the region.[21-23] This study shows that it is necessary to implement a global esophageal cancer program in Mozambique and at MCH, aiming to increase the imaging stage and treatment of esophageal cancer with radical surgery, chemoradiotherapy, neoadjuvant and palliative chemotherapy, and endoscopic palliation of dysphagia (Table 2). Soon, the use of radiotherapy will also be available in this hospital, which, together with the nutrition and clinical psychology departments, will have an important role in cancer treatment and prehabilitation of surgical patients.[24] The African Esophageal Cancer Consortium, the call to action in esophageal cancer by von Loon et al,[22] could be a useful platform to implement multisite investigation in this field and capacity building and to share research in treatment and palliative care, including the palliation of dysphagia. However, risk factors associated with esophageal carcinogenesis in Mozambique and East Africa are largely unknown.[25] In sub-Saharan Africa, case-control studies have shown that low economic level, consumption of local fermented beverages and foods cooked with charcoal, and smoking may be associated with a high risk of esophageal cancer.[8,24-29] Deficits of selenium and zinc in foods also seem to have an important role in malignant transformation of esophageal epithelium in this region.30 In Kenya, endoscopic evaluation of asymptomatic individuals living in regions with high esophageal cancer rates revealed that 14.4% of patients had dysplastic lesions.[31] In this study, the identification of risk areas was performed using Lugol's solution. However, endoscopy using narrow band imaging seems more effective for those with an early diagnosis of esophageal cancer.[32] In Mozambique, there is currently no information on these issues; therefore, it is necessary to study the local risk factors and promote early diagnosis in high-risk areas (in which the provinces of Gaza, Inhambane, and Maputo seem to be included according to the present data), possibly using endoscopy through narrow band imaging, because this technical resource is available at the MCH gastroenterology department, with Lugol staining improving diagnosis. However, large tumors that prevent endoscopic stent placement, as well as the difficulty of managing the reported feeding gastrostomies, suggest that the role of palliative surgery associated with chemotherapy or radiotherapy should be evaluated in a scientific way in this context.[33]

This study has several limitations that need to be addressed. For 25% of our patients, the diagnosis was on the basis of clinical diagnosis only, endoscopy, or death certificate. The data obtained are from those patients for whom clinical records were available. As a retrospective study, there is a lack of information on some variables (eg, staging, time of follow-up, and outcomes for some patients). In order to overcome these limitations, MCH is currently implementing a hospital-based cancer registry comprising data from 2014 onward that integrates relevant sources of information. [13] However, the quality of information produced by the registry is dependent not only on the organization and availability of the hospital records but also importantly on the quality of the information included in such records. For example, information about disease stage was not present in almost all clinical records and was only available for patients undergoing surgery with curative intent, when this information referred to pathologic stage. A recent study conducted in Tanzania[34] reported a clinical profile and problems similar to those described in our study, which reinforces the need for a regional intervention in this disease.

Esophageal cancer is a commonly diagnosed and treated condition at MCH, but diagnosis is made at an advanced stage of disease, which is related to poor prognosis. This study reveals the necessity for the development of a comprehensive program to combat esophageal cancer in Mozambique.

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AUTHOR CONTRIBUTIONS

Conception and design: Jotamo Come, Clara Castro, Carla Carrilho, Lúcio Lara Santos
Administrative support: Lúcio Lara Santos
Collection and assembly of data: Jotamo Come, Atílio Morais, Matchecane Cossa, Prassad Modcoicar, Satish Tulsidás, Lina Cunha, Vitória Lobo, Alberto Gudo Morais, Lúcio Lara Santos
Data analysis and interpretation: Jotamo Come, Clara Castro, Nuno Lunet, Sofia Cotton, Carla Carrilho, Lúcio Lara Santos
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Jotamo Come
No relationship to disclose

Clara Castro
No relationship to disclose

Atílio Morais
No relationship to disclose

Matchecane Cossa
No relationship to disclose

Prassad Modcoicar
No relationship to disclose

Satish Tulsidás
No relationship to disclose

Lina Cunha
No relationship to disclose

Vitória Lobo
No relationship to disclose

Alberto Gudo Morais
No relationship to disclose

Sofia Cotton
No relationship to disclose

Nuno Lunet
No relationship to disclose

Carla Carrilho
No relationship to disclose

Lúcio Lara Santos
No relationship to disclose

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Affiliations

Jotamo Come, **Atílio Morais**, **Matchecane Cossa**, **Prassad Modcoicar**, **Satish Tulsidás**, **Lina Cunha**, **Vitória Lobo**, **Alberto Gudo Morais**, and **Carla Carrilho**, Hospital Central de Maputo; **Carla Carrilho**, Universidade Eduardo Mondlane, Maputo, Moçambique; **Clara Castro**, **Sofia Cotton**, and **Lúcio Lara Santos**, Instituto Português de Oncologia; **Clara Castro** and **Nuno Lunet**, Universidade do Porto; **Sofia Cotton**, Project ESTIMA-01-0145-FEDER-000027; **Sofia Cotton** and **Lúcio Lara Santos**, Grupo de Patologia e Terapêutica Experimental; **Lúcio Lara Santos** Universidade Fernando Pessoa, Porto, Portugal.

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CHAPTER V - SQUAMOUS CELL CARCINOMA OF THE ESOPHAGUS IN MOZAMBIQUE: MOLECULAR ASPECTS, PRELIMINARY DATA

The etiology of ESCC is multifactorial. The risk factors reported worldwide comprise several lifestyle and environmental and genetic facts [1]. Modifiable risk factors potentially related to ESCC carcinogenesis have been studied in Mozambique (Chapter III). The clinical profile was studied in 522 patients and the pathological characteristics was evaluated in 391 patients (Chapter IV). In order to know the molecular profile of squamous cell carcinoma of the esophagus in Mozambique, we think as soon as we get funding, carry out a study involving our series of patients. However, we carried out a preliminary study in 27 of the cases of our series admitted and treated consecutively at MCH.

5.1. Genetic characteristics of the MCH series (preliminary study)

5.1.1 Introduction

Methods for molecular diagnostics for esophageal cancer

Before the era of molecular biomarkers, esophageal cancer has been classified by histological criteria identifying two different entities, squamous cell carcinoma and adenocarcinoma. However, the molecular oncology arena has evolved tremendously in the last decade. New assays (immunohistochemistry, proteomic, PCR assays, etc) to detect and measure biomarkers that can predict the response to chemotherapy are being developed. Recently Exome sequencing, also known as whole exome sequencing (WES), is a genomic technique for sequencing all of the protein-coding region of genes in a genome (known as the exome). It consists of two steps: the first step is to select only the

subset of DNA that encodes proteins. Genome wide association studies (GWAS) are hypothesis-free methods for identifying associations between genetic regions (loci) and traits (including diseases). Statistical analysis is after carried out to indicate how likely a variant is to be associated with a trait.
A genome-wide association study is an approach that involves rapidly scanning markers across the complete sets of DNA, or genomes, of many people to find genetic variations associated with a particular disease. These techniques have been used to assess the molecular characteristics of ESCC in Africa.

Esophageal cancer genetics in Sub-Saharan Africa

Several studies have been carried out in order to know the genetic ESCC profile in Sub-Saharan Africa. Simba H et al. [2] carried out a literature search on all published African ESCC studies up to April 2019 to systematically assess the evidence on genetic variants associated with ESCC in African populations. They found 25 Single nucleotide polymorphisms (SNPs) in 20 genes namely: ADH1B, ADH3, ALDH2, AR, CASP8, CHEK2, CP, CYP2E1, CYP3A5, GSTT2B, MGMT, MLH3, MSH3, NAT2, PTGS2 (also known as COX-2), PLCE1, PMS1, RUNX1, SLC11A1, and TP53. The associations with all 25 SNPs were identified in South African populations, while none were found in the Sudanese population. All the genes encode for proteins. Three of the genes, ADH1B, ADH3, and ALDH2, are involved in alcohol metabolism [3;4]. Three mismatch repair genes, MLH3, MSH3, and PMS1, play a role in genomic integrity [5]. They are reported to also play a role in carcinogenesis. MGMT is involved in cell defence against mutagens, and mutations in the gene are reported to be associated with cancer formation [4]. NAT2 and GSTT2B play a role in the activation and deactivation of drugs and carcinogens, with reports of mutations being associated with carcinogenesis [6]. Genes regulating cell apoptosis are TP53, CHEK2, and CASP8 [4;7;8;9]. TP53 and CHEK2 are also involved in gene expression and DNA repair. Regulation of gene expression is facilitated by PLCE1 and SLC11A1 [10;4] The AR gene regulates the sex hormones, androgens [11], while CYP2E1 and CYP3A5 are involved in steroid, cholesterol, and lipid synthesis [12;13;14]. CYP2E1 also metabolizes drugs and has been implicated in carcinogenesis. CP facilitates transportation of iron from

organs into the blood cells; RUNX1 plays a role in hematopoiesis and PTGS2 in inflammation and mitogenesis [4; 15; 16]. In terms of HPV infection and its relationship to EC in South Africa, HPV DNA could only be isolated from 9% of ESCC patients. HPV infection appears to only play a minor role in EC [17]. The only multi-omics analysis conducted in an African cohort [18] concluded that the genomic landscape of oesophageal tumours arising in Malawi does not differ from that reported for other high-incidence regions. However, perhaps an undisclosed aetiologic cause of EC in this country might exist, since a new mutational signature was identified and those associated with tobacco use, aflatoxin and mismatch repair genes were absent [19]. It is clear that EC aetiology in Africa is yet poorly defined, and large efforts are needed in order to perform more comprehensive genomics studies in these populations.

5.1.2. Material and Methods

Study sample and DNA collection of esophageal tissue samples were obtained from 27 patients recruited at MCH, Mozambique, between 2012 and 2016. The study is part of the protocol approved by the Mozambican National Bioethics Committee for Health in Mozambique. Cases were histologically confirmed as esophageal squamous cell carcinoma by pathologists at both the MCH and IPO-Porto. Clinical data was collected by review of medical records. Tumour tissues were obtained from surgical resections. Total DNA was extracted from paraffin slides using the QIAamp DNA FFPE Tissue Kit (Qiagen), according to the manufacturer's instructions. From the 27 cases, only 20 had normal adjacent tissue, totalising 47 DNA samples from both tumour and normal tissue.

Targeted deep sequencing

Two out of 47 samples from normal tissue did not have enough quality DNA to proceed with the analysis and were excluded. DNA library was prepared for 45 samples resorting to Ion Chef System (Thermo Fisher Scientific, San Francisco, CA, USA) according to the manufacturer's instructions. Barcoded libraries were generated using Ion 510 & Ion 520 & Ion 530 Kit-Chef (Thermo Fisher Scientific) and Ion Ampliseq Cancer Hotspot Panel v2 (Thermo Fisher

Scientific). This panel consists of a single pool of primers which allows the amplification of 207 amplicons covering 2855 COSMIC mutations in 50 well-established oncogenes and tumour suppressor genes (ABL1, AKT1, ALK, APC, ATM, BRAF, CDH1, CDKN2A, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAS, GNAQ, HNF1A, HRAS, IDH1, JAK2, JAK3, IDH2, KDR, KIT, KRAS, MET, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, SRC, STK11, TP53, and VHL). Ion Sphere Particles (ISP) were used to allow the clonal amplification of the constructed libraries by emulsion PCR, in the Ion Chef System, following the manufacturer's instructions. Finally, after loading the enriched ISPs onto 510 and 530 chips, amplicons were sequenced using Ion S5 System (Thermo Fisher Scientific). This means that the methodology used is limited when compared to the NGS and that many genes studied and found in other studies carried out in African EC samples were not studied by us.

Variant calling

Low-quality sequence reads were removed, and those passing the quality control (QC) step were filtered, trimmed and aligned to the reference genome GRCh37/hg19, using Torrent Suite™ Software 5.12. The threshold for quality control score was ≥20 somatic. The variant caller used was Torrent Variant caller (TVC) v5.12, a plugin integrated in the Torrent Suite™ Software 5.12 that accepts the BAM files resulting from the alignment step directly as input.

Variants were called in both tumour and matched-normal pairs and the resulting VCF files were merged using BCFtools. The identification of somatic variants was made by comparison between tumour and normal determined genotypes. For the VarScan [20] analysis, SAMtools pileup-format files (the file format accepted by the algorithm) were generated from each sample's tumour and normal BAM files. VarScan then applies a holistic approach for genotype detection independently on both samples, in those positions which meet minimum coverage and quality criteria. For somatic detection, the algorithm directly compares both tumour and normal calls based on the number of read counts supporting each allele by Fisher's exact test. Two output files are created in the analysis, a SNVs file and an indels file. The two data files were

then concatenated and those variants with the “SOMATIC” label in the INFO field of the resulting VCF file were selected. Using BCFtools, somatic variants located within enriched positions in the Ion Ampliseq Cancer Hotspot Panel v2 were selected.

Somatic Sniper [21] reads tumour and normal BAM files and determines differences at a genotype-level between these two. Through the implementation of the MAQ model for genotype likelihoods, the algorithm calculates the probability that the tumour and normal genotypes are different. The output VCF file was then filtered, using BCFtools, by those somatic variants located in the regions covered by the Ion Ampliseq Cancer Hotspot Panel v2. The identified somatic variants were then annotated using wANNOVAR [22]. All single nucleotide variants were examined for previously reported hotspot somatic mutations reported in the Catalogue of Somatic Mutations in Cancer (COSMIC) database.

5.1.3. Results and discussion

Samples clinical data

Our cohort (Table 5) included 17 females (63%) and 10 males (37%). The higher percentage of females ESCC patients is usual at MCH but does not reflect the pattern generally observed in other countries of the AECC, where the incidence of ESCC is higher in males [23].

Clinical information regarding age at diagnosis, residence area, alcohol and tobacco consumption, vegetables fruit intake and use of charcoal and/or wood was available for all patients, except for one individual (Table 5).The mean age at diagnosis was 53,2 years (standard deviation 9,0 years; range 33-70). Two patients were aged between [20-39] years, 16 were aged [40-59] and the remaining eight patients were over 60 years old. The average age at diagnosis was lower than the median age in other ESCC hotspot regions of Sub-Saharan Africa [2]. The majority of ESCC patients lived in urban settings except for three individuals living in rural areas. Six patients reported being frequent alcohol consumers. All five individuals that informed being tobacco users also consumed alcoholic beverages. The remaining 15 individuals stated no alcohol or tobacco use. Twenty patients reported the frequent use of charcoal or wood

for cooking, while 5 rarely used these methods and another reported never to use them in cooking. Vegetables and cereals consumption was frequent for 24 individuals, rare for one patient and non-existent for another.

Somatic mutations

DNA was successfully isolated for 45 samples for which the DNA library was prepared. Nevertheless, 12 tumour-normal pairs were excluded due to insufficient DNA amount or low sequence depth of one of the sample pairs. For the remaining 15 tumour- normal matched samples, the estimated mean depth was roughly 7,525x in all amplicons. The genomic DNA obtained from the 15 ESCC Mozambican patients was screened for somatic mutations in the 50 oncogenes and tumour suppressor genes. Only mutations found over 15% and 20% were considered for normal and tumour tissue, respectively. In total, 13 somatic variants were detected in seven different genes, from which 11 were previously reported in COSMIC and two were novel mutations. Of the somatic mutations, nine are non-synonymous, one non-frameshift, one splicing and two stop variants. No deletions or insertions were observed (Table 5, Figure 7). The median number of somatic mutations per sample pair was one (rage, 0-3).

Most mutated genes presented only one variant each that occurred only once in the tumour-normal pairs cohort (Table 6) and few tumours presented more than one gene mutated. Genes VHL, CTNNB1 and TP53 were mutated in sample ESO11, resulting in mutations c.C286T:p.Q96X, c.C125T:p.T42I and c.C265T: p.P89S, respectively. The mutation in VHL gene was reported in COSMIC but not described in the esophagus or in squamous cell carcinomas. c.C125T:p.T42I in CTNNB1 gene was reported in vulvar squamous cell carcinoma. Tumour ESO23 showed somatic mutations in genes TP53 and FGFR3, not yet described in the esophagus. Yet, mutation c.C746G:p.S249C in FGFR3 was reported in upper aerodigestive tract squamous cell carcinomas (UADTSCC; mouth, pharynx, larynx, head and neck or tonsil). Tumour ESO27 presented somatic mutations in genes CDKN2A and TP53, resulting in mutations c.C247T:p.H83Y and c.G351T:p.R117S, respectively. CDKN2A mutation was reported in ESCC and UADTSCC, as well as EAC. Finally, mutation in gene PIK3CA was also already largely described in ESCC and UADTSCC in 19 and 69 tissue samples, respectively. TP53 gene is as

expected the most mutated gene in this cohort with seven variants, all in different patients. Previous studies have already reported a significant association of TP53 mutations in ESCC [24] . Six TP53 mutations were missense and located in exons 1, 3 and 4 (Table 6). Locus Chr17:7577534 was mutated in two different individuals but the different nucleotide substitutions resulted in the same amino acid change (c.G351C:p.R117S and c.G351T:p.R117S). All these six TP53 somatic mutations were already reported in COSMIC and almost all in the esophagus, namely in ESCC patients, except for c.G351C:p.R117S. Interestingly, all TP53 mutations including the former not reported in esophagus were reported only in the upper aerodigestive tract (UADT), specifically in squamous cell carcinomas. Frameshift substitution c.356_357AA: p.I119K also occurred in TP53 and was not yet described in COSMIC. Tumour ESO08 shows one of the novel mutations, a non-frameshift mutation in gene TP53 and located in exon 3 (c.356_357AA: p.I119K). The other novel mutation occurs in tumour ESO25, a splicing mutation in gene NPM1. Due to the small sample size and limited clinical data available, no significant association between molecular and clinical features may be concluded.

Table 5. Patient's clinical characteristics and sociodemographic features of the patients

Feature	Category	Cohort (n=27)*
Age	Mean	53
	Range	33-70
Sex	Male	10
	Female	17
Residence	Rural	3
	Urban	23
Alcohol	Yes	11
	No	15
Smoking	Yes	5
	No	21
Wood/Charcoal	Never	1
	Rare	6
	Frequent	20

Cereals/Vegetables	Never	1
	Rare	1
	Frequent	24

*One individual had no available information regarding age at diagnosis, residence area, alcohol and tobacco consumption, vegetables fruit intake and use of charcoal and/or wood.

Table 6. Somatic mutations reported in the ESCC Mozambican cohort.

Mutations listed in this table were identified by deep targeted sequencing using Ion 'Ampliseq Cancer Hotspot Panel v2'. Only mutations over 15% for normal tissue and 20% for tumour tissue were considered. All variants were surveyed for previously reported hotspot somatic mutations in COSMIC database, including occurrence in esophageal tissue with histological subtype of squamous cell carcinoma.

Gene	Exon	Position	Nucleotide	Protein	COSMIC	Reported in ESCC (n)
TP53	3	Chr17:7577528	AA*	I119K	-	No
TP53	3	Chr17:7577579	C/A	Y102X	COSM5047027	Yes (1)
VHL	1	Chr3:10183817	C/T	Q96X	COSM17658	No
CTNNB1	3	Chr3:41266128	C/T	T42I	COSM5696	No
TP53	4	Chr17:7578548	C/T	P89S	COSM44397	Yes (1)
PIK3CA	10	Chr3:178936082	G/A	E542K	COSM760	Yes (19)
TP53	1	Chr17:7578403	G/T	C44F	COSM10645	Yes (20)
FGFR3	7	Chr4:1803568	C/G	S249C	COSM715	No
TP53	3	Chr17:7577534	G/C	R117S	COSM10785	No
TP53	1	Chr17:7578526	G/A	C3Y	COSM10801	Yes (2)
NPM1	-	Chr5:170837525	splicing	-	-	No
CDKN2A	2	Chr9:21971111	C/T	H83Y	COSM12504	Yes (1)
TP53	3	Chr17:7577534	G/T	R117S	COSM10817	Yes (7)

*nonframeshift substitution

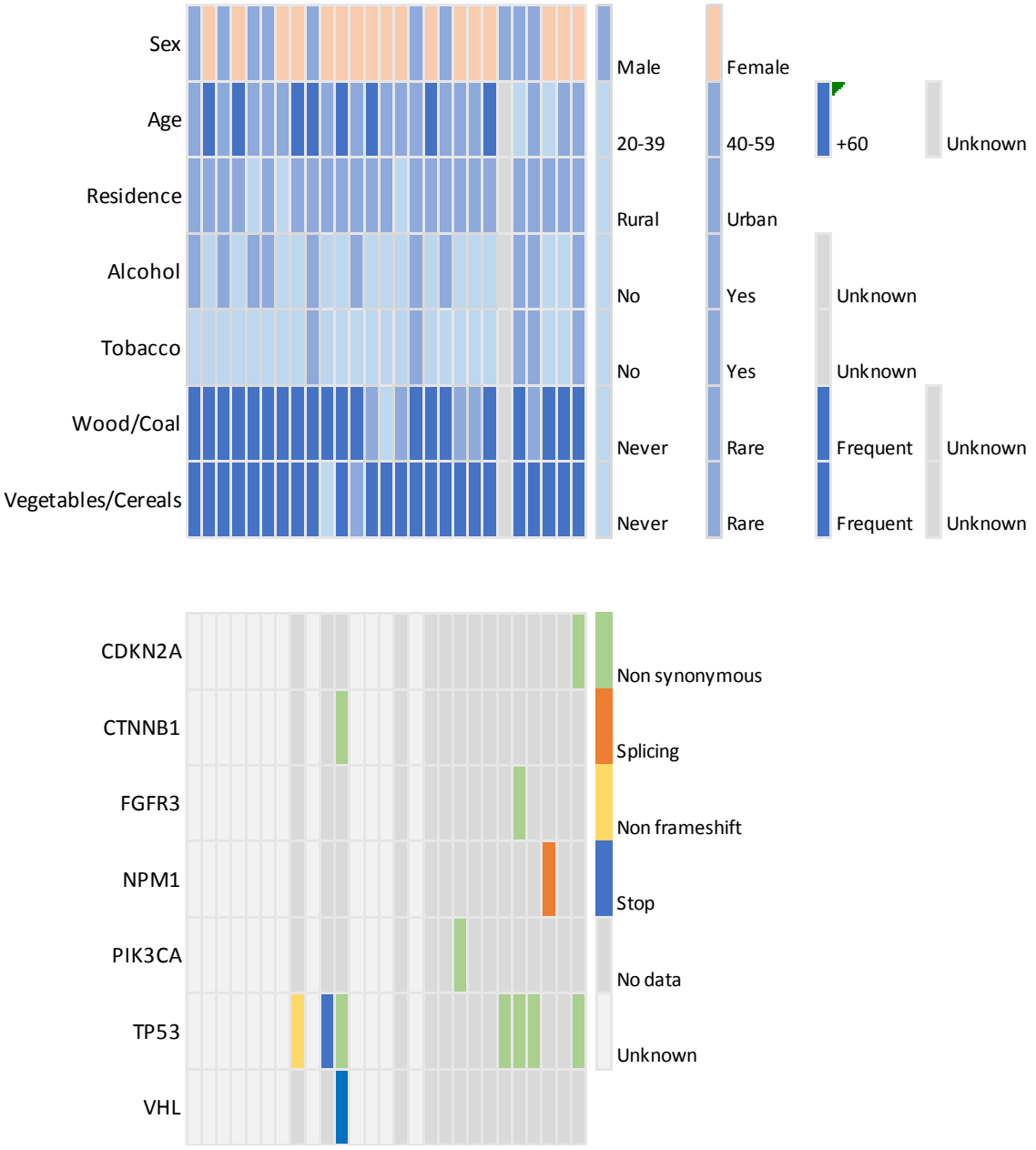

Figure 7. Clinical and molecular data of the 27 ESCC Mozambican patients. Each column represents an individual.

In the Figure above, clinical and environmental features are represented in the upper matrix, where each row is a specific characteristic followed by the corresponding colour scheme. Somatic mutations of each tumour-normal matched pair are represented in the lower matrix, where each row represents a

gene. Each mutation is colour coded, corresponding to a single mutation. Fifteen patients had the tumour-normal matched pairs characterised.

5.2 Tumor biomarkers in the MCH series (preliminary study)

5.2.1. Introduction

There is growing evidence that tumor biomarkers may be useful in diagnosis and prognosis in esophageal squamous cell carcinoma (ESCC). We decided to study a panel of biomarkers that can be studied by immunohistochemistry (techniques that can be performed in Mozambique) and with prognostic significance already demonstrated in our series. The studied biomarkers and their meaning are referenced below.

Glucose transporter-1 (GLUT-1) is a membrane receptor that mediates the passive export of glucose across plasma membranes and its expression increases in hypoxic conditions due to reduced oxidative phosphorylation and hypoxia inducible factor-1 (HIF-1) induction [25]. GLUT-1 facilitates the metabolic adaptation of cells to hypoxia and is essential for survival and proliferation of glycolytic metabolism [26] Patients with a high GLUT-1 expression had significantly poorer local control and lower recurrence-free survival [27]. Carbohydrate antigen 19-9 (CA19-9), also known as sialyl-Lewis A, is a tetrasaccharide which is usually attached to O-glycans on the surface of cells. It is known to play a vital role in cell-to-cell recognition processes. It is also a tumor marker used primarily in the management of pancreatic cancer [28]. CA19–9 is a prognostic indicator for poor overall survival at ESCC CA19–9 is a prognostic indicator for poor overall survival in ESCC on the other hand ESCC patients with low CA19–9 may be more likely to benefit from the postoperative chemotherapy [29]. High Carbonic anhydrase IX (CA IX) is a transmembrane glycoprotein related to hypoxia. CA IX expression is associated with shorter survival in SCC esophageal cancer, and the hypoxic phenotype seems to be preserved at least during formation of lymph node metastasis [30]. Hypoxia-inducible factor-1 (HIF-1) is a regulatory protein, mainly responsible for

maintaining oxygen homeostasis in response to reduced oxygen concentration in cells and tissues. HIF-1α promotes metastasis of ESCC by targeting specific protein 1 involved in regulation of cell proliferation, apoptosis in a hypoxic microenvironment [31]. Sialyl-Tn antigen (STn) is a short O-glycan containing a sialic acid residue α2,6-linked to GalNAcα-O-Ser/Thr and is related with poor prognosis in SCC esophageal cancer [32; 33]. KI-67 is a nuclear protein that is associated with cellular proliferation. High Ki-67 LI is correlated with lymph node metastasis and can be used as an independent prognostic factor for ESCC patients [34]. Strong p53 expression is correlated with unfavourable prognosis in esophageal cancer and represents an independent poor prognostic in SCC [35]. Lysyl oxidase (LOX) enzyme has attracted attention due to its involvement in tumor progression in various cancers. LOX expression was associated with poor prognosis in ESCC and was regulated epigenetically by genome-wide hypomethylation. It could serve as a prognostic biomarker in ESCC patients, and therapeutically targeting LOX could reverse the progression of esophageal cancer [36]. Lysyl oxidase-like 4 (LOXL4), a member of the LOX family proteins, catalyzes oxidative deamination of lysine residues in collagen and elastin, which are responsible for maintaining extracellular matrix homeostasis. The analyses based on the protein–protein interaction network depicted the expression of LOXL4 and its associated proteins as well as their functions, suggesting that LOXL4 and its associated proteins may serve a significant role in the development and progression of ESCC [37].

5.2.2 Materials and methods

The screening of relevant clinical markers was performed retrospectively in a series of 26 formalin-fixed paraffin-embedded (FFPE) esophageal tumor tissues obtained from archived paraffin blocks from patients recruited at MCH, Mozambique, as described above (5.1.2). Esophageal tumors were removed from 9 men and 17 women, ranging from 33 to 70 years of age (median 51±9 years), admitted and treated in the institution between 2014 and 2016. The study is part of the protocol approved by the Mozambican National Bioethics Committee for Health in Mozambique

Formalin-fixed paraffin embedded (FFPE) EC tissue sections were screened by immunohistochemistry for p53, hypoxia-related markers (HIF1 alpha and CAIX), proliferation (KI-67), GLUT1, LOX and LOXL4. Additionally, glycosylation markers used in clinical serological analysis were also incorporated, namely Sialyl Lewis A and Sialyl Tn.

Briefly, three micrometers gastric tumour sections were deparaffinized, rehydrated and incubated for 15/20min with boiling citrate buffer (Vector) or EDTA 1mM pH 9, after pre-heating of the solution at maximum power rating for 5 min. Sections were incubated with 3% hydrogen peroxide (Leica) for 5 min, blocked with Protein Block® (Leica) and incubated in a wet chamber with the different antibodies. Experimental conditions for antigen analysis were summarized on Table 7. After washing with PBS-Tween, biotinylated secondary antibody (Post-primary -Leica) was added to tissue sections, before incubation with polymer (Leica). Antibodies' binding was detected by incubation with 3,3′-diaminobenzidine (ImmPACT™ DAB, Vector Laboratories, SK-4105) for 5 min. Nuclei were counterstained with hematoxylin for 1 min. Positive and negative control sections were tested in parallel. Negative control sections were performed using 5% BSA-PBS devoid of primary antibody. Positive controls consisted of known positive tumor tissues for the antigen in study. Tumors were classified as positive when immunoreactivity was observed by microscopic presence of marked brown chromogenic product in tumor cells and negative when the presence of brown chromogenic product was absent or weak (according to extension and/or intensity). Antibodies' staining was assessed double-blindly by two independent observers and validated by an experienced pathologist. Whenever there was a disagreement, the slides were reviewed, and consensus was reached.

Statistical analysis

Statistical data analysis was performed with IBM Statistical Package for Social Sciences–SPSS for Windows (version 25.0). Mann–Whitney U test were used. Chi-square analysis was used to compare categorical variables. Comparison of

estimates was done using log-rank test. A 95% significance threshold for the null hypothesis was considered $p < 0.05$.

Table 7. Antibody properties and experimental conditions employed in immunohistochemistry procedures.

Antibody	Manufacture	Reference	Clonality	Clone	Host	Experimental conditions
Anti-STn (Sialyl Tn)	Abcam	ab199002	Monoclonal	B72.3 + CC49	Mouse	0,5µg/mL, 4ºC overnight
Anti-CA19.9 (Sialyl Lewis A)	Abcam	ab116024	Monoclonal	CA19.9-9-203	Mouse	1:100, 4ºC overnight
Anti-LOX	Abcam	ab174316	Monoclonal	EPR4025	Rabbit	1:250, 4ºC overnight
Anti-LOXL4	Abcam	ab262890	Polyclonal	-	Rabbit	1:100, 4ºC overnight
Anti-KI67	DAKO	M7240	Monoclonal	MIB-1	Mouse	1:150, 1h room temperature
Anti-p53	DAKO	M7001	Monoclonal	DO-7	Mouse	1:250, 1h room temperature
Anti-HIF1 alpha	Abcam	ab51608	Monoclonal	EP1215Y	Rabbit	1:600, 30’ room temperature
Anti-GLUT1	Abcam	ab196357	Monoclonal	EPR3915	Rabbit	1:750, 30’ room temperature
Anti-CAIX	Thermo Fisher Scientific	MA5-15737	Monoclonal	2D3	Mouse	1:500, 1h room temperature

5.2.3. Results and discussion

The screening of above-mentioned antigens was performed on 26 EC FFPE tissue sections, being the immunohistochemical results summarized in Figure 8A. In summary, LOXL4 was negative in all cases (Figure 8B) and by the other hand GLUT1 was strongly and diffusely expressed in all cases (Figure 8C). Remain antigens presents either negative or positive phenotypes (Figure 8D and 8E). STn, SLeA, and GLUT1 were predominantly found at the cell surface

of tumour cells, while Ki-67 and p53 showed positive nuclear expression, not being identified any particular pattern of staining. HIF1 alpha, CA IX and LOX were mainly found in the cytoplasmic compartment of tumour cells. The expression of HIF1 alpha and CA IX was mainly found in ischemic areas as expected according to literature.

A

Antigen	Positive cases (%)	Negative cases (%)	Total
P53	21 (81)	5 (19)	26 (100)
HIF1 alpha	12 (46)	14 (54)	26 (100)
CA IX	15 (58)	11 (42)	26 (100)
LOX	11 (46)	13 (54)	24 (100)
LOXL4	0 (0)	26 (100)	26 (100)
GLUT1	26 (100)	0 (0)	26 (100)
KI67	20 (77)	6 (33)	26 (100)
STn	10 (39)	16 (61)	26 (100)
SLeA	10 (39)	16 (61)	26 (100)

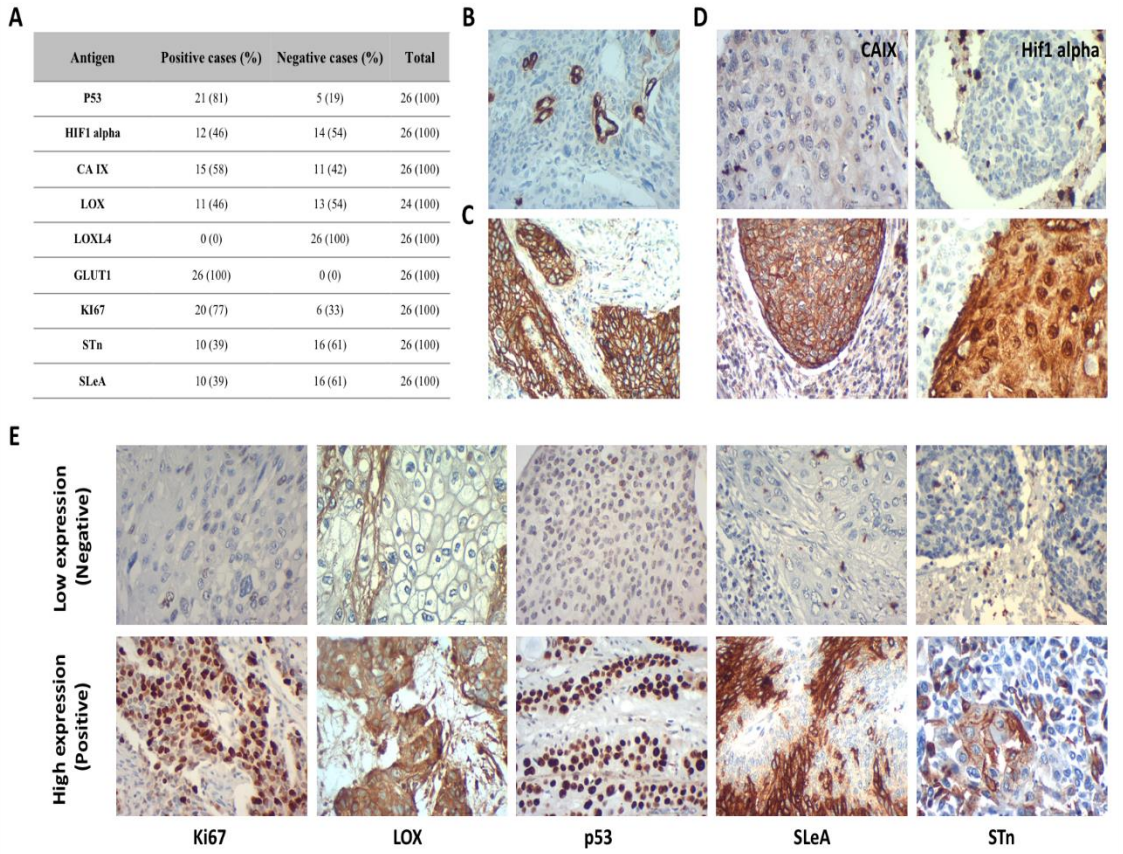

Figure 8. Frequencies of antigen expression in Mozambique EC cases (A). Illustrative representation of LOXL4 negative phenotype (B) and GLUT1 positive phenotype (C) in EC tumour sections. Representation of HIF1 alpha and CAIX, hypoxia-related proteins, in Mozambique EC tumours (D). STn, SLeA, LOX, p53 and KI67's pattern of expression in tumour tissues (E).

The expression of each antigen is independent of the expression of the remain analyzed antigens, being only observed a concomitant expression of STn and SLeA, both markers of aberrant glycosylation in several tumour models (Table

8, *p*=0,001). Further, the correlation of STn and SLeA expression with relevant clinicopathological variables, namely clinical stage, presence of metastasis, histological type and others, should be stablished to propose an implantation of this classical serological markers into the clinical routine to predict treatment response, tumour aggressiveness and prognosis. These two markers, as well as the remain studied antigens that segregate distinct groups of patients, shown potential to be integrated into stablished stratification systems and improve medical care in Mozambique oncologic practice.

Previous studies demonstrate that immunohistochemical expression of P53 is associated with severity of histological alterations and can be useful to stratify a group of asymptomatic smokers and alcohol drinkers with normal mucosa or chronic esophageal inflammation with higher risk to progress to ESCC, that could benefit from surveillance [38,39]. No correlation between antigen expression and lifestyle was observed in this retrospective study, namely with exposure to coal, consumption of vegetables/ cereals, tobacco and alcohol.

More-in-depth studies should be conducted to disclosure the impact of lifestyle factors in the carcinogenic process of Mozambique EC patients and its reflection in the expression of relevant cancer-related antigens.

Table 8. Correlation between STn expression and SLeA expression among EC cases.

		SLeA		
		Negative n (%)	Positive n (%)	*p* value
STn	Negative n (%)	14 (87.5)	2 (12.5)	***0.001***
	Positive n (%)	2 (20)	8 (80)	

Most of the cases studied express biomarkers that are associated with poor prognosis. Unfortunately, it was not possible to study the survival of these patients in this preliminary study. In the future with the survival data we will be

able to assess the significance of these biomarkers in the ESCC in Mozambique.

5.3 TP53 mutations in MCH series (preliminary results)

TP53 gene is, as expected, the most mutated gene in MCH cohort with seven variants, all in different patients (Table 6, above). In our series tumour ESO08 shows one of the novel mutations, a non-frameshift mutation in gene TP53 and located in exon 3 (c.356_357AA: p.I119K). Frameshift substitution c.356_357AA: p.I119K also occurred in TP53 and was not yet described in COSMIC.

Structural organization for the human TP53 gene is showed in Figure 9. Classical exons 1 to 11 are shown in green in the figure below, with exon 1 which is noncoding. Exons 2 to 11 are translated into a full-length TP53 protein (P1, 393 residues). Exons 9 beta (red) and gamma (blue) are used via an alternative splicing leading to different transcripts (see below). Exons beta and gamma contain STOP codons and lead to truncated proteins missing part of the carboxy terminus of the protein. Translation of exons 9 beta and gamma adds 10 residues and 15 residues, respectively.

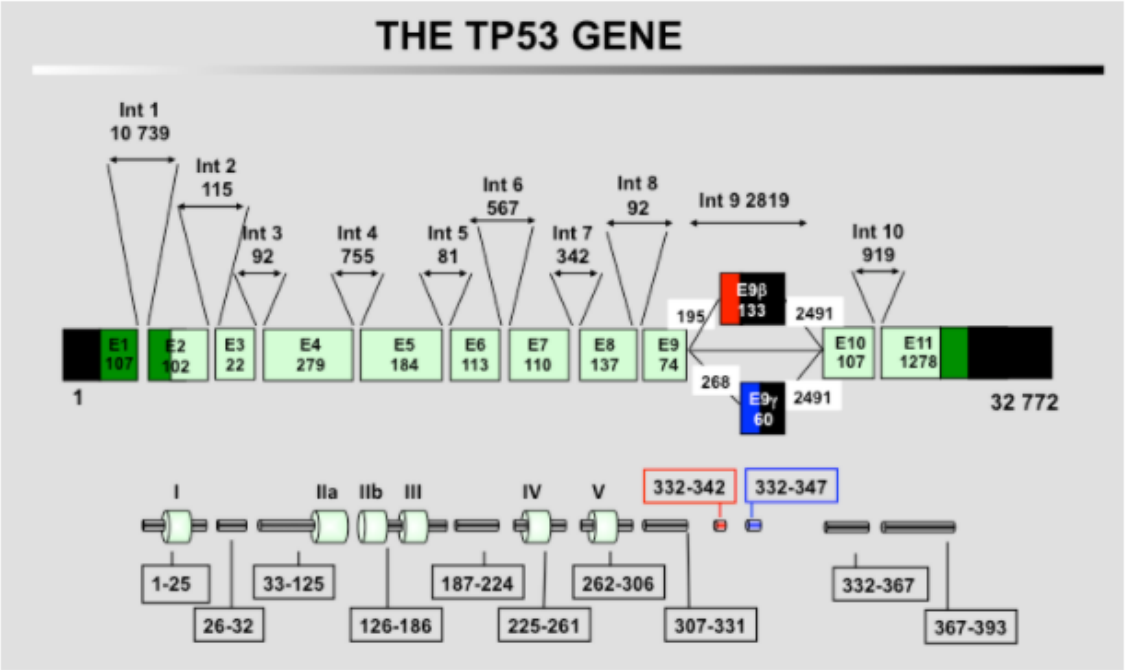

Figure 9. Structural organization for the human TP53 gene (https://p53.fr/tp53-information/tp53-knowledge-center/26-knowledge-center/4-the-tp53-gene)

As can be seen in the Figure 10, the TP53 gene mutations observed in our series are found in the less frequently mutated codons in the series studied by Hjortsberg and Soussi [40] and corresponded to 4 transversions (C>A;G>T; G>C; G>T) and 2 transitions (C>T; G>A).

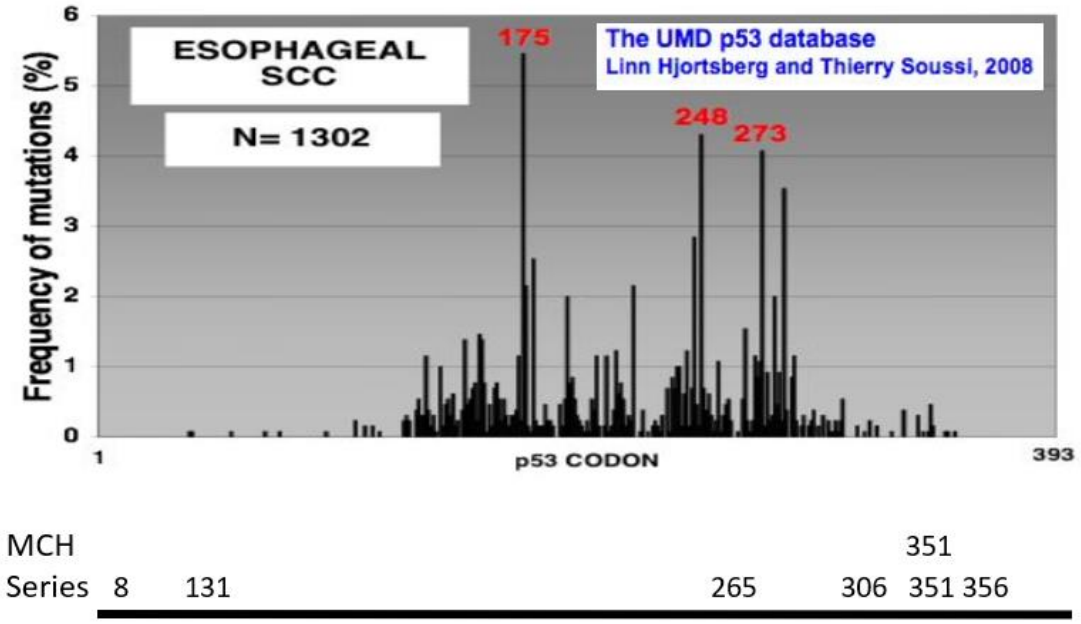

Figure 10. p53 codon hotspots

Usually G > T transversion is present in tumors of smokers and may be generated by benzo(a)pyrene 7,8-diol-9,10-epoxide adduct formation at guanosine [41]. In our series with TP53 mutations only 3 patients had smoking and heavy alcohol habits but all used charcoal to cook.

Additionally, a correlation between TP53 mutational status and p53 protein expression was established. All cases with TP53 mutations in the DNA mutational analysis were positive in the immunohistochemical studies, showing the potential of DO-7 clone in the detection of frequent P53 mutations in Mozambique EC. Nevertheless, DO-7 clone also detects native p53 protein, limiting the application of DO-7 clone antibody in the detection of mutant p53 isoforms.

This preliminary study allows us to understand the need to conduct the study of the ESCC's genetic profile in Mozambique and to provide pathology services with resources that make it possible to study predictive biomarkers of early diagnosis, poor prognosis or response to therapy.

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CHAPTER VI - PREVENTION, EARLY DETECTION PROGRAM AND BEST TREATMENT APPROACH

The Ministry of Health and the First Lady of Mozambique have launched national cancer control plan (NCCP) 2019-2029. During the launch, the First Lady underlined the importance of education, prevention and health promotion [**1**]. NCCP is a effective instrument for prevention and early detection, since it integrates existing health systems and related services, the different programs that ensure systematic and equitable implementation of control strategies across the continuum of prevention, early detection, treatment and palliative care as set out in WHO's guidelines. "Key Prevention and Control Interventions for Reducing Cancer Burden in the WHO African Region" is a useful WHO publication to help in organizing cancer prevention and early detection national program [2]. The goal of primary prevention is to reduce or eliminate exposure to cancer-risk factors, which include modifiable factors. The National Strategic Plan (NSP) for the prevention and control of NCDs in Mozambique has as its aim to create a positive environment to minimise or eliminate the exposure to risk factors and guarantee access to care [3]. The main objective of early detection is to detect precancerous changes or early stage cancers when they can be treated most effectively. Therefore, ESCC cancer morbidity and mortality could be prevented by implementing evidence-based interventions to reduce cancer risk factors, increase early detection of cancer and ensure the best treatment [4]. In this chapter we summarize the different aspects that were relevant during the realization of this thesis which we suggest that they be included in a comprehensive program to control esophageal cancer in Mozambique to be developed by the Ministry of Health. We cannot fail to mention that the formation of the multidisciplinary team is relevant as an instrument of competent and proficient action in the application of that program. This team involves specialists in the field of epidemiology, medical oncology, gastroenterology, pathology, surgical oncology, radio-oncology, pharmacy,

nursing, psycho-oncology as well as the primary health structures and their teams that already work with populations in HIV programs, breast and cervical cancer. In this sense the recently approved National Curriculum to Advance Surgical Oncology in Mozambique is a fundamental instrument in the training of surgical teams, as well as the resident training program in radioncology and medical oncology [5]. The surgical complexity of treating esophageal cancer requires that patients be prepared prior to surgery. In this sense, a pre-qualification program is necessary [6]. All the aspects referred should be part of the *prevention, early detection and best treatment approach program*, including the interventions that are listed below.

6.1 Recommended interventions

- The formulation of a national EC cancer policy and strategic plan as well as advocacy and cancer awareness;
- Provide a global education about EC cancer risks and prevention and to incorporate these concepts into the school curriculum;
- Disseminate information on EC cancer prevention to the general public, including environmental and domestic occupational carcinogen;
- Advocate for behaviors favoring EC cancer prevention such as healthy diet, oral hygiene, reduce alcohol intake, stop smoking, adopt policies that allow providing good drinking water, rural well ventilated grain silos, distinct energy alternatives to PAHs and allow food preservation contaminated, create conditions for better population housing and direct educational programs at schools and workplaces on esophageal cancer;
- Avoid chronic consumption of drinks or food at elevated temperatures;
- Educational/training programs for health providers regarding EC, which include learning about modifiable risk factors, and clinical signs that ensuring an early diagnosis;
- Promote early diagnosis by creating increased awareness of early signs and symptoms of detectable and curable tumours that have high prevalence in the community, such as breast, cervical, prostate and esophageal cancers.

- To include dysphagia alert program that includes dysphagia awareness, an episode of dysphagia in people over 39 years of age or living in high-risk places (of any age), or anyone with episodes of recurrent dysphagia should be clinically investigated;
- Train gastroenterologists in the diagnosis of premalignant esophageal lesions;
- The establishment / reinforcement of reliable and sustainable sources of EC data collection, particularly cancer registries (hospital or population based). Identifying the regions where EC is most prevalent in Mozambique;
- Integrate into this program and collaborate with other health programs, e.g. malaria, HPV, schistosomiasis, AIDS control, hepatitis B, etc., for strengthening their results;
- Application of effective tobacco control measures; Mozambique have signed the WHO Framework Convention on Tobacco Control;
- Application of effective alcohol abuse control measures;
- Application of effective food safety control measures;
- Application of effective drink water safety plan;
- Develop a clear policy for diagnosis, referral, treatment and follow-up of all cancer cases (including EC);
- Application of multidisciplinary EC treatment approach;
- Application of the best treatment approach according to existing resources;
- Train surgical oncologists according to the recently approved National Curriculum to Advance Surgical Oncology;
- Optimize the resources of imaging, endoscopic, laboratory, radiotherapy, chemotherapy, target therapies and oncological facilities;
- Identify the genetic changes and the relevant biomarkers in the identification of risk EC populations and stratification of the prognosis;
- Identify research priorities for EC in the country;
- Mobilize funds from sustainable sources for funding researches;
- Facilitate capacity building in EC research at various levels of the health system, including genetics;

- Promote collaboration between various stakeholders involved in EC research;
- Enhance EC research capacity in the country.
- Promote a creation of surgical oncologic unit in the MCH and implementation of esophageal cancer pathway as proposed in the attachments

We believe that the program should be built with maximum speed and should be implemented in Mozambique, in order to reduce exposure to esophageal cancer risk factors, to raise people awareness , adopt healthy lifestyles and best treatment protocol shown in the Figure 11.

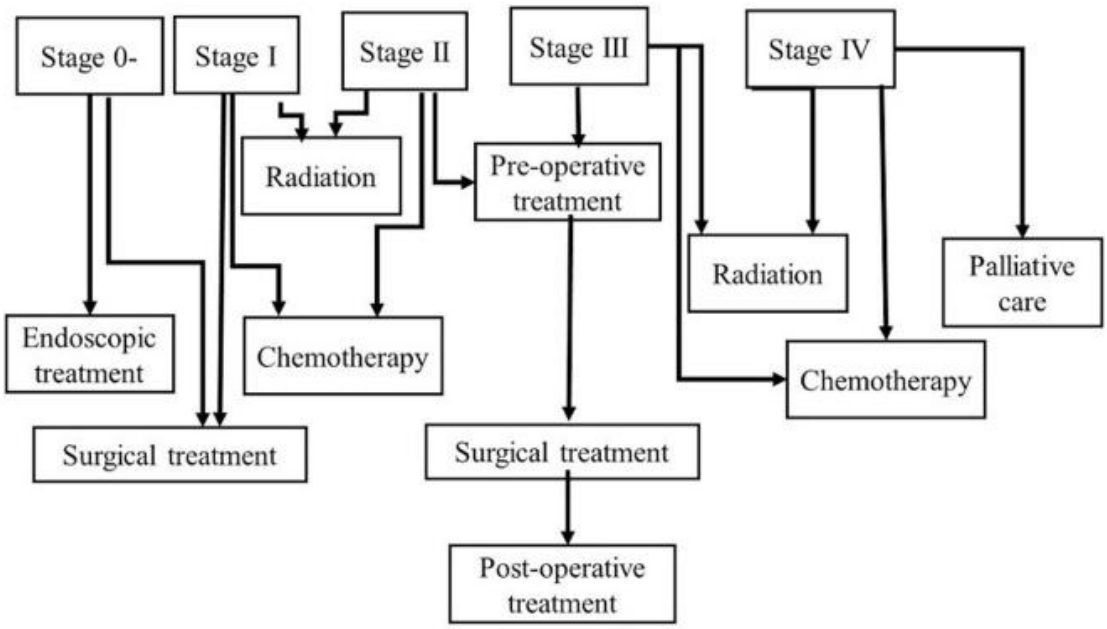

Figure 11.Treatment of oesophageal cancer at different stages (adapted from NCI protocols).

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CHAPTER VII – CONCLUSIONS AND FUTURE PERSPECTIVES

Conclusions

Esophageal cancer is one of the most common cancers in Mozambique, and the number of cases is predicted to more than double simply as a result of demographic changes . Its diagnosis is late taking many lives. Modifiable risk factors associated with this cancer and identified in African populations are also present in the country. A prevention and early detection program must be set up. This must be combined with a treatment standard that includes best practices according to the country's resources.

Future Perspectives

In future we hope to implent a project/study of prevention and early detection strategy to challenge the burden of esophageal cancer in Mozambique. The main goal of this project will be achieved by:

Characterisation of the oral microbiome by Next Generation Sequencing (NGS) of the 16S ribosomal RNA (rRNA) that affords information for secure identification and quantification of genera inhabiting the human mouth.*[7*-9]

Characterisation of plasma metabolome by Mass spectrometry (MS)-based metabolomics, providing both qualitative and quantitative data [10] of ~100 metabolites (proteins, carbohydrates, lipids, vitamins and minerals) associated with African population’s genetic markers [11-14], nutrients and minerals associated with OC risk [15-20, 21, 22] and mycotoxin FB1 [23].

1. Assessment of the EC genetic susceptibility of Mozambican versus Angolan populations by genotyping nearly 1 million informative markers using the population-optimized Axiom AFR 1 Array (Affymetrix Inc.) in patient and control samples through a genome-wide association test.

2. Identification of informative risk biomarkers from different backgrounds by statistical integration of all datasets: genetic + metabolite + oral microbiome + natural micronutrient deficits + harmful lifestyle and diet behaviours. Implementation of an easy and cheap risk assessment tool, incorporating the calculation of EC risk. Local testing in Mozambique for identification of individuals and/or regions with higher risk of EC. This strategy will launch the roots of an informed prevention campaign with tailor-made actions focused on populations at higher risk of developing the disease. These actions may consist in providing fortified/enriched food, supplements use and/or fortified fertilizers. OHANA-nkumi awareness strategy will be a concerted action in hospitals and pilot Mozambican villages. A population-based awareness strategy for EC risk factors also needs to promote good health practices (e.g. better nutrition habits; discourage smoking and alcohol consumption) as a primary preventive intervention, with particular focus on women.
3. OHANA-nkumi will test the possibility of immunotherapy treatment for advanced EC African patients. We will target PD-1/PD-L1 complex, which inhibition demonstrated tumour shrinkage and overall improved survival in patients with other cancer types [15-17]. We will begin by in vitro testing of this therapy in EC cell lines of African ancestry, checking its expression in Mozambican EC cases.

Soon, we also perspective to implement pathways and protocolos to improve and standatize the esophageal cancer aproch in terms of management and clinical tratament over the country.

CHAPTER VIII – ATTACHMENTS

8.1 ATTCHMENT I - Scientific publications related to thesis as co-author

ecancermedicalscience

Identifying barriers and finding solutions to implement best practices for cancer surgery at Maputo Central Hospital, Mozambique

Atílio Morais[1], Matchecane Cossa[1], Adriano Tivane[1], Jotamo Come[2], Volodimir Venetsky[2], Fernando Torres[2], Victor Pacheco[3], Miguel Reyes[3], Germano Pires[4], Mariana Peyroteo[5], Satish Tulsidas[6], Ellen Baker[7], Moshin Sidat[8,9], Maria do Rosário O Martins[9], Lúcio Lara Santos[5,10,11]

[1]Thoracic Surgery, Surgical Department, Maputo Central Hospital, Av Agostinho Neto n° 164, Maputo 1164, Mozambique
[2]Breast Surgery, Surgical Department, Maputo Central Hospital, Av Agostinho Neto n° 164, Maputo 1164, Mozambique
[3]Colorectal Surgery, Surgical Department, Maputo Central Hospital, Av Agostinho Neto n° 164, Maputo 1164, Mozambique
[4]Heath National Institute, Av Eduardo Mondlane, Maputo 264, Mozambique
[5]Surgical Oncology Department, Portuguese Institute of Oncology, Dr António Bernardino de Almeida Street, Porto 4200-072, Portugal
[6]Medical Oncology Department, Maputo Central Hospital, Av Agostinho Neto n° 164, Maputo 1164, Mozambique
[7]UT MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030, USA
[8]Department of Community Health, University Eduardo Mondlane, Av Julius Nyerere, Maputo 257, Mozambique
[9]Global Health and Tropical Medicine, Institute of Hygiene and Tropical Medicine, Universidade Nova de Lisboa, Junqueira Street 100, Lisboa 1349-008 Portugal
[10]Experimental Pathology and Therapeutics Research Group, Surgical Oncology Department, Portuguese Institute of Oncology, Dr António Bernardino de Almeida Street, Porto 4200-072, Portugal
[11]ONCOCIR—Education and Care in Oncology—Lusophone Africa, Quires Street 168, Moreira da Maia 4470-643, Portugal

Correspondence to: Lúcio Lara Santos. Email: llarasantos@gmail.com

Research

Abstract

Purpose: The aim of this study was to assess the surgical resources and surgical oncology team skills at the Surgical Department of Maputo Central Hospital (MCH) in Mozambique in order to define an educational program to support surgical oncology practice.

Methods: From January 2017 to December 2017, a general evaluation of the resources of MCH was carried out, as well as its offerings in oncological care in different services. Data were obtained by reviewing documents, visiting surgical services and interviewing key-informants and others informally. In addition, a group of seven surgeons of the Surgical Department of MCH answered a questionnaire about the quality of the cancer units (The Cancer Units Assessment Checklist for low- or middle-income African countries). Subsequently, surgical, anaesthesiology and intensive care facilities were evaluated according to the Portuguese-speaking African Countries Assessment of Surgical Oncology Capacity Survey (PSAC-Surgery). All the data were triangulated in order to identify gaps, develop an action plan and define an educational program.

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Results: Breast, oesophagus and colorectal cancers were the most commonly treated neoplasms in MCH. A range of technical and resource needs as well as the gaps in knowledge and skills were identified. All surgeons recognised the need to create a training program in oncology at the undergraduate level, specific training for residents and continuing oncological education for general surgeons to improve the practice of surgical oncology. It was evident that all these interventions needed to be formalised, appropriately certified and count for professional career progression. Based on the local epidemiological data and on these study findings, oncology education programs were developed for surgeons.

Conclusions: The findings of this study contributed to the development of an educational program in surgical oncology, considered essential to the training of surgeons at MCH. The cancer educational programs and the mobilisation of adequate resources will ensure the provision of adequate surgical oncology treatments for MCH. The training requirements should be tailored to suit the local needs based on the most prevalent malignancies diagnosed in the region. In our view, this methodology may apply to other countries with similar realities in the formation of surgical oncologists.

Keywords: *training, surgical oncology, curriculum development, Mozambique*

Introduction

Cancer is a major public health problem in Sub-Saharan Africa because of population aging, as well as increased prevalence of key risk factors [1]. The resources available for cancer control are less than adequate in Africa; therefore, standardised cancer treatment for low- and middle-income African countries (LMIACs) is still a major concern and surgery plays an important role in the diagnosis, staging and treatment of cancer [2, 3]. In most LMIACs, as Mozambique, surgery remains the only locoregional treatment and represents the best hope for the cure, thus a careful evaluation of surgical team skills is critical to improving cancer management [4]. In addition, the quantity, quality and functionality of equipment and supplies, availability of running water and electricity, access to safe blood transfusion services, chemotherapy and radiation, the presence of postoperative facilities as well as the number, type and qualification of healthcare personnel should be included in the assessment of cancer surgery quality [4]. Noncommunicable diseases, including cancer, have been considered a major public health problem by the Ministry of Health of Mozambique since 2008 [5].

According to Lorenzoni *et al* [6], in males, the most common cancers are prostate, Kaposi sarcoma (KS), liver and oesophagus. In females, the most frequent cancers are cervix, breast, KS and oesophagus.

The report on human resources needed for cancer control in low- and middle-income countries, performed by the National Cancer Institute suggested that surgical oncologists, medical oncologists and radiation oncologists are an unmet need in Mozambique. In addition, support staffs such as onco-pharmacists, pharmacy technicians, oncology nurses and palliative care specialists are also needed. Recently, an article evaluating the cancer plans for Mozambique the authors revealed that there are only seven oncologists in the country and no oncological surgeons [7, 8]. Thus, an educational cancer program is crucial to existing surgical teams and medical students. According to Snyder *et al* [9], 14% of the causes of death in the Maputo Central Hospital (MCH) surgery department are oncological diseases and neoplasms accounted for 9% of all surgical discharge diagnoses [9]. The cancer registry of the MCH in 2015–16 pointed out that the malignant tumours most often operated by general and thoracic surgery are breast, oesophageal and colorectal cancer [10].

In order to develop and implement a Cancer Education Program (CEP) and introduce best practices for cancer surgery at MCH, Mozambique, we assessed current surgical resources and surgical oncology skills.

Methods

This cross-sectional study was conducted between January 2017 and December 2017 in MCH, a tertiary level hospital in Mozambique. An evaluation of the general capacity of MCH and its offer in oncological care in the different services was done through documental analysis, visits to the services and informal interviews to the providers of oncological care in those services. In addition, a questionnaire was administered to the seven general surgeons of the surgical Department of MCH, who have the main role in the surgical treatment of the most frequent types of cancer, namely, breast (three surgeons), colorectal (two surgeons) and oesophageal cancer (two surgeons).

The questionnaire used was the cancer units assessment checklist for low- or middle-income African countries [11]. Subsequently, surgical, anaesthesiology and intensive-care facilities were evaluated according to the Portuguese-speaking African Countries Assessment for Surgical Oncology Capacity Survey (PSAC-Surgery) in order to identify gaps.

This second instrument assesses the capacity of the hospital to perform surgical oncological procedures; infrastructures available; specific resources to perform breast, oesophageal and colorectal cancer surgery; workforce available, and the number of surgical oncology procedures per year.

The institutions that train health professionals in Mozambique were also evaluated in order to understand the formative capacity and the potential integration of a CEP in their curriculum.

After the visits, interviews, documental analysis and questionnaires assessment, the main needs in general oncology and surgical oncology were identified, in particular, for residents and fellows in general surgery. Moreover, a set of recommendations for the training of general surgeons at MCH Surgical Department were also suggested.

Questionnaires were collected on paper and the data were entered into an electronic database. Descriptive statistics were performed. This study was approved for the Mozambican National Bioethical Committee.

Results

MCH, a tertiary unit level (1500 beds), is the referral centre for all of the complex surgical care in the country. MCH provides a complete emergency service with advanced diagnostic capacities, inpatient wards for complex medical and surgical care (325 beds in the surgical unit), three fully equipped operating rooms, a fully equipped delivery room, three recovery rooms, an intensive-care unit, two high dependency care units and rehabilitation therapy facilities. The centre is also equipped with respirators, oxygen supply devices, intravenous fluids, blood products, basic microbiology equipment, the main pharmaceuticals (anaesthetics, analgesics, antibiotics) and the main surgical materials (drapes, gowns, dressings, gloves) as well as other consumables (disposable equipment and devices).

Human resources for health at MCH include: nurses—700, operating room nurses—30, anaesthetists nurses—15, anaesthesiologists—20, general physicians—125, obstetricians/gynaecologists—20, general surgeons—10, orthopaedic surgeons—7, pharmacy assistants—40, pharmacists—10, radiology technicians—12, radiologists—3, physiotherapists—2, neurosurgeons—5, thoracic surgeons—3, reconstructive surgeons—3, urologists—4, medical oncologists—3, radio-oncologist—1, medical physics—3, gastroenterologists—4 and pathologists—10.

The first questionnaire was fulfilled by seven surgeons. Cancer diagnosis pitfalls were related with fragile imaging resources and low capacity to perform biopsies guides per image. The specific needs to properly diagnose and treat the most common tumours are summarised in Table 1.

For treatment capacities, the following needs were identified: existence of a safe oncology pharmacy with access to essential oncology drugs according to the WHO list; the improvement of the day hospital facilities; to start the activity of the radiotherapy unit; to endow the surgical services with the missing resources and to produce guidelines to ensure good practice in oncology. Other needs include pre-rehabilitation programs for frail patients, rehabilitation and palliative care. At present, there are no surgeons with formal training and certification in surgical oncology.

All surgeons recognised the need to create a training program in oncology at the undergraduate level. Table 2 shows the training capacity of health professionals in Mozambique where this training should take place. Moreover, a specific training for residents and fellows (wherever possible with practical training in high workload centres) and continuing oncological education for general surgeons to improve the practice of surgical oncology was also recommended (Table 3). Surgeons also underlined that their participation in multidisciplinary decision-making meetings (locally known as the tumour Commission) is infrequent; they also stressed the need to improve the oncological knowledge about the most frequent tumours, scientific research and how to strength external collaborations.

Since the most frequently diagnosed tumours surgically treated at MCH are breast, oesophageal and colorectal cancer and based on the gaps mentioned by the seven surgeons, a Global Curriculum in Surgical Oncology was elaborated and will be tested at the MCH (Table 4) [12].

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Table 1. Specific resources lacking to perform breast, colorectal and esophageal cancer surgical treatment.

	Breast cancer	Colorectal cancer	Oesophageal cancer
Diagnostic	imaging resources and to perform core needle biopsy of the breast	Rigid sigmoidoscope	Imaging resources
Surgery	Patent Blue V for sentinel lymph node biopsy	Bookwalter retractor system	Surgical Clips and Clamps
	Fluorescence navigation with indocyanine green for detecting sentinel lymph nodes	Saint Mark's pelvic retractor	Circular and linear staplers
	Surgical clips	Surgical stapler for rectum	Harmonic scalpel consumables
Post-operative care	Aspiration drainage	Provide information and advice on diet to stoma patients	Oesophageal stents

Table 2. Training capacity of Mozambique in surgery, anaesthetist and nurses.

Health professional	Academic level (degree)	Years of duration (academic degree, training + residency)	Rotation in oncology during studies	Number of certifying universities/ institutions	Number graduates per year	Educational institutions*	
						Public sector	Private sector
Nurse	BSc	3 years	No	4	60	4	4
Nurse Anaesthetist	BSc, AN	2 + 3 years	No	1	10	1	
General practitioner	MD	6 years	Yes	8	200	4	4
Anaesthetist	MD + R	6 + 5 years	Yes	1	3	1	
Gynaecologist	MD + R	6 + 5 years	Yes	1	5	1	
Orthopaedist	MD + R	6 + 4 years	No	1	3	1	
Urologist	MD + R	6 + 5 years	Yes	1	1	1	
General surgeon	MD + R	6 + 5 years	Yes	1	4	1	
Thoracic surgeon	MD + R	6 + 5 years	Yes	1	1	1	
Plastic Surgeon	MD + R	6 + 5 years	Yes	1	1	1	

BSc = Bachelor of Science in Nursing; AN = Anaesthetic Nursing. MD = Medical Doctor graduation, R = Residency
*According to the Mozambican catalogue of higher education (www.mzformativa.com)

Table 3. MCH CEP (Modules).

Undergraduate level	General surgery residents	General surgeons*
Basics of oncology	Basics of oncology	
	Breast cancer	Breast cancer
	Colorectal cancer	Colorectal cancer
	Oesophageal cancer	Oesophageal cancer
	Surgical oncology fellowships	Advanced surgical oncology training

*According their oncological field and surgical expertise

Table 4. Modules and core content of the MCH CEP.

Module 1—Basics of oncology
Dimensions of the cancer problem in Mozambique; cancer registry; Carcinogenesis; The diagnosis in oncology; cancer prevention, cancer staging; surgical oncology; chemotherapy; radiotherapy; personalised treatment in oncology; multidisciplinary therapeutic approach for cancer; follow-up of the cancer patient; best supportive care; research in oncology; quality in oncological care
Module 2—Breast cancer
Breast cancer epidemiology; breast imaging and diagnostics, including screening; The molecular basis of breast cancer and pathological phenotypes; breast cancer classification; management of BRCA (breast cancer) gene carriers; treatment of ductal carcinoma in situ (DCIS); surgery: standards of care; sentinel node biopsy: technical aspects, intraoperative nodal assessment and localisation techniques; axillary lymph node dissection: indications and technical aspects; oncoplastic breast surgery; reconstructive surgery; Radiation therapy: standard of care; therapies in HR+ breast cancer; therapies in HER2+ breast cancer; therapies in triple negative breast cancer; management of advanced disease. Follow-up
Module 3—Colorectal cancer
Incidence and epidemiology; symptoms; diagnosis; pathology and molecular biology (RAS and BRAF mutational status); histopathology; staging and risk assessment; management of local and locoregional disease; colon and rectal surgery; laparoscopy approach; neoadjuvant and adjuvant treatment; Selection between short-course preoperative radiotherapy and long-course chemoradiotherapy; management of advanced/metastatic disease; treatment of liver and lung metastasis; management of local recurrence; follow-up
Module 4—Oesophageal cancer
Incidence; symptoms; pathogenesis; histological classification; diagnosis and staging; treatment of premalignant lesions with endoscopic therapy or esophagectomy; selection of appropriate treatment; pre-habilitation program; the selection of surgical approach; radiation treatment; combined chemo-radiation; postoperative adjuvant chemotherapy or chemoradiation; endoscopic palliative therapy; chemotherapy for metastatic disease; nutritional support; follow-up

Discussion

MCH, the referral centre for all complex surgical care in Mozambique, provides primary surgical care for its local population and is also a teaching hospital. In MCH, the top three solid tumours treated by general and thoracic surgery are breast, oesophageal and colorectal cancer.

This study was particularly helpful to define the global needs of cancer education at the national level and, in particular, at MCH. The results from the first questionnaires revealed a shortage of both technical and material resources and skills/formative deficits. In addition, direct observation through the visit to operating rooms, intensive care and ward facilities was critical in order to identify existing resources and needs. Based on the second questionnaire results, it was possible to assess the needs for quality cancer surgery. We realised the current organisational level of cancer care, all human resources of MCH will be fundamental in the organisation of oncology but most of them, including de surgeons, need specific training. We agree that is also important and crucial to creating a training program in oncology at the undergraduate level. The challenge is how we can do it effectively, taking into account local realities.

The complex nature of cancer makes oncological surgeries highly technically demanding and, therefore, outcomes are improved when surgery is undertaken by experienced multidisciplinary teams of specialists at high-workload centres and with adequate resources [13].

Our results show that surgeons participate erratically in the multidisciplinary therapeutic decision meeting. Multidisciplinary programs provide many benefits for the physicians involved [14, 15]. The attendance of multiple specialists from each discipline in this type of meetings leads to dynamic discussion and learning opportunities for all, especially students and trainees [16]. Multidisciplinary team management is associated with improved outcomes after surgery for breast, colorectal and oesophageal cancer [17, 18].

Stefan *et al* [19] address the various areas of oncology in a very interesting article on education and training for the future in cancer research at the East African Regional Meeting. Unfortunately, in their work, the training of surgical oncologists was not discussed [19]. However, the College of Surgeons of East, Central and Southern Africa developed a fellowship in surgical oncology and the average length of time it takes to train surgical oncologist ranges from 11 to 17–19 years [20]. However, this training is long and does not cover the immediate needs of Mozambique. It is necessary to combine a formal training with a fast-track program (2 years). Thus, in order to improve the quality

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of care in MCH, the creation of a comprehensive CEP to address the educational needs of medical students, interns, residents, fellows, nurses and allied health staff is crucial. However, cultural and university education differences of the Lusophone African countries must be taken into account. It is important that training in oncology and surgical oncology considers these differences.

Despite different interventions to train Mozambique oncologists involving Brazilian hospitals, the Calouste Gulbenkian foundation from Portugal, MD Anderson Cancer Center from USA and others, according to the respondents, there were no substantial changes in the practice of surgical oncology at MCH. It seems clear that without integrating these training efforts into an official and formal CEP at different levels (namely, pre-graduate and continuing medical education), the results will continue to be fragile and these activities will be erratic [21, 22].

A surgical oncologist should possess in-depth knowledge of malignancies involving each specific disease site. Thus, our proposal takes into account local resources, the nosological profile, the critical actors on the ground and the need to first create a group of competent trainers who later disseminate oncology training in general and oncology surgery in particular.

We follow the recommendations of Are *et al* [12], regarding the training and certification of surgical oncologists, namely: *The training period should be shortened, if possible. The training requirements can be tailored to suit the local needs based on the most prevalent malignancies diagnosed in the region. LMIACs can proactively partner with foreign countries that offer surgical oncology fellowships. Emphasis also needs to be placed on continuing medical education to remain abreast of the current standards of treatment. Training should include the basic principles of chemotherapy and other disciplines of oncology. Surgeons should also be equipped with knowledge on pain, palliative care in order to improve the quality of life for cancer patients* [20].

In the definition of the contents suggested in our programme, we adopted the global curriculum in surgical oncology suggested by Are *et al* [12], the recommendations of Mozambican surgeons, but we prioritise the surgical treatment of the most prevalent malignancies in Mozambique, as had already been done in other Lusophone African countries with success [23].

As a whole, during the 2 years surgical oncology fellowship programme (during surgical residency), the clinical rotation includes: 12 months spent in the surgical oncology department of a high-volume centre, focusing on complex oesophageal and gastrointestinal malignancies, pancreatic and hepatobiliary malignancies, breast cancer, melanoma, head and neck cancer, bone and soft-tissue sarcoma and foregut malignancies; 1 month in a radiation therapy unit; 1 month in a pathology unit and 1 month in a medical oncology department. The remaining 9 months will be spent in Mozambique conducting oncological research activity.

For formal general and thoracic surgeons, MCH should offer advanced training opportunities in surgical oncology (3 months) who have before completed the basic oncology training program and are interested in an additional intensive experience in advanced surgical oncology related to their specific area, namely, breast cancer, colorectal or oesophageal cancer. In our view, this methodology may apply to other countries with similar realities, daily activity and difficulties encountered should become teaching and research issues in order to overcome them properly. Specific topics, such as assessment based on decision making on assignment as trusted professional activities, is probably a useful methodological option in this context [24].

Thus, the proposal of our study should be discussed by the country's health and university authorities and by the college of surgeons in order to integrate this Mozambican program for training surgical oncologists and then evaluate its effectiveness.

Similar programs should be implemented in other African countries, demonstrating their capacity to adapt to their specific reality and conditions. Those responsible for the training of surgical oncologists from countries with high resources should, according to the difficulties we have studied, develop specific and adapted courses that can be used as a complement of the training capacities of developing countries. For surgical oncology fellows in higher-resource settings specifically, the learning benefits of rotations in LMICs are even greater since they learn to deal with cancer patients at advanced stages or different cancer biology, in countries having fewer screening options, fewer imaging options, as well as limited perioperative care [25].

Conclusions

The findings of this study contributed to the development of an educational program in surgical oncology considered essential for the training of surgeons and residents of surgery at MCH. Undergraduate medical training programmes should incorporate oncology education.

Cancer education should fully integrate all healthcare professionals involved in cancer care. The educational cancer program and the mobilisation of adequate resources will ensure the provision of adequate surgical oncology treatments at MCH. The training requirements should be tailored to suit the local needs based on the most prevalent malignancies diagnosed in the region. In our view, this methodology may apply to other countries with similar realities in the training of surgical oncologists.

Conflicts of interest

Ellen Baker is the director of Project ECHO, MD Anderson, USA.

For the other authors, there are no conflicts of interest to disclose and no financial or commercial relationships.

Authors' contributions

This study was conceptualised, designed and written by Atílio Morais and Lúcio Lara Santos. Acquisition of data was carried out by Atílio Morais, Adriano Tivane, Matchecane Cossa, Jotamo Come, Volodimir Venetsky, Fernando Torres, Victor Pacheco, Miguel Reyes, Germano Pires. Analysis and interpretation of data were done by Atílio Morais, Mariana Peyroteo and Lúcio Lara Santos. Ellen Baker, Satish Tulsidas, Maria Rosário Martins, Moshin Sidat and Lúcio Lara Santos revised the article for important intellectual content. All authors read and agreed to the final version of this manuscript. Atílio Morais and Lúcio Lara Santos equally contributed to this study.

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8.2 ATTACHMENT II - Scientific publications related to thesis as co-author

PanAfrican Medical Journal

Open Access

Essay

Prehabilitation program for African sub-Saharan surgical patients is an unmet need

Antero do Vale Fernandes[1,2], Daniel Moreira-Gonçalves[1,3], Jotamo Come[4], Nilton Caetano Rosa[5], Victor Costa[6], Lygia Vieira Lopes[7], Paulo Matos da Costa[8,9], Lúcio Lara Santos[1,10,11,&]

[1]Experimental Pathology and Therapeutics Group of Portuguese Institute of Oncology of Porto Francisco Gentil, E.P.E (IPO-Porto), Portugal, [2]Intensive Care Service of Hospital Garcia de Orta, E.P.E, Almada, Portugal, [3]Research Centre in Physical Activity, Health and Leisure (CIAFEL), Faculty of Sport, University of Porto, Porto, Portugal, [4]Surgical Department of Maputo Central Hospital, Maputo, Mozambique, [5]Surgical Oncology Department of Angolan Institute Against Cancer, Luanda, Angola, [6]Surgical Department of Agostinho Neto Hospital, Praia, Cape Verde, [7]Oncological Unit of Sagrada Esperança Clinic, Luanda, Angola, [8]General Surgery Service, Hospital Garcia de Orta, E.P.E, Almada, Portugal, [9]Faculty of Medicine of the University of Lisbon, Lisbon, Portugal, [10]Surgical Oncology Department of Portuguese Institute of Oncology of Porto Francisco Gentil, E.P.E (IPO-Porto), Portugal, [11]ONCOCIR, Education and Care in Oncology, Lusophone Africa, Angola

[&]Corresponding author: Lúcio Lara Santos, Experimental Pathology and Therapeutics Group of Portuguese Institute of Oncology of Porto Francisco Gentil, E.P.E (IPO-Porto), Portugal

Keywords: Africa, patients, postoperative, surgery, risk

Domain: Surgical oncology

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Abstract

Approximately 4.2 million people worldwide die within 30 days of surgery each year. Half of these deaths occur in low- and middle-income countries. Postoperative deaths account for 7.7% of all deaths globally, making it the third-highest contributor to deaths, after heart disease and stroke. In sub-Saharan Africa, there is a higher rate of mortality following postoperative complications compared to high-income countries. The WHO has tools to help countries provide safer surgery. However, implementation remains poor in most African countries. Interventions focused on intraoperative or postoperative measures to improve perioperative prognosis may be too late for high-risk patients. Poor preoperative cardiorespiratory functional capacity, poor management of pre-existing comorbidities and risk factors and no assessment of the patient´s surgical risk is associated with adverse postoperative outcomes, including mortality, complications, slower recovery, longer intensive care stay, extended hospital length of stay and reduced postoperative quality of life. To significantly decrease morbidity and mortality following surgery in Africa, we propose the implementation of a comprehensive preoperative intervention, that must include: i) risk assessment of surgical patients to identify those at greater risk of postoperative complications for elective surgery; ii) increase the preoperative functional reserve of these high-risk patients, to enhance their tolerance to surgical stress and improve postoperative recovery; iii) anticipate postoperative care needs and organize tools, resources and establish simple workflows to manage postoperative complications. We believe this approach is simple, feasible and will significantly reduce postoperative burden for both patients, hospitals and society.

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Essay

An African continental study of 25 low- and middle-income countries was recently published by Biccard BM *et al.* characterizing perioperative outcomes of 11193 surgical patients. The patients were 66.4% women, 87.3% classified with an American Society of Anesthesiologists (ASA) score of I and II, 29.7% undergoing major surgery and 57.1% urgent/emergency surgery [1]. Arterial hypertension (16.3%), diabetes mellitus (6.8%) and HIV positive/AIDS (11%) were the main comorbidities of the operated patients. Non-communicable diseases (NCD) were the most frequent indication for surgical treatment (42.2%), followed by caesarean section (27.3%), trauma (17.8%) and acute infection (12.7%). Despite being younger, presenting a lower risk profile and low complication rates, surgical patients in Africa were twice as likely to die after surgery in comparison to the global average. Indeed, in hospital mortality was 2.1%, with 18% developing postoperative complications (POC) and 9.5% of the patients died following POC [1]. When considering elective surgery (ES) only, mortality occurred in 1% of 4658 patients, with an incidence of postoperative complications of 13.4% and death after POC of 4.8%.

A greater incidence of POC and death were reported following surgery for NCD (37.3% and 40.3%, respectively), infection (20.2% and 26.5%, respectively) and trauma (20.5% and 25.5%, respectively). Infectious, cardiovascular and respiratory complications were the most prevalent [1]. The factors contributing to POC and death are multifactorial and may include insufficient medical staff, poor infrastructure, low procedural volumes and failure to identify and/or treat POC by health professionals [2,3]. Intensive care admission should also be scheduled in advance. In their study, Biccard BM *et al.* [1] identified that only 16.3% of patients who developed POC, the vast majority after ES, were admitted to intensive care units (ICU) to prevent and treat early complications. The lack of immediate postoperative surveillance and intervention is responsible for many deaths in many African countries. Therefore, acute care surgery (ACS) services should be implemented even in a low-resource setting [4].

In Rwanda, the implementation of an ACS service resulted in decreased length of hospital stay [5]. Thus, while surgical care is a major need for African countries, surgical outcomes will remain poor unless effective perioperative care based on affordable resources is made universally available. Perioperative care is a multicomponent intervention implemented by a multidisciplinary team with the purpose to provide safe surgery, accelerate recovery and reduce morbidity and mortality (Figure 1). While the intra- and postoperative care have already received some attention, the potential of the perioperative period remains poorly explored in African countries [6-11]. This time frame represents a major opportunity for decreasing postoperative morbidity and mortality through appropriate surgical risk stratification and patient optimization [12].

Risk assessment of surgical complications in sub-Saharan Africa: in an environment with limited resources for postoperative care, the early identification of high-risk patients for POC is likely to be a key factor to consider. Several tools are available to estimate perioperative risk for both planned and emergency surgeries in high-income countries [13,14]. However, their use in low-income countries is often limited because the pattern of risk for poor outcomes differs from high-income countries and due to the lack of resources, the access to biochemical and imagological tests required by more sophisticated tools is reduced [15]. Recently, Kluyts H-L *et al.* proposed the use of the ASOS surgical risk calculator as a simple tool to identify African surgical patients at risk for in-hospital postoperative mortality and severe complications and thus, to identify those patients in greater need for enhanced postoperative surveillance [16]. However, its external validation needs to be assessed before. To predict complications and risk of death before surgery, other African authors have conducted relevant studies, including the use of online tools, provided that this tool has wide distribution [17,18]. This is a field that deserves further research effort as it may greatly contribute to save lives.

Estimating the risk of complications and mortality of surgical patients before surgery can be helpful: risk stratification of patients is supposed to support better decisions by informing about the risks and benefits of proceeding with surgery, about discussing treatment alternatives and guide the use of available resources, with the ultimate purpose of improving postoperative outcomes. Ntobeko Ntusi, a South African cardiologist, in a recent editorial in the South African Medical Journal, asked: "does the preoperative evaluation of patients improves surgical outcomes?". He found that the data on the effect of preoperative medical consultation on cost measures is conflicting [19]. While some studies reveal a decrease in-hospital stay after preoperative evaluation and care of patients [20,21], other studies have shown an increase in costs and a similar length of stay for consulted patients [22-24]. He also points that while medical teams can successfully identify conditions that may affect surgical outcomes, it is not clear if they make evidence-based recommendations to target

those conditions and assuming they make it, it is also not clear if the consultative recommendations are implemented [19]. With this data and his experience, Ntusi argued that an experienced perioperative medicine physician should be able to identify the pertinent medical problems, anticipate potential perioperative problems, recommend evidence-based interventions to optimize the patient and communicate and work effectively with all the preoperative team members (e.g. nursing, physiotherapist, medical, surgical and anesthetic) [19]. Thus, to deal with the problem of postoperative morbidity and mortality, the perioperative care, particularly the potential of the pre-surgical period to optimize the patient for surgery, needs to be taken more seriously. These patients need and deserve better care and prehabilitation programs can make the difference once incorporated in the routine practice of surgical teams.

Prehabilitation to prepare for surgery: the impact of surgery leads to significant homeostatic disturbance which, together with reduced functional capacity (physical, nutritional and psychological status) and poor medical optimization (e.g. unappropriated management of chronic diseases, anemia, hypertension, hyperglycemia and smoking), act as risk factors for negative surgical outcomes [25]. Prehabilitation is a multimodal strategy implemented in the preoperative period, aiming to increase preoperative functional reserve and leading to better postoperative functional recovery and reduced incidence of complications. In practice, prehabilitation programs may include cardiovascular and resistance training exercises, nutritional advice designed to support an increase in lean body mass, the introduction of coping strategies to deal with surgical anxiety, smoke cessation support, treating preoperative anemia and other modifiable risk factors [26]. An increasing number of studies support the safety, feasibility and efficacy of multimodal prehabilitation to improve surgical outcomes in cancer patients undergoing major abdominal and cardiothoracic surgery [27]. The benefits range from lower rate of postoperative complications, to less deterioration of physical function and better quality of life [28]. However, this evidence comes mainly from high-income countries and thus, there is an urgent need to test the potential of prehabilitation programs in African countries.

Conclusion

Decreasing morbidity and mortality following surgery in Africa will require adequate perioperative optimization and better postoperative care planning. Preoperative diagnosis of comorbidities and social habits that are considered risk conditions for surgery should be identified throughout appropriate risk assessment tools. Patients considered to be at high-risk for complications following surgery should be proposed for prehabilitation to increase their preoperative functional reserve and enhance recovery following surgical treatment. The knowledge about most common surgical complications should be used to anticipate postoperative burden, care needs and organize available resources in advance. We believe that this approach to perioperative care will play a decisive role in sub-Saharan Africa in changing surgical morbidity and mortality for better.

Competing interests

The authors declare no competing interests.

Authors' contributions

AVF and LLS were responsible for the primary conception and design of the article with input from co-authors; AVF, DMG and LLS prepared initial drafts of the article. Additions, modifications and critical revisions for the relevant intellectual content of the report were performed by AVF, DMG, JC, NCR, VC, LVL, PMC and LLS, including final approval of the version to be published. All the authors have read and agreed to the final manuscript.

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Figure

Figure 1: stratification of measures to decrease perioperative morbidity and mortality in surgical patients

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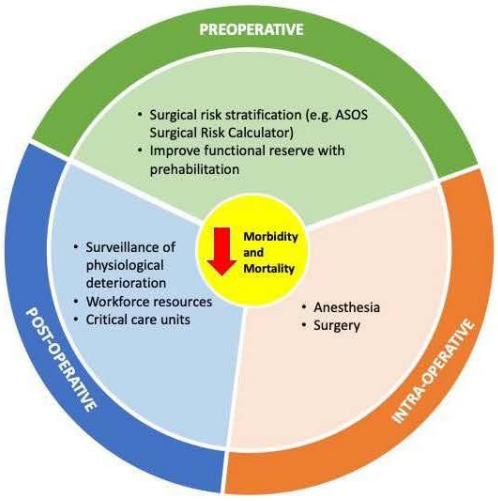

Figure 1: stratification of measures to decrease perioperative morbidity and mortality in surgical patients

8.3 ATTACHMENT III - Scientific publications related to thesis as co-author

ARTICLE IN PRESS

ORIGINAL REPORTS

Designing a National Curriculum to Advance Surgical Oncology in Mozambique: A Delphi Consensus Study

*Atílio Morais, MD,[*] Manuel Simão, MD,[†] Matchecane Cossa, MD,[*] Jotamo Come, MD,[‡] Carlos Selemane, MD,[§] Adriano Tivane, MD,[*] Satish Tulsidás, MD,[‖] Cesaltina Lorenzoni, MD, PhD,[¶] Jéssica Rodrigues, MSc,[#] Luís Antunes, PhD,[#] Donzilia Brito, MD,[**,††] Manuel João Costa, PhD,[‡‡] Moshin Sidat, MD, PhD,[§§] Maria do Rosário O Martins, PhD,[‖‖] and Lúcio Lara Santos, MD, PhD[**,¶¶,##],*

[*]Department of Surgery, University Eduardo Mondlane, Thoracic Surgery, Surgical Department, Maputo Central Hospital, Maputo , Mozambique; [†]College of surgeons, Mozambican Medical Association, Maputo, Mozambique; [‡]Life and Health Sciences Research Institute, School of Medicine, University of Minho, Braga, Portugal; [§]Colorectal Surgery, Surgical Department, Maputo Central Hospital,Maputo, Mozambique; [‖]Medical Oncology Department, Maputo Central Hospital, Maputo, Mozambique; [¶]Department of Pathology, Maputo Central Hospital, Maputo, Mozambique, Faculty of Medicine, Eduardo Mondlane University, Maputo, Mozambique; [#]Cancer Epidemiology Group, IPO Porto Research Center (CHPOP), Portuguese Oncology Institute of Porto (IPO Porto), Porto, Portugal; [**]Surgical Oncology Department, Portuguese Institute of Oncology, Porto, Portugal; [††]European Union of Medical Specialists (UEMS) of Portuguese College of General Surgery; [‡‡]Medical Education Unit, School of Medicine University of Minho, Portugal; [§§]Department of Community Health, University Eduardo Mondlane, Maputo, Mozambique; [‖‖]Global Health and Tropical Medicine, Institute of Hygiene and Tropical Medicine, Universidade Nova de Lisboa, Lisboa, Portugal; [¶¶]Experimental Pathology and Therapeutics Research Group, Surgical Oncology Department, Portuguese Institute of Oncology, Porto, Portugal; and [##]ONCOCIR—Education and Care in Oncology—Lusophone Africa, Moreira da Maia, Portugal

OBJECTIVE: Mozambique is currently experiencing an increase in chronic diseases including cancer. There is a large unmet need for cancer surgery in Mozambique. The aim of this study was to define the content and the design of a training program for practicing surgeons in surgical oncology that would be consensually regarded as adequate to care for oncological patients requiring surgical interventions.

DESIGN & SETTING: A 3-round modified-Delphi approach was implemented to obtain consensus on surgical oncology training curriculum. The participants were purposefully selected experts in surgical oncology working in Mozambique. In round 1, participants answered a questionnaire with open-ended questions regarding the content of the curriculum and the timing and venue of training. In round 2, answers from the first round were presented to a purposeful selected sample of nationally recognized experts in oncology and surgical oncology, including members of the Mozambican College of Surgeons and leadership of the Ministry of Health. A final round was carried out to discuss the draft version of the training program aiming to achieve a predetermined consensus level of 80%.

PARTICIPANTS: Fifteen of 23 experts (65.2%) responded to round one.The response rate for round 1 and 3 was 80% (12 of the 15 participants in round one).

RESULTS: The responses collected in the first round were analyzed and revealed that basic principles of oncology and basic principles of surgical oncology should be included in the curriculum of surgical residency in Mozambique (80% of the experts agree; Cronbach $\alpha = 0.93$); a 24-months fellowship in surgical oncology should take place after residency in the surgical field (86.6% of experts agree; Cronbach $\alpha = 0.97$); and should occur at Maputo Central Hospital and at comprehensive cancer centers abroad (100% agree). In round 2 the proposal for the program of surgical oncology fellowship obtained a strong agreement amongst the experts

This investigation had no financial support.

Correspondence: Inquiries to Lúcio Lara Santos, MD, PhD, Instituto Português de Oncologia. Rua Dr. Bernardino de Almeida, 4200-072, Porto, Portugal; e-mail: llarasantos@gmail.com

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(97.3%). The final proposal for the program was divided into the following structure: (1) theoretical components; (2) duration; (3) location; (4) methodology; (5) technical skills in oncology; and (6) competency and paid particular attention to the oncological diseases prevalent in Mozambique. The agreement amongst the experts was 97.3%.

CONCLUSIONS: The experts reached a consensus regarding the general structure for a cancer surgery postgraduate training program in Mozambique, which should be a 24-months fellowship after residency in surgical disciplines. This fellowship should mostly take place in Mozambique, but it should also include dedicated internships in recognized cancer hospitals abroad. Such curricula embrace the Global Curriculum in Surgical Oncology including in particular the oncological nosology of Mozambique and should advance the quality of oncology surgical care provided in the country. (J Surg Ed 000:1–8. © 2020 Published by Elsevier Inc. on behalf of Association of Program Directors in Surgery.)

KEY WORDS: surgical oncology, curriculum, Mozambique, training, Delphi method

COMPETENCIES: Patient Care, Medical Knowledge, Practice-Based Learning and Improvement

BACKGROUND

The epidemiological profile in Africa is changing. Amidst a persistently high burden of communicable diseases, chronic noncommunicable diseases are increasing.[1] Cancer incidence and mortality are also growing rapidly in sub-Saharan Africa[2] where access to safe, effective and timely surgery is extremely limited.[3] Most patients in sub-Saharan Africa present with advanced-stage disease. Furthermore, most sub-Saharan African low-income countries lack surgeons or hospitals adequately equipped to care for oncological patients.[4]

Mozambique is currently experiencing an increase in cancer and other chronic diseases.[5] As the country was not prepared for this epidemiological transition, the treatment of cancer patients is potentially suboptimal and there is referral for oncology care abroad.[6] These circumstances emphasize the importance of rapidly upscaling the preparedness of the country's healthcare system in surgical oncology. In this regard, health authorities and universities have improved services within existing capacity, but there are unmet needs in terms of human resources, material resources and knowledge to deal with the increase of cancer diseases.

In Mozambique, patients with suspected diagnosis of a cancerous condition are referred to secondary or tertiary hospitals where diagnosis is confirmed and, if possible, treatment is implemented. Maputo Central Hospital (MCH) is the only hospital equipped with oncology ward and clinical and surgical capacity to care to a certain extent for patients with oncological conditions and also to provide palliative care. Oncological conditions requiring complex treatments such as radiotherapy and complex chemotherapy are referred abroad (for example, to the Republic of South Africa, India, or Portugal depending on existing agreements). MCH is the referral center for complex surgical care in Mozambique including surgical oncology. With 1,500 beds, MCH provides a complete emergency service with advanced diagnostic capacities, inpatient wards for complex medical and surgical care, 3 fully equipped operating rooms and delivery room, 3 recovery rooms, an intensive-care unit, 2 high dependency care units and rehabilitation therapy facilities. MCH is also the country's most important postgraduate medical training center for resident doctors.[7] The uniqueness of this Hospital in the national health system makes it a key player and an important context to study the evolution of oncological care in Mozambique.

In 2018, we studied barriers and suggested solutions to the implementation of best practices for cancer surgery at MCH.[8] Our study identified the prevalent surgical cancers – esophagus, colorectal and breast – and concluded that there were deficits in resident's surgical oncology knowledge.[8] There are currently no formally specialized surgeons in oncology. The introduction of medical treatment and surgical care protocols at MCH relies frequently on the aid of international experts, due to the inexistence of formally trained surgical oncologists in the institution. In Mozambique, currently, the undergraduate training in oncology is fragmented and, at the postgraduate level, there is limited and occasional exposure of medical and surgical residents to oncological conditions. As there are yet in the structured curriculum or formal specific oncology residency programs, training needs to proceed abroad. Unfortunately, only 5 Mozambican Oncologists returned to the country after being trained abroad.The enhancement of postgraduate training in surgical oncology is needed to overcome the pitfalls of cancer surgery in Mozambique. In accordance, there have been successive internationally sponsored interventions to train Mozambique oncologists involving Brazilian hospitals, the Calouste Gulbenkian Foundation in Portugal and the Portuguese Institute of Oncology, MD Anderson Cancer Center from US and others in the last 4 years. The models include training of Mozambican surgeons in these hospitals or on-the-job training in Mozambique with international visiting mentors. So far, these models have had little impact on the practice of surgical oncology in Mozambique.[7-9]

The ability to develop a sustainably adequate surgical oncology workforce depends on the presence of robust educational systems that provide training in all oncologic domains and help maintain competency for those in clinical practice.[10] According to the European Union of Medical Specialists (UEMS), surgical oncologists are oncologists who also possess the expertise to perform operative procedures and interventions. As such, they are specialists that are expected to possess the required knowledge of basic principles and tenets of multidisciplinary cancer management: epidemiology, screening, diagnostic pathology, medical imaging, chemotherapy and radiation therapy, palliative care, interventional radiology and endoscopy.[11] Therefore, the development of an official and formal surgical oncology residency or fellowship holds great potential to in delivering an impressive and lasting impact on oncology surgery in Mozambique, since the transmission of knowledge is based on continued practice in an organized manner, it is not episodic and allows training of generations of surgical oncologists.

The goal of this study was to develop a fellowship curriculum (24-months) though a consensus methodology, suitable to be implemented by the Mozambican College of Surgeons regardless of clinical training and practice site. The impetus for the study was that the provision of educational leaders with such a curriculum would induce educational change, create coherent practical surgical training opportunities and ultimately minimize the gaps in surgical oncology of fellows. To achieve this aim the Delphi consensus methodology was implemented in order to develop expert consensus regarding the key program required to graduate Mozambican surgical oncologists.

METHODOLOGY

Study Design

To reach consensus on the surgical oncology fellowship curriculum, we used a modified Delphi method to develop a consensual definition of the core surgical oncology competencies (knowledge, skills, and attitudes) for surgery residents and young surgeons in Mozambique. The Delphi process is a systematic technique to collect and transform individual expert opinions in a group consensus, through multiple rounds of surveys.[12] This methodology has been used widely in health-related research including surgical education research.[13] The Delphi method avoids the possibility that the highest positioned expert is the most influential in reaching consensus and, secondly, prevents that an expert will adjust to the group opinion regardless of the evidence that supports his/her own opinions.[14]

Participant Selection for the Delphi Consensus

Twenty-three individuals (surgeons, medical oncologists, radio-oncologists, pathologists from Mozambique Central Hospitals; members of the Mozambican College of Surgery; and the Ministry of Health) with expertise in oncology care, surgery or health policy were purposefully invited to participate in this study. These experts were chosen because of their extensive experience with Mozambican healthcare context and national leadership role in academic surgery and oncology, their potential to influence and implement education changes at their institutions and their ability to influence policy and practice within the Ministry of Health in Mozambique. A 3-person steering group (SG) was formed to guide the process consisting of experienced surgical oncologists (AM, DB, LLS, 2 of whom working in a comprehensive cancer center). The SG did not participate in the expert panel.

Questionnaire Development and Implementation

A 3-round modified Delphi process was conducted between February and November 2019, in order to reach consensus among experts on issues related to the design a Mozambican surgical oncology fellowship curriculum. The SG was responsible for organizing the questionnaires and conducting the study. The Delphi structure for data collection and analysis is presented in Figure 1.

A questionnaire was created covering the global surgical oncology training curriculum items and formed the basis of the Delphi survey. The items were measured on a 5-point Likert scale (1 - "strongly disagree" and 5 - "strongly agree"). The SG contacted potential participants by email to ensure interest and agreement to participate in the project. Reminder invitations were sent twice. Participation in the panel was voluntary. The process was conducted in a "quasi" anonymous manner. Respondents' identity was only known by the SG to allow reminders and provide feedback in subsequent rounds. The participants' judgments and opinions remained strictly anonymous to members of the expert panel.

Round One

The items presented in round one were:

1- Regarding the preparation of Mozambican surgeons in surgical oncology what oncologic items do you think surgical residents and young surgeons should be knowledgeable about and masters of?

A. Basic principles of oncology,
B. Basic principles of surgical oncology,
C. National oncological program,
D. Disease site specific oncology surgical treatment of dominant oncological pathologies in the country (themes are not mutually exclusive).

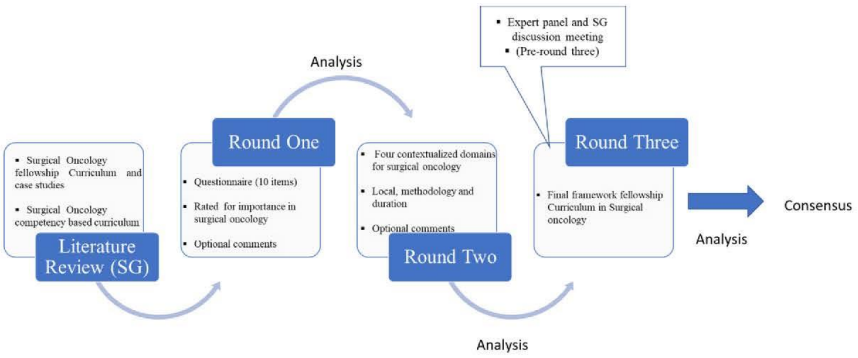

FIGURE 1. Delphi study structure for data collection and analysis. Building a surgical oncology fellowship curriculum with a three round modified survey. SG, Steering group).

2- At what time should the surgical oncology fellowship take place?

A. During of residence,
B. End of residence,
C. After residence

3- Where should this surgical oncology fellowship take place and with whom?

A. MCH,
B. Abroad,
C. Combining the 2 locations but mostly in the country.

The results were analyzed by the SG, and a second questionnaire was constructed including the important items obtained in this round.

Round Two

For the second round, the SG used the information provided by the experts and a curriculum proposal was built (Table 1). Experts were asked if they agreed or not with the proposal and a consensus ≥80% was reached.

Round Three

The results of the last 2 rounds were presented, and the final proposal (Table 2) was discussed with the expert panel in the consensus session held at the African Organization for Research and Training in Cancer meeting, in Maputo on November 2019. In order to provide educational leaders, with a framework to develop competency and milestones, create didactic, and build practical surgical opportunities to minimize gaps in surgical oncology in Mozambique, the proposal, presented by the SG, also included the following items: knowledge and attitudes in oncology, duration, location and methodology for its implementation and technical. The SG provided additional information that supports the final proposal in the meeting, namely: the Global Curriculum in Surgical Oncology, the European Society of Surgical Oncology Core Curriculum and the Surgical Oncology Competency Based Curriculum Goals and Objectives that was profiled by the experts was those adopted at the General Surgery Residency at East Carolina University.[15-17] One of the most important aspects of the meeting was to give an additional weight in the curriculum to the dominant oncological pathology in Mozambique and to adapt the training taking into account the existing diagnostic and treatment resources according to the philosophy to maintain good practices according to the existing resources.[18]

After the meeting, the participants were requested to only answer anonymously whether they agreed with the final proposal program or not. Consensus on this round was established when agreement with the program was ≥80%.

Data Analysis

Statistical analysis was performed with SPSS 24. The responses of each round were ranked, and their frequency evaluated. For the Delphi questionnaire (round 1) a measure of internal consistency (Cronbach's alpha) was calculated.

RESULTS

Mozambican Surgical Oncology Delphi

Of the 23 experts contacted, 15 (65.2%) participated in the Mozambican surgical oncology Delphi (round 1) The

TABLE 1. Final Proposal for the Mozambican Core of Surgical Oncology Fellowship Curriculum

Components (Agree %)	**Location (Agree %)**	**Methodology (Agree %)**	**Duration (Agree %)**
Basic principles of oncology Carcinogenesis Epidemiology of cancer The profile of cancer diseases in Mozambique Cancer prevention Radiation biology Principles of chemotherapy and target therapy Paliative care Clinical trials Communications skills	Mozambique	Theoretical	During the first 6 months of fellowship
91.6% *Generic clinical skills* Radiology interpretation Preoperative assessment and prehabilitation Perioperative care Postoperative care and rehabilitation The role of the MDT	**91.6%** Mozambique	**83.3%** Theoretical and Practice	**83.3%** During the first 6 months of fellowship
83.3% Disease site specific oncology* Breast cancer Colorectal cancer Upper gastrointestinal cancer (Esophageal, Gastric, GIST, Small Bowel) Thoracic cancer Hepatopancreatobiliary cancer Skin cancer and Melanoma Urological malignancies Endocrine malignancies (thyroid, parathyroid, adrenal and pancreatic endocrine) Sarcomas Gynaecological malignancies Peritoneal surface malignancies Surgical aspects of HIV-associated malignancies	**100%** Mozambique and abroad	**100%** Theoretical and Practice	**83.3%** During the next 17 months of fellowship
100% *Examination in surgical oncology* **100%**	**100%** Mozambique **100%**	**100%** Theoretical and Practice **91.6%**	**91.6%** In the last month of fellowship **100%**

MDT, multidisciplinary team; GIST, gastrointestinal stromal tumor; HIV, human immunodeficiency virus.
*The surgical oncology fellows should receive direct operative training by experienced and accredited trainers in minor, intermediate, major and complex major surgery as their experience progresses.

response rate for round 2 and 3 was 80% (12 of the 15 participants in round 1).

After the first round data was analyzed and revealed that basic principles of oncology and basic principles of surgical oncology should be included in the curriculum of surgical residency in Mozambique (80% of the experts agree; Cronbach's $\alpha = 0.93$); a 24-months fellowship in surgical oncology should take place after residency in the surgical field (86.6% of experts agree; Cronbach's $\alpha = 0.97$); and should occur at MCH and at comprehensive cancer centers abroad (100% agree). In Round two the proposal for the program of surgical oncology

TABLE 2. Final Proposal for Mozambican Core of Surgical Oncology Fellowship Curriculum

Component **Generic clinical skills**	**Location** **Mozambique**	**Methodology** **Theoretical and Practice**	**Duration** **During the first 3 months of fellowship**
Competencies	Know how to interpret CT scans of head, chest, and abdomen, other radiographic studies, mammograms, laboratory values including tumor markers. Have a working knowledge of evaluating a surgical oncology patient. Know indications to obtain studies and utilize these studies when evaluating a patient. Be able to complete a comprehensive history and physical for a surgical oncology patient. Be able to do appropriate pre and postoperative care for a surgical oncology patient. Management of: 1. Daily surgical care 2. Organization of teaching conferences and rounds. Make daily management decisions. Organize and direct resuscitation of critically ill postoperative surgical oncology patients. Fellows will understand and adopt available clinical practice guidelines and recognize the limitations of these guidelines. They will work with patient care managers, discharge coordinators and social workers to coordinate and improve patient care and outcomes.		
(Agree %)	83.3%		
Basic principles of oncology	Mozambique	Theoretical	During the second 3 months of fellowship
Competencies	Understand tumor kinetics including biology of tumor growth and some therapeutic regimens including chemotherapy, radiotherapy, immunotherapy. Understand the basic principles of surgical therapy for cancer. Be able to outline a basic treatment strategy for treatment of common types of cancer based upon stage. This should be both surgical and chemotherapy if indicated. Be able to outline a unified plan of care for common cancers based upon stage, type of cancer, location and potential for resection. Know the principles and approach to common cancers including a detailed understanding of the surgical approach. Have a basic understanding of common cancers, i.e. breast, soft tissue, hepatobiliary, pancreatic, and gastro-intestinal.		
(Agree %)	91.6%		
Disease site specific oncology	Mozambique and abroad	Theoretical and Practice	During the next 17 months of fellowship
Competencies	Have a working knowledge of anatomy and how surgical resection for tumors is influenced by the stage of cancer and the location of the cancer. Be able to perform the following operative procedures: 1. Breast biopsy 2. Perform a sentinel node biopsy 3. Mediport (portacath) placement and removal. Be able to perform the following procedures: 1. Mastectomies 2. Colonic resection 3. Gastric resection. Deal with complex surgical problems in the surgical oncology patient. Deal with the oncological diseases prevalent in Mozambique that require surgical treatment. Will assume major responsibility on the service and have achieved complex technical skills required for the management of: 1. Major complex gastrointestinal surgery 2. Hepatic resection 3. Major cancer resections 4. Pancreatic operations. Assigning resident staff to operative procedures. Deal with complex surgical problems in the surgical oncology patient. They will strive to create ethically sound relationships with patients, the physician team, the care team and the supporting hospital personnel. They will effectively communicate through accurate and complete notes on the electronic medical record. They will exhibit listening skills appropriate to patient-centered interviewing and communication. Fellows will be able to communicate with patients concerning end-of life decisions. All fellows will demonstrate integrity, accountability, respect, compassion, patient advocacy, and dedication to patient care that super cedes self-interest. Fellows will demonstrate a commitment to excellence and continuous professional development. Fellows will be sensitive to health care costs while striving to provide quality care. They will begin to understand the place of appropriate consultation for the best care of their patients. Teaching medical students and junior residents Organizing conferences		
(Agree %)	100%		
Examination in surgical oncology	Mozambique	Theoretical and Practice	In the last month of fellowship
(Agree %)	100%		

fellowship obtained a strong agreement amongst the experts (97.3%). The final proposal for the program was divided into the following structure: (1) theoretical components; (2) duration; (3) location; (4) methodology; (5) technical skills in oncology; and (6) competency. The agreement amongst the experts was 97.3%.

DISCUSSION

The aim of this study was to design a curriculum for the Mozambican fellowship in surgical oncology, using the Delphi methodology. The resulting curriculum represents consensus of experts in oncology including surgical oncology from MCH, Ministry of health and Mozambican College of Surgery. To our knowledge this is the first study that uses the Delphi method for this purpose in Africa.

The percentage of response rates in all three rounds were high, affirming support and adding credibility to our results.

The inclusion of Good Clinical Practice experiences and the experience of other African countries has been taken into account. Thus, Schweitzer RC et al. recommendations regarding the Essential Elements of Surgical Oncology Training, other curricular experiences in surgical oncology and the experience of Malawi supported the final curriculum propose and ensuring their quality and effectiveness.[15-17, 19,20]

A consensus for a 24-months surgical oncology fellowship that will take place in Mozambique with participation of cancer surgeons experienced in Mozambique activities, internship periods at volume referral cancer centers abroad and the creation of a functional surgical oncology unit in the department of surgery of the MCH was achieved during AORTIC meeting consensus session. In this context, a surgical oncology unit was organized at the MCH.[8]

The least consensual aspect was the importance given, in the second round, in the training curriculum to the treatment of prevalent malignant tumors in Mozambique. The operative rate, in Mozambique, is deficient across all cancer types, including common cancers (breast/cervical/prostate/colorectal/esophagus). Cancer is becoming more prevalent in Mozambique, and there is an increase in new types and body locations, therefore, cancer-specific surgical capacity must be increased to meet all these new needs. This vision was included in the final proposal that achieved high consensus. A similar situation occurs in Ghana, so a Benchmarking Global Surgical Oncology study was carried out defining the unmet need for cancer surgery.[21]

The expert panel also took into account that optimal delivery of cancer therapy requires coordination across disciplines; therefore interdisciplinary training for cancer specialists was considered.[22] At the MCH therapeutic decisions are already multidisciplinary for breast, esophagus, head and neck, colorectal cancer and urogynecological tumors.[23]

Finally, the training program in surgical oncology (fellowship) designed throughout this Delphi process was submitted to the Mozambican College of Surgery for approval. This program was approved and is expected to start in 2021.

CONCLUSIONS

The training in cancer surgery in Mozambique, according to the local experts' consensus, will be based on a 24-months fellowship after residency in surgical disciplines. The curriculum embraces the Global Curriculum in Surgical Oncology and the local epidemiological, prevalent oncological diseases, resources and developmental characteristics. It will mostly take place in Mozambique but also includes dedicated internships in comprehensive and recognized cancer hospitals. The Delphi methodology was crucial to determine this consensus.

ACKNOWLEDGMENTS

We thank everyone who accepted to answer the questionnaires and be part of the panel of experts.

AUTHORS' CONTRIBUTIONS

This study was conceptualized, designed and written by Atílio Morais and Lúcio Lara Santos. Atílio Morais, Donzilia Brito and Lúcio Lara Santos formed the Steering Group. Acquisition of data was carried out by Atílio Morais, Manuel Simão, Adriano Tivane, Matchecane Cossa, Jotamo Come, Carlos Selemane, Satish Túlsidas Cesaltina Lorenzoni. Analysis and interpretation of data were done by Jessica Rodrigues, Luis Antunes and Lúcio Lara Santos, Manuel João Costa, Maria Rosário O. Martins, Moshin Sidat and Lúcio Lara Santos revised the article for important intellectual content. All authors read and agreed to the final version of this manuscript. Atílio Morais and Lúcio Lara Santos equally contributed to this study.

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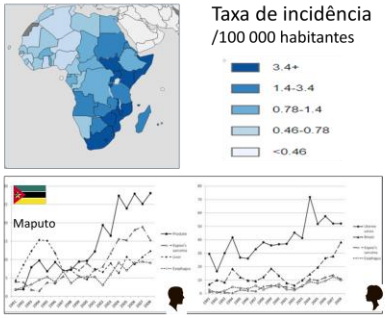
8.4 ATTACHMENT IV - Scientific publication (poster) related to the thesis

Perfil clínico e patológico dos tumores malignos do esófago em Moçambique. Estudo preliminar de 235 casos consecutivos admitidos no Serviço Cirurgia do Hospital Central de Maputo.

Jotamo Come[1]; Atílio Morais[1]; Prassad Modcoicar[1]; Satish Tulsidas[1]; Lúcio Lara Santos[2] e Carla Carrilho[1]

1 - Hospital Central Maputo, Moçambique ; 2 - IPO - Porto



Introdução

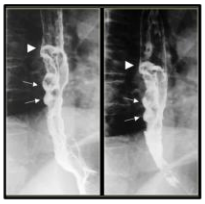
A taxa de incidência do cancro do esófago no continente africano é elevada e está associada à perda de qualidade de vida e elevada mortalidade. Em Moçambique, segundo registo oncológico de Maputo, o cancro do esófago é o 4º tumor mais frequente em ambos os géneros. Porém não se conhece com rigor o perfil clínico e patológico desta patologia no momento do diagnóstico.

Objetivo

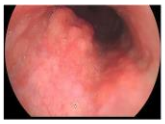
Estudar o perfil clínico e patológico dos doentes com cancro do esófago admitidos no Hospital Central de Maputo (HCM).

Metodologia

Foram estudadas, retrospetivamente, as características clínicas e patológicas de 235 doentes com cancro do esófago admitidos e tratados consecutivamente no HCM desde 1 de Janeiro de 2012 a 31 de Dezembro de 2015.



HCM



Resultados

A idade mediana foi de 56 anos (dos 22 aos 92 anos). A maioria dos doentes eram do género feminino (Mulheres 144 casos e Homens 91 casos), 98,7% dos casos eram de raça negra e em 94% os doentes eram provenientes das regiões do sul do país. Em 47,9% o tumor localizava-se ao terço médio, 37,4% ao terço inferior e em 14,7% ao terço superior. O carcinoma espinocelular foi o mais prevalente (97,4%). Em apenas 14 doentes (5,9%) o estádio da doença permitiu tratamento cirúrgico com intenção curativa nomeadamente esofagectomia subtotal ou total. Em 55 casos (23,4%) o tratamento realizado foi quimioterapia com intenção paliativa. A gastrostromia de alimentação foi realizada em 76 doentes (32,3%), nos restantes 145 doentes beneficiaram de cuidados paliativos. Não foi possível obter dados relativos à sobrevivência global.

Conclusão

Em Moçambique o cancro do esófago é uma entidade frequente. É necessário desenvolver ações de diagnóstico precoce e otimizar o tratamento. Impõem-se, por estes motivos, um programa global de combate a esta neoplasia maligna no país.

8.5 ATTACHMENT V - Strategy, protocols and pathway proposed for esophageal cancer better management in the country

Hospital Central do Maputo

Proposta para criação de uma Unidade Funcional de Oncologia Cirúrgica

I. Preambulo

O crescimento do número de casos de cancro diagnosticados no Hospital Central de Maputo(HCM) bem como os casos referenciados por outros hospitais nacionais, coloca um desafio importante pois há necessidade de melhorar a capacidade de resposta da unidade hospitalar de referência nacional para este tipo de doenças. Os cancros do colo uterino, próstata, mama, esófago e do cólon e rectal são os mais prevalentes ao nível desta unidade sanitária, adicionalmente os cancros associados a infeção pelo VIH são também frequentes.

A Unidade Funcional de Oncologia Cirúrgica congrega os conhecimentos e habilidades dos serviços de cirurgia, sendo detentora de recursos especializados no tratamento de doentes com cancro, constituído por um conjunto de profissionais com formação específica e treinados na abordagem integral desse tipo de doentes. Esta unidade é membro da Unidade de Oncologia do HCM, subordinando-se administrativamente ao Departamento de Cirurgia.

II. Justificação

A cirurgia constitui uma parte essencial no tratamento de doentes oncológicos. Há evidência que para obter os melhores resultados é necessário, para além do recurso a técnicas cirúrgicas mais ou menos complexas, a intervenção sequencial e coordenada de Oncologistas Cirúrgicos, Oncologistas Médicos, Radio-Oncologistas e um conjunto alargado de profissionais especializados na abordagem multidisciplinar de doentes com cancro e capazes de desenvolver planos de tratamento individualizados.

III. Missão

A missão da Unidade funcional de Oncologia Cirúrgica do Departamento de Cirurgia é proporcionar o tratamento adequado e célere a cada doente oncológico segundo o estádio, com o recurso às técnicas inovadoras, suportadas pela melhor evidência científica, contando com a experiência de equipas de cirurgiões dedicados a cada tipo

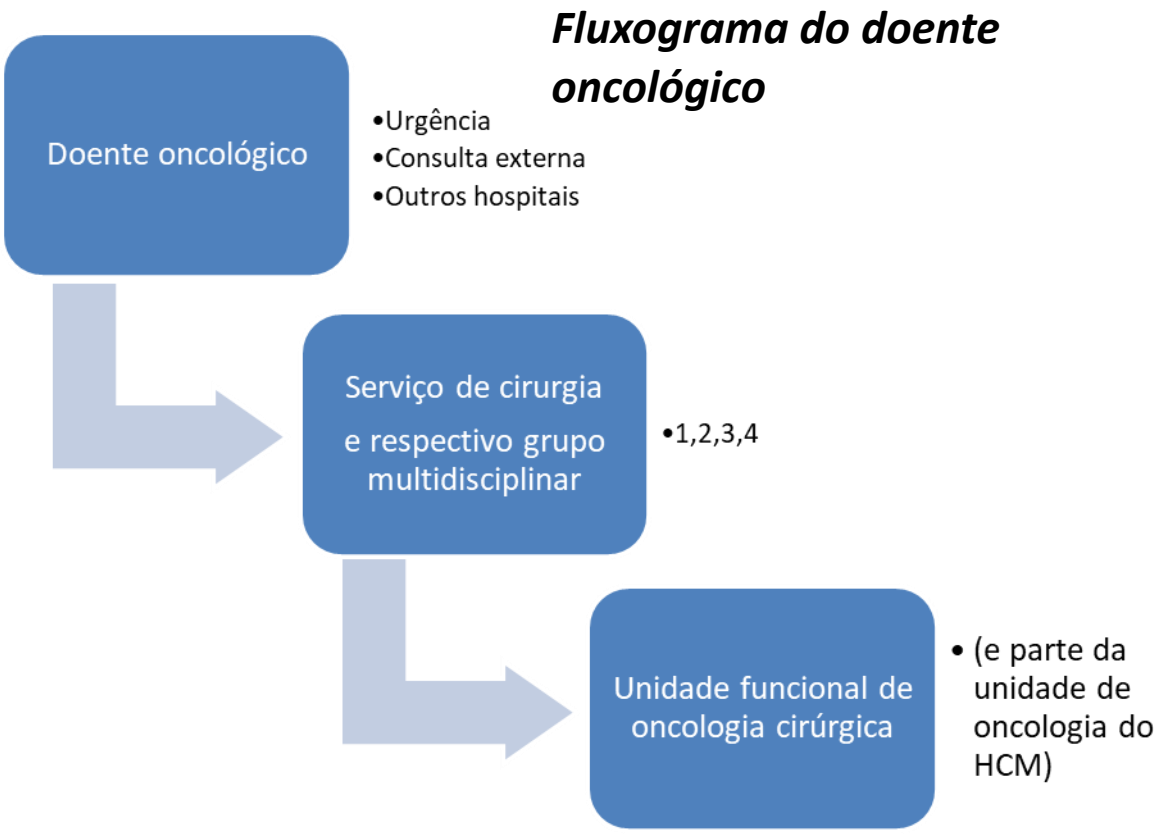
de cancro, com o objetivo de maximizar a sobrevivência e melhorar a qualidade de vida dos doentes, mas tendo sempre em conta as suas necessidades como pessoa e o respeito pela sua dignidade.

A unidade irá melhorar o registo oncológico, especialmente em cirurgia, permitindo dessa forma, auditorias técnico-científicas e promovendo o ensino de oncologia.

IV. Abordagem

O objetivo desta unidade funcional será prestar um serviço de elevada qualidade, personalizado e humano, com a oferta dos recursos técnicos mais avançados. A educação e a formação de estudantes de medicina, médicos dos internatos complementares e especialistas, constituem um dos pilares da actividade do serviço, através da atualização do conhecimento científico e disseminação das melhores práticas no diagnóstico e tratamento do cancro, estimulando a investigação clínica e enfatizando a importância da multidisciplinaridade na moderna terapêutica do cancro.

Nesta unidade os doentes serão tratados numa perspetiva multidisciplinar, beneficiando da discussão do seu plano de tratamento em reuniões multidisciplinares, com aplicação de protocolos de tratamento inovadores e de eficácia comprovada e inclusão em ensaios clínicos. Estas reuniões multidisciplinares serão realizadas com a periodicidade necessária, por área de patologia, sendo a pedra angular da actividade oncológica do hospital, onde serão discutidos os casos complexos, definidos os planos de tratamento para cada doente, determinação dos recursos necessários, revistos os resultados da cirurgia, selecionados os tratamentos complementares e analisados os casos com indicação para inclusão em ensaios clínicos.



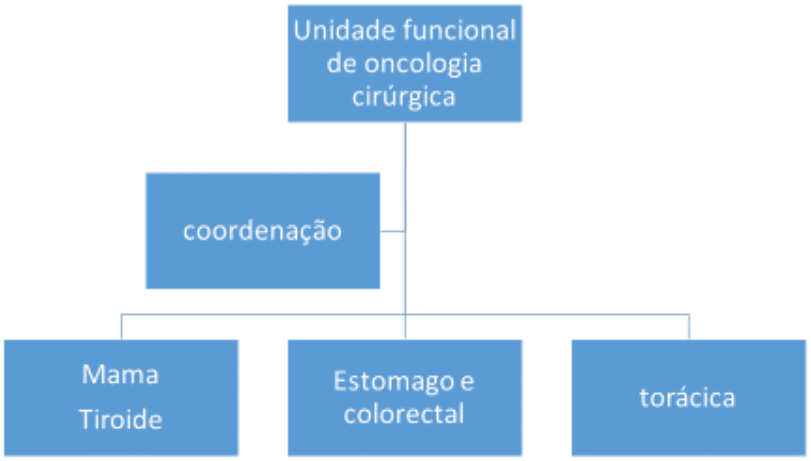
V. Equipes organizadas por patologias

Tendo em conta a perfil de doenças cirúrgicas mais comuns no país bem como a escassez de cirurgiões e de outros quadros especializados torna-se necessário programar e realizar a preparação de especialistas de hospitais secundários e terciários no sentido de aprenderem e realizarem a assistência e referenciação dos doentes de Oncologia Cirúrgica. Serão definidos nos serviços de cirurgia do HCM as equipas responsáveis pelos cuidados de oncologia cirúrgica.

Na actualidade é de amplo consenso, de que os melhores resultados são obtidos quando as equipas estão constituídas por profissionais treinados e especialmente dedicados a uma determinada patologia congregando os distintos saberes no momento em que se trata um doente oncológico; a Unidade Funcional de Oncologia Cirúrgica seria formada pelos especialistas de cada tipo de patologia, designadas Áreas de Patologia. Neste sentido, os médicos desenvolverão as suas actividades, de acordo com as respectivas áreas de especialização, oferecendo aos doentes tratamento cirúrgico baseado no uso das técnicas mais avançadas e executadas com os melhores padrões qualidade. Inicialmente, a Unidade Funcional de Oncologia do

Hospital Central do Maputo, concentraria a sua atenção nas seguintes áreas de Patologia:

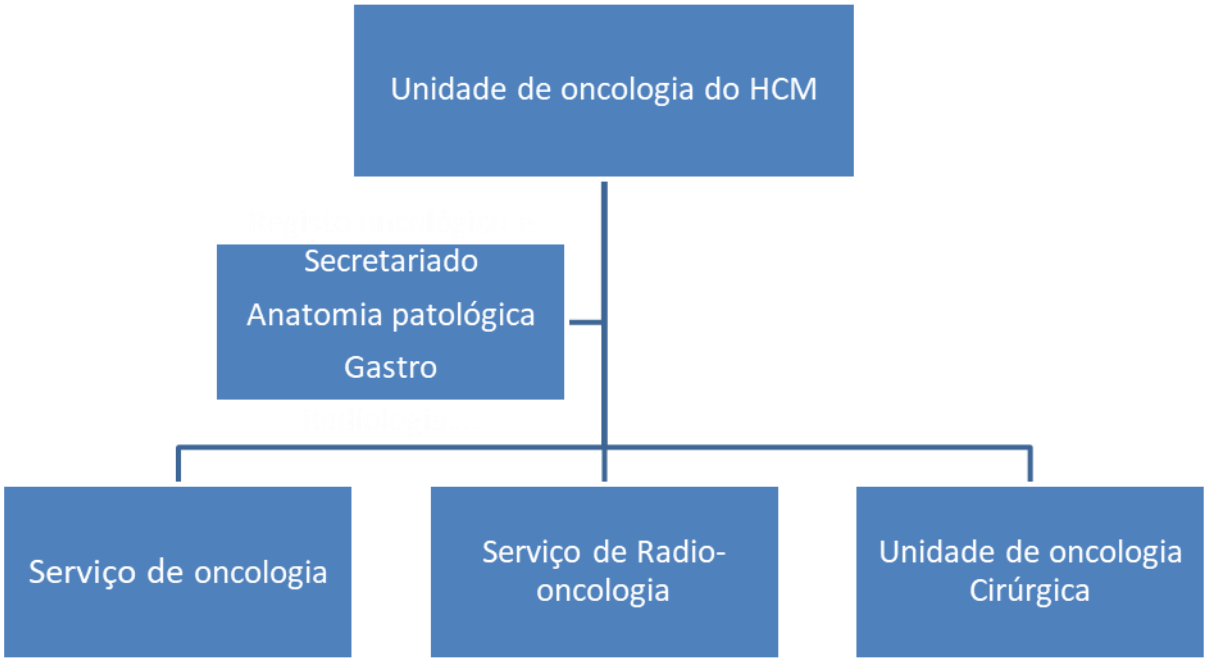
1) Cirurgia do cancro da mama
2) Cirurgia do cancro do esófago e do estômago
3) Cirurgia do cancro do cólon e do reto
4) Outras que mostrarem relevantes



VI. Instalações, recursos e gestão

As áreas referidas como constituintes da Unidade de Oncologia Cirúrgica, são basicamente unidades funcionais não implicando necessariamente a sua transformação ou sua criação como unidades físico-estruturais. As áreas de patologia funcionarão nas actuais enfermarias ou serviços fortalecendo-se em termos técnicos, na padronização das abordagens terapêuticas e na investigação científica, apostando para tal, na formação dos respectivos profissionais e na sua modernização.

Seria discutido um modelo de coordenação técnica desta unidade funcional tendo em conta que ela seria basicamente constituída por cirurgiões que tem formação específica em oncologia cirúrgica, oncologistas médicos, radio-oncologistas e outras relacionadas



VII. Ganhos para o SNS

Esta abordagem irá potenciar e expandir com maior brevidade a capacidade do sistema na assistência de doentes oncológicos no Pais em geral, incluindo em regiões onde as subespecialidades específicas que lidam com alguns tumores são raras ou ausentes. As acções de formação de profissionais em oncologia realizada de uma forma mais sistematizada irão permitir para além de maior divulgação a adopção das melhores abordagens e inovações no manejo dos doentes oncológicos no País.

VIII. Apoio logístico para actividades iniciais (2 anos)

As primeiras acções para operacionalização da criação Unidade Funcional de Oncologia Cirúrgica se enquadraram no programa para a melhoria de Atenção Integrada ao Doente Oncológico no Hospital Central do Maputo e Reforço da Capacidade Institucional, que conta com o apoio logístico da Fundação Calouste Gulbenkian.

No entanto, outras iniciativas que possam estar alinhadas terão uma contribuição valiosa no processo.

Jotamo Come

8.6. ATTACHMENT VI

Hospital Central do Maputo

Departamento de Cirurgia

Unidade de Oncologia Cirúrgica

Protocolo para o manejo dos doentes cancro esofágico

Conceito

O cancro do esófago, é o tumor maligno do tubo digestivo mais vezes diagnosticado no Hospital Central do Maputo e é a quarta neoplasia maligna mais comum no País em ambos os géneros. Mais de 95% são carcinomas espinocelulares; os adenocarcinomas são menos frequentes.

Diagnóstico

Na sua maioria, sobretudo nas fases precoces são assintomáticos, frequentemente o diagnóstico é realizado quando o tumor se encontra num estágio avançado.

Os principais sintomas incluem essencialmente a disfagia de início para sólidos e depois para líquidos, perda de peso e a dor retrosternal. A disfagia como já referimos é progressiva obrigando a mudança de hábitos alimentares com perda progressiva de peso. A dor retrosternal ou torácica, pode manifestar-se como uma dor ou desconforto no tórax médio e pode ser descrita como uma sensação de pressão ou ardor no peito. A perda de peso decorre da insuficiência alimentar e pode estar associada à anorexia e ao catabolismo associado ao tumor. Outros sintomas mais raros incluem a rouquidão, tosse persistente, vômitos, soluços, dor óssea e por vezes de Hemorragia digestiva.

O exame de diagnóstico é a biopsia da neoplasia obtida por endoscopia digestiva alta.

Exames complementares do diagnósticos

Laboratoriais

Devem ser solicitados os exames de laboratório, seguintes:

1) Hemograma Completo
2) Provas da função hepática
3) Função renal

4) Estudo de coagulação

Imagiológicos

1) Outros exames incluem radiografia simples do tórax e ecografia abdominal para avaliar possível metástase.
2) Esofagograma ou esófagogastroscopia.

   Este exame é fundamental para localizar a neoplasia, mas será também utilizado para diagnosticar potenciais fístula traqueo-esofágicas. A endoscopia tem um papel crucial, pois além de permitir o diagnóstico macroscópico, permite a colheita de amostra para estudo histológico, a avaliação da permeabilidade do lumen, bem como a colocação de stents.
3) Tomografia Computadorizada

   A tomografia computadorizada será solicitada para avaliar se existe disseminação para outros órgãos ou para gânglios. Poderá ser realizada sem e com contraste dependendo a função renal do doente ou eventuais alergias.

Endoscópicos

1) Broncoscopia

É um procedimento invasivo, será solicitado para avaliar o envolvimento da via respiratória contígua ao esófago, traqueia e brônquios. Será realizada com paciente sedado e serão colhidas amostras das secreções brônquicas para estudo citológico e microbiológico.

Avaliação cirúrgica

1) Laparoscopia de estadiamento

A laparoscopia geralmente será solicitada após diagnóstico do cancro de esófago e será realizada antes da cirurgia para confirmar se o cancro de esófago ainda está confinado apenas ao esófago e se pode ser completamente removido cirurgicamente, fundamentalmente nos tumores do esófago inferior ou nos adenocarcinomas e sempre que a tomografia computorizadas sugira envolvimento peritoneal.

2) Toracoscopia

A toracoscopia será utilizada para a realização de biópsia nos casos de presença de lesões pulmonares periféricas suspeitas, e doentes considerados inoperáveis.

Nota: Ecoendoscopia Digestiva Alta

A Ecoendoscopia digestiva alta, permitirá estadiar a neoplasia. Porém ela não é útil nos tumores estenosantes pois não permite a passagem do aparelho. Deve ser utilizada se possível.

Exame anatomopatológico

O diagnóstico do cancro de esófago será confirmado por uma biópsia cuja amostra será obtida essencialmente na altura da endoscopia digestiva alta. Deve-se constituir com as amostras um bio-banco de tecidos para estudos posteriores.

Estadiamento do Cancro do Esófago

O estadiamento irá avaliar a disseminação ou metastização para outros órgãos do corpo. Conhecer o estádio do tumor irá ajudar na definição do tipo de tratamento e a prever o prognóstico do paciente.

Sistema de Estadiamento TNM

Para o estadiamento do cancro de esófago irá obedecer o sistema TNM da American Joint Committee on Cancer.

TNM é abreviatura de tumor (T), linfonodo /gânglio (N) e metástase (M) e será adicionado G, do grau de diferenciação.

a) Tumor Primário (T)

TX. Tumor não pode ser avaliado.

T0. Ausência de tumor.

Tis. Carcinoma *in situ*.

T1a. O tumor desenvolveu-se na lâmina própria ou mucosa.

T1b. O tumor desenvolveu-se nas outras camadas e dentro da submucosa.

T2. O tumor está crescendo na muscularis própria.

T3. O tumor está crescendo na adventícia.

T4a. O tumor está crescendo na pleura, no pericárdio ou no diafragma.

T4b. O tumor está crescendo na traqueia, aorta, espinha dorsal ou outras estruturas importantes.

b) Linfonodos /Gânglios Regionais (N)

NX. Linfonodo regional não pode ser avaliado.

N0. Ausência de linfonodo comprometido.

N1. Até 2 gânglios regionais comprometidos.

N2. De 3 a 6 gânglios regionais comprometidos.

N3. Mais de 7 gânglios regionais comprometidos.

C) Metástase à Distância (M)

M0. Metástase à distância não pode ser avaliada.

M1. Metástase à distância.

d) Grau

GX. O grau não pode ser avaliado.

G1. As células são bem diferenciadas.

G2. As células são moderadamente diferenciadas.

G3. As células são pouco diferenciadas.

G4. As células são indiferenciadas.

e) Estágios para o Carcinoma de Células Escamosas

Estágio 0. Tis, N0, M0, GX ou G1; qualquer localização.

Estágio IA. T1, N0, M0, GX ou G1; qualquer localização.

Estágio IB. T1, N0, M0, G2 ou G3; qualquer localização; T2 ou T3, N0, M0, GX ou G1; localização no terço inferior.

Estágio IIA. T2 ou T3, N0, M0, GX ou G1; localização média ou superior; T2 ou T3, N0, M0, G2 ou G3; localização no terço inferior.
Estágio IIB. T2 ou T3, N0, M0, G2 ou G3; localização no terço médio ou superior; T1 ou T2, N1, M0, qualquer G; qualquer localização.
Estágio IIIA. T1 ou T2, N2, M0, qualquer G; qualquer localização; T3, N1, M0, qualquer G; qualquer localização; T4a, N0, M0, qualquer G; qualquer localização.
Estágio IIIB. T3, N2, M0, qualquer G; qualquer localização.
Estágio IIIC. T4a, N1 ou N2, M0, qualquer G; qualquer localização; T4b, qualquer N, M0, qualquer G; qualquer localização; Qualquer T, N3, M0, qualquer G; qualquer localização.
Estágio IV. Qualquer T, qualquer N, M1, qualquer G; qualquer localização.

f) Estágios para o Adenocarcinoma (raros)

Estágio 0. Tis, N0, M0, GX ou G1.
Estágio IA. T1, N0, M0, GX, G1 ou G2.
Estágio IB. T1, N0, M0, G3; T2, N0, M0, GX, G1 ou G2.
Estágio IIA. T2, N0, M0, G3.
Estágio IIB. T3, N0, M0, qualquer G; T1 ou T2, N1, M0, qualquer G.
Estágio IIIA. T1 ou T2, N2, M0, qualquer G; T3, N1, M0, qualquer G; T4a, N0, M0, qualquer G.
Estágio IIIB. T3, N2, M0, qualquer G.
Estágio IIIC. T4a, N1 ou N2, M0, qualquer G; T4b, qualquer N, M0, qualquer G; Qualquer T, N3, M0, qualquer G.
Estágio IV. Qualquer T, qualquer N, M1, qualquer G.

g)Tumor Ressecável verso Não Ressecável

Durante o estadiamento é fundamental que em consulta de decisão multidisciplinar que envolverá os especialistas abaixo mencionados se avalie a ressecabilidade da neoplasia. Nos tumores localmente avançados a possibilidade de terapêutica pré-operatória deve ser equacionada. Outras opções terapêuticas como *bypass* associado ou não a quimioterapia serão opções terapêuticas a realizar, mas incluídas em protocolo de investigação clínica.

Como regra geral, todos os cancros do esófago nos estágios 0, I e II são potencialmente ressecáveis. A maioria dos tumores no estágio III é potencialmente ressecável, mesmo quando há disseminação ganglionar. Serão considerados irressecáveis os tumores que se associem a invasão da traqueia, aorta, coluna vertebral ou outras estruturas próximas importantes.

Consulta multidisciplinar

O tratamento de doentes com cancro do esófago é definido em consulta multidisciplinar que incluirá as seguintes especialidades:

- Cirurgia, oncologia médica, radio-oncologia, anatomia patológica, gastroenterologia, anestesia, nutrição e imagiologia. Outras disciplinas devem ser convocadas sempre que necessário. A decisão deve ser tomada e publicada em documento próprio que fará parte do processo clínico do doente.

Tratamento

1-Tratamento /Estágio

O tratamento do cancro de esófago será baseado no seu estágio. No entanto, outros fatores, como estado de saúde geral do paciente, também irão influenciar na escolha das opções de tratamento.

Estágio 0

No estágio 0, as opções para tratamento irão incluir ressecção endoscópica da mucosa e a esofagectomia. Quando a ressecção endoscópica for realizada, os pacientes terão seguimento a longo prazo com endoscopias anuais ou semestrais em função do grau da displasia observada. No entanto, a opção da remoção da lesão por esofagectomia, será discutida com o doente como sendo vantajosa uma vez que não necessitará de acompanhamento com endoscopia ao longo da vida.

Estágio I

Neste estágio, o tumor atingiu uma das camadas mais profundas da parede do esófago, mas não atingiu os gânglios linfáticos ou outros órgãos.

Tumores T1. A esofagectomia para remoção da parte do esófago que contém a doença, será realizada para a maioria dos pacientes com tumores T1. Se não forem obtidas margens suficientemente livres da doença, a quimioterapia e irradiação serão indicada após a cirurgia.

Tumores T2. A cirurgia por si só será uma opção para tratamento. Se o tumor se encontra próximo ao estômago, apenas a quimioterapia será administrada antes da cirurgia.

Se o tumor se localizar na parte superior do esófago será indicada a radioquimioterapia como tratamento principal, em vez da cirurgia, seguido de um acompanhamento endoscópico para detectar uma possível recidiva da doença.
Os pacientes do estágio I que não podem fazer a cirurgia por outros problemas de saúde ou que não querem fazer a cirurgia, podem serão recomendados para possíveis tratamentos endoscópicos em centros estrangeiros ou apenas serão oferecidos quimioterapia/ou radioterapia.

Estágio II e III

O estágio II inclui tumores que invadiram a camada muscular do esófago ou o tecido conjuntivo fora do esófago, por vezes também com invasão ganglionar, 1 ou 2 gânglios linfáticos próximos.
O estágio III inclui alguns tipos de cancros que cresceram através da parede do esófago para a camada externa e para tecidos ou órgãos próximos incluindo gânglios adjacentes.
O tratamento para esses tipos de cancro será quimioterapia e radioterapia, seguida por cirurgia.
Pacientes que não podem fazer a cirurgia, por outros problemas de saúde serão normalmente tratados com quimioradioterapia.

Estágio IV

A cirurgia não será opção terapêutica. Uma gastrostomia ou jejunostomia será realizada com intuito de alimentação. A radioterapia ou outros tratamentos podem ser realizados para aliviar a dor ou melhorar a deglutição.

Quimioterapia para Câncer de Esófago

De forma geral, a quimioterapia será administrada por via endovenosa e em ciclos.

Dependendo do tipo e do estágio da doença, a quimioterapia será utilizada nos seguintes termos:

a. Antes da cirurgia, normalmente junto com a radioterapia, para reduzir o tamanho do tumor, sendo denominado neoadjuvante.
b. Após a cirurgia, normalmente junto com a radioterapia, para destruir as células tumorais remanescentes, sendo neste caso adjuvante.
c. No tratamento de tumores avançados com disseminação, por exemplo, para o fígado. A quimioterapia será usada para diminuir o tamanho do tumor e aliviar os sintomas da doença.

Os medicamentos quimioterápicos mais utilizados serão:

1) Carboplatina e paclitaxel (que podem ser associados com radioterapia).

2) Cisplatina e 5-fluorouracilo (muitas vezes associados com radioterapia)
3) ECF: epirubicina, cisplatina e 5-FU (especialmente para tumores da junção gastroesofágica).
4) DCF: docetaxel, cisplatina e 5-FU.
5) Cisplatina com capecitabina.
6) Adriamicina e ciclofosfamida

Nos casos com HER2+, a quimioterapia será ser administrada junto com trastuzumabe.

Efeitos Colaterais da quimioterapia

Pelo mecanismo de acção dos quimioterápicos, espera-se efeitos colaterais significativos, dependendo do tipo e das doses do medicamento bem como da duração do mesmo.
Os efeitos colaterais comuns à maioria das drogas quimioterápicas incluem: Náuseas e vômitos; perda de apetite; perda de cabelo; inflamações na boca; diarreia ou obstipação; diminuição das taxas sanguíneas; infecção devido a diminuição dos glóbulos brancos; hemorragia ou hematomas, devido a diminuição das plaquetas; fadiga, devido a diminuição dos glóbulos vermelhos.

No entanto, alguns medicamentos quimioterápicos podem provocar outros efeitos colaterais menos comuns, como:

- ***Síndrome mão-pé.*** A capecitabina ou o 5-FU, quando administradas como uma infusão, pode provocar vermelhidão que pode progredir para dor e sensibilidade nas mãos e pés, provocando bolhas ou descamação da pele.

- ***Neuropatia.*** É um efeito colateral comum da cisplatina, docetaxel e paclitaxel. Os sintomas incluem alterações motoras, formigamento e até dor em mãos e nos pés.

- ***Reações alérgicas.*** Alguns pacientes podem ter reações alérgicas a uma das drogas referidas e os sintomas podem incluir erupção cutânea, aperto no peito, dificuldade para respirar, dor nas costas ou sensação de tontura, devendo o provedor atento às queixas dos doentes.

- ***Diarreia.*** Pode surgir a diarreia que deve ser tratado imediatamente para evitar desidratação.

Embora se espera que estes efeitos colaterais sejam de curto prazo e desapareçam após o

término do tratamento, caso ocorrerem efeitos colaterais graves, a dose da quimioterapia será reduzida ou suspensa por um período de tempo.

Radioterapia

Considerando o estádio em que a maioria dos pacientes se apresentam, muitas vezes, a radioterapia será combinada com outros tipos de tratamento, como quimioterapia ou cirurgia.

A radioterapia será utilizada os seguintes termos:

a. Pacientes que não podem ser submetidos a cirurgia devido a co-morbilidades, como o tratamento principal do cancro do esófago, ou junto com a quimioterapia.

b. Neoadjuvante, antes da cirurgia, geralmente junto com a quimioterapia, para diminuir o tamanho do tumor antes da cirurgia.

c. Adjuvante, após a cirurgia, geralmente junto com a quimioterapia, para destruir as células cancerígenas remanescentes.

d. Paliativo, para aliviar os sintomas de câncer de esófago avançado, como dor, sangramento ou dificuldade de deglutição.

Dependendo das indicações e/ou da cirurgia, será usado uns dois tipos de radioterapia nomeadamente:

- ***Radioterapia Convencional (Radioterapia Externa).*** O tratamento será realizado cinco vezes por semana com a duração específica definida pelo radiooncologista.

- ***Braquiterapia (Radioterapia Interna).*** Na braquiterapia o material radioativo será colocado, confirme indicado e/ou disponível e uma vez terminado o tratamento o material será localmente retirado. A braquiterapia será utilizada em tumores de estágio avançado, no intuito de reduzir o tamanho para que o paciente possa engolir com mais facilidade.

Imunoterapia para Cancro de Esófago

Não sendo disponível, este tipo de tratamento será indicado quando os pacientes manifestem possibilidade de adquiri-lo fora do sistema.

Tratamento para Recidiva

O tratamento da recidiva dependerá da localização e dos tratamentos já realizados anteriormente. Se o tumor tiver sido tratado inicialmente com ressecção endoscópica da mucosa, na maioria das vezes a recidiva será no esófago. Este tipo de recidiva será tratado com esofagectomia. Se o paciente não tem condições clínicas para a cirurgia, a doença será tratada com quimioterapia, radioterapia ou ambos.

Se a recidiva for nos gânglios linfáticos, após a cirurgia de remoção do esófago, será administrada radioterapia e/ou quimioterapia. A radioterapia não será uma opção se já foi administrada no início do tratamento. No caso de a quimioterapia ter sido administrada inicialmente, ainda será possível realizar novamente, com os mesmos medicamentos ou com outras drogas.

Se a recidiva for local após quimioradioterapia, a esofagectomia será uma opção se o paciente tiver condições clínicas para o procedimento. Se a cirurgia não é possível, as opções de tratamento irão incluir quimioterapia ou outros tratamentos para prevenir ou aliviar os sintomas.

O cancro de esófago avançado será tratado como um cancro no estágio IV. Estes doentes, com cancro de esófago avançado receberão tratamentos destinados a prevenir ou aliviar os sintomas da doença.

Tratamento Paliativo

O objetivo principal desse tipo de tratamento será melhorar o conforto e a qualidade de vida do paciente.

Prótese esofágica

A colocação da prótese esofágica será uma opção para manter o canal esofágico viável por mais algum tempo. Este procedimento será realizado em gastroenterologia.

Radioterapia

A radioterapia será realizada para aliviar alguns dos sintomas do cancro de esófago avançado, como dor e problemas de deglutição. Também será administrada na doença que se disseminou para o cérebro ou coluna vertebral. A radioterapia não será repetida em áreas já irradiadas anteriormente. Nesses casos, a opção será a braquiterapia para aliviar o esófago obstruído.

Quimioterapia e Terapia Alvo

A quimioterapia e a terapia alvo será administrada no tratamento do cancro avançado, como forma de retardar o crescimento do tumor e aliviar os sintomas da doença. Nos tumores sensíveis, HER2+ a terapia alva será usará o trastuzumabe isoladamente ou em combinação com a quimioterapia.

Controle da Dor

O controlo da dor é uma preocupação importante para os pacientes com cancro. Os doentes serão manuseados em função do protocolo do manejo da dor no Hospital.

Unidade de Patologia do Digestivo

Etiqueta de identificação do doente

Data da Admissão: _____/_____/_____

PROGRAMA (*PATHWAY)* DE CUIDADOS INTEGRADOS NA ESOFAGECTOMIA

1. Referenciação Inicial

(Centro de Saúde, Hospital Público ou Privado, Médico Privado, Outro Serviço do HCM)

2. Primeira Consulta

História Clínica e Exame Objetivo

Fornecer informação educacional ao paciente

Exames Complementares de Diagnóstico

- Hemograma, bioquímica, estudo da coagulação, marcadores tumorais
- Rx Tórax; ECG
- TC torácico e abdominal
- Ecocardiograma

Consulta de psicologia

Consulta de Nutrição

Calcular *Score HCM* para definição de Risco Cirúrgico

3. Consulta de Grupo Multidisciplinar para Tratamento

Cirurgia

Quimioterapia

Quimioradioterapia

Radioterapia

Cuidados de Suporte

4. Consulta de Planeamento Cirúrgico

Marcação da data da cirurgia

Assina consentimento informado

Reserva de sangue

Consulta de Anestesia

Consulta de MFR

5. Internamento

Confirmação de reserva de sangue

Pedido de Consulta Interna de MFR

6. Bloco Operatório

Anestesia: Geral

Cirurgia

8.7 ATTACHMENT VII - Scientific publications Awarded

ENCONTROS 2017 DA PRIMAVERA ONCOLOGIA
ONCOLOGY SPRING MEETING

CERTIFICADO

O Poster
Perfil clínico e patológico dos tumores malignos do esófago em Moçambique. Estudo preliminar de 235 casos consecutivos admitidos no Serviço Cirurgia do Hospital Central de Maputo

Elaborado por Jotamo Come; Atílio Morais; Prassad Modcoicar; Satish Tulsidas; Lucio Lara Santos; Carla Carrilho

foi apresentado nos Encontros da Primavera, que decorreram de 20 a 22 de Abril de 2017, em Évora, tendo sido distinguido com o 3º Prémio na classificação de Posters Médicos.

Ana Castro *Ana Pais* *Hélder Mansinho* *Joana Augusto*
Margarida Damasceno *Miguel Barbosa* *Noémia Afonso* *Sandra Bento*

Steering Committee

EACCME
European Accreditation Council for Continuing Medical Education

Certificate

Encontros da Primavera 2017
Évora, Portugal (20.–22.04.2017)

has been accredited by the European Accreditation Council for Continuing Medical Education (EACCME) to provide the following CME activity for medical specialists.

Encontros da Primavera 2017
is designated for a maximum of, or up to 15 European CME credits (ECMEC).

Jotamo Come

(Portugal)

Each medical specialist should claim only those credits that he/she actually spent in the educational activity.
The EACCME is an institution of the European Union of Medical Specialists (JEMS), www.uems.net.
Through an agreement between the European Union of Medical Specialists and the American Medical Association, physicians may convert EACCME credits to an equivalent number of AMA PRA Category 1 Credits™. Information on the process to convert EACCME credit to AMA credit can be found at: www.ama-assn.org/go/internationalcme.
Live educational activities, occuring outside of Canada, recognized by the UEMS-EACCME for ECMEC credits are deemed to be Accredited Group Learning Activities (Section 1) as defined by the Maintenance of Certification Program of The Royal College of Physicians and Surgeons of Canada.

8.8 ATTACHMENT VIII - Conferences and scientific meetings

VIII. 1. The 11th International Conference on Cancer in Africa, 7 - 10 November 2017 Kigali, Rwanda. Speaker at the PALOP session: **Clinical and pathological profile of esophageal tumors in Mozambique - a study of 496 consecutive cases admitted to surgery at the Central Hospital of Maputo.**

VIII. 2. Encontros da Primavera, 20 a 22 de Abril de 2017, em Évora, Portugal. Poster Presentation: **O Poster Perfil clínico e patológico dos tumores malignos do esófago em Moçambique. Estudo preliminar de 235 casos consecutivos admitidos no Serviço Cirurgia do Hospital Central de Maputo**

VIII. 3. IIIº Congresso Aortic PALOP sobre cancro, 11 a 15 de Junho de 2018, Praia, Cabo Verde.
Speaker: **Mozambique Esophageal and gastric tumors prevalence**

VIII. 4. IVº Congresso PALOP Aortic, 29 a 31 Julho de 2020, Luanda, Angola.
Moderator: **Breast Cancer - The current situation in the PALOP**

VIII. 5. Encontros da Primavera 2018 - da evidência à clínica, 11 a 14 de Abril 2018, Évora, Portugal. Participant.

VIII. 6. 15º Congresso Nacional de Oncologia, 22 a 24 de Novembro 2018, Coimbra, Portugal. Participant

VIII.7. The 25th Global Meet on Cancer Research & Oncology, 20 May 2019. Rome, Italy . Participant

VIII.8. Aortic Conference 2019, 5 – 8 November 2019, Maputo, Mozambique

Poster presentation: C**aracterísticas clínicas do cancro da mama no Hospital Central do Maputo**

8.9 ATTACHMENT IX - Relevant documents

Exmo. Senhor
Prof. Doutor Artur Águas
Diretor do Doutoramento em Ciências Médicas

Jotamo José Come, Estudante n.º com o Passaporte n.º 15AK32019, Residente em Maputo, Moçambique, Av. Paulo Samuel Kankomba n.º 1568.Telefone n.º +258 21 319000 e-mail, jotacome@gmail.com, Telemóvel n.º +258843233002;
Tendo sido admitido/a como Estudante de Doutoramento em 02/01/17, vem solicitar a realização da prova pública para conclusão do ano probatório do Doutoramento em Ciências Médicas do Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto.

Data 30 de Setembro de 2017

Pede deferimento

DESPACHO (reservado à Direcção e Serviços):

APP

8.10 ATTACHMENT X - Relatório de actividades realizada no âmbito do Ano Probatório

Outubro 2017

Nome: Jotamo José Come
N˚ de Estudante: 201608911

Preâmbulo

Em Moçambique, apesar de relativa melhoria na prestação cuidados de saúde à população em geral, a atenção ao doente oncológico constitui um desafia de grande destaque. O carcinoma do esófago é o sexto cancro mais incidente no mundo e a oitava causa de mortalidade. Dados disponíveis relevam que o cancro do esófago pode ser o quarto tumor mais frequente em ambos os géneros e o tumor mais frequente do tubo digestivo em Moçambique. Neste ano probatório o estudante esteve envolvido em actividades que visam perceber melhor a situação actual do carcinoma do esófago no País.

Objetivo

O presente documento visa descrever, em forma de síntese, as actividades que foram realizadas de Janeiro a Outubro de 2017 como parte do processo de doutoramento em ciências médicas, em que o relator está matriculado.

Descrição das actividades desenvolvidas

1 - Levantamento de dados sobre os doentes atendidos com disfagias nos diversos serviços do hospital central do Maputo (livros de registo e movimento de internamento).

Esta actividade decorreu os meses de janeiro e fevereiro e consistiu na identificação dos serviços onde os são atendidos doentes que acorrem ao HCM com disfagia, considerando que grande parte de doentes com tumores do esófago se apresentam nessa fase. Desta forma foram identificados como locais de atendimento:

a) Serviço de urgência
b) Serviço de gastroenterologia
c) Serviço de cirurgia geral

d) Serviço de cirurgia torácica
e) Serviço de otorrinolaringologia
f) Serviço de oncologia
g) Serviço de anatomia patológica
h) Consultas externas

Com base nos livros e/ou sistema eletrónicos de registo dos doentes, foram identificados apenas 283 doentes atendidos com a disfagia no período de 2012 – 2016 nos serviços acima referidos

2 - Selecção de doente com tumor de esófago com base nos registos clínicos (processos clínicos).
Durante os meses de Março, Abril e Maio, procedeu se o levantamento dos processos clínicos dos doentes atrás identificados e selecção dos doentes cuja disfagia tinha como causa um tumor do esófago. Para tal no processo clinico, para além da informação clinica deveria contar um exame complementar confirmativo, maioritariamente a endoscopia ou apenas exame anátomo-patológico. Neste processo foram selecionados apenas 235 que serviram de base para um estudo preliminar.

3 - Selecção dos doentes com carcinoma do esófago confirmado por anatomia patologia (base de dados da anatomia patológica).
Durante o mês de junho, os dados clínicos atrás recolhidos foram cruzados com os dados da base de registo do serviço de anatomia patológica. Desta base foi possível identificar doentes não incluídos nas fases anteriores, mas confirmação histológica de carcinoma do esófago

4 - Elaboração de um estudo com dados preliminares sobre a situação dos tumores de esófago em Moçambique.
Como trabalho de meio-termo foi elaborado resumo que incluiu 235 doentes com carcinoma seleccionados com base nos registos clínicos antes do seu cruzamento com a base de registo do serviço de anatomia patológica. O referido estudo como título “Perfil clínico e patológico dos tumores malignos do esófago em Moçambique. Estudo preliminar de 235 casos consecutivos

admitidos no Serviço Cirurgia do Hospital Central de Maputo" descreve de forma sumária as características da doença no Pais. Apresentado nas jornadas de primavera Évora/2017 em forma de póster foi permeado com 3º Prémio na classificação de Posters Médicos.

5 - Revisão artigos sobre a doença no mundo, concepção e elaboração do protocolo de investigação que irá nortear a formação do candidato nas diferentes etapas. Esta actividade continua em curso tendo tipo maiores progressos nos meses de Agosto e Setembro.

Neste protocolo destaca-se a definição dos estudos que serão realizados no processo de formação, variáveis a serem estudadas, forma de análise dos mesmos bem como os possíveis artigos que serão publicados. Neste contexto serão realizados 2 estudos nomeadamente sendo o primeiro retrospectivo, de análise de base dados com recurso a dados secundários de cancro do Serviço de Anatomia Patológica (SAP), do Serviço de Cirurgia II e da base de óbitos, do Hospital Central de Maputo (HCM) dos doentes com carcinoma do esófago, admitidos e/ou tratados consecutivamente de 1 de Janeiro de 2012 a 31 de Dezembro de 2016 no HCM; e outro prospectivo de análise de blocos de amostras armazenadas em parafina.

Fases cumpridas e por cumprir na formalização do protocolo:

a) Revisto e aprovado pela comissão científica da Faculdade de Medicina da Universidade Eduardo Mondlane
b) Revisto e aprovado pela comissão Ética conjunta da Faculdade de Faculdade e Hospital Central do Maputo
c) Atualmente em processo de revisão para aprovação pela comissão Nacional de Bioética

6 - Para além dos referidos no protocolo, o candidato para participa noutro estudo sobre factores de risco para cancro de esófago em Moçambique liderado pelo serviço de gastroenterologia

7 - Preparação de um artigo final para a publicação já com amostra de 496 casos de tumores de esófago atendidos no Hospital Central do Maputo no

período de 2012-2016. De referir que o HCM é o hospital de referência nacional e os melhores meios para diagnóstico e tratamento disponíveis no País.

Em Anexo:

- O abstrat apresentado sob forma de póster nas jornadas de primavera, Évora/17.
- Versão do protocolo de investigação aprovado pela Comissão Cientifica da Faculdade e pela comissão conjunta da Faculdade e HCM de Ética
- Artigo preliminar para ser publicado
- Premio de Évora/17
- Certificado da EACCME

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Registo do tema de tese

Exmos.(as) Senhores(as) membros da Comissão Científica do Programa Doutoral em _____

Nome: JOTAMO JOSÉ COMO

Estudante nº: 201608911

Vem requerer a V. Exª. autorização para o registo do seguinte tema de tese proposto nos termos do n.º 1 do artigo 12.º do *Regulamento Geral dos Terceiros Ciclos de Estudos da Universidade do Porto*:

_____

_____

(por favor preencher com letra maiúscula)

Para o efeito e de acordo com o n.º do já referido artigo, dá(ão) parecer favorável o orientador e coorientador(es) (se aplicável):

Orientador Carla Maria Zamith de Franco Carrilho

(por favor preencher nome completo)

Documento de Identificação n.º _____

Instituição Faculdade Medicina - UBM Categoria Prof. Associado

Assinatura: _____ Data 12/12/17

Coorientador Lúcio José de Sousa Lobo

(se aplicável; por favor preencher nome completo)

Instituição ICBAS/ Categoria Prof Afreg

Assinatura: _____ Data 20/11/2017

Coorientador José Manuel Nunes dos Santos

(se aplicável; por favor preencher nome completo)

Instituição ICBAS Categoria Prof. Auxiliar

Assinatura: _____ Data 20/11/2017

Coorientador _____

(se aplicável; por favor preencher nome completo)

Instituição _____ Categoria _____

Assinatura: _____ Data ___/___/____

Porto, 12 de 12 de 2017

O(a) Estudante: Jotamo José Como

Recebido por: _____ em ___/___/_____

Legislação aplicável: Regulamento Geral dos Terceiros Ciclos de Estudos da U.Porto

INFORMAÇÃO DA ORIENTADORA

Carla Maria Eugénia Zamith de Franco Carrilho, Professora Associada da Faculdade de Medicina da Universidade Eduardo Mondlane, na qualidade de orientadora geral e de Moçambique do estudante do Ano Probatório de Doutoramento Jotamo José Come, com o projeto "Perfil clínico, patológico e molecular dos carcinomas do esófago em Moçambique", vem por este meio confirmar as actividades constantes no seu relatório de actividades decorrentes no ano probatório, e declarar que a evolução do trabalho realizado por este até ao momento, com qualidade e responsabilidade, faz prever a concretização dos objetivos do seu projeto de doutoramento.

Maputo, 27 de outubro de 2017

A orientadora

Carla Carrilho, MD, MSc, PhD

Cancro do esófago em Moçambique. Caracterização da doença para a definição de um programa de ação proficiente

ACTIVIDADES REALIZADAS NO ANO LECTIVO 2017/18

Comunicações realizadas

1. Clinical and pathological profile of esophageal tumors in Mozambique

   Jotamo Come
   AORTIC 11th Conference, 7 – 10 November 2017
   Kigali- Rwanda

2. Cancro do esófago em Moçambique. Caracterização da doença.

   Jotamo Come
   III Congresso da AORTIC para países PALOP, 11 a 15 Junho 2018
   Cidade da Praia- Cabo Verde

3. Perfil clínico de doentes admitidos no Hospital Central do Maputo entre 2012 e 2016.
   II Curso nacional sobre o esófago em Moçambique
   Faculdade de Medicina da Universidade Eduardo Mondlane, 2 a 4 de Julho 2018
   Maputo, Moçambique

Artigos elaborados e em curso:

1. Clinical and Pathologic Profiles of Esophageal Cancer in Mozambique: A Study of Consecutive Patients Admitted to Maputo Central Hospital
   Jotamo Come et all, J Glob Oncol 3. © 2018 by American Society of Clinical Oncology

2. Identifying barriers and finding solutions to implement best practices for cancer surgery at Maputo Central Hospital, Mozambique
   ecancer 2018

3. Artigo de revisão sistemática sobre factores de risco o cancro do esófago em Africa (em curso) – Realizada a selecção dos artigos para referenciação na descrição do artigo numa base ampla de mais de 506 pesquisados.

4. Estudo anátomo-patológico e molecular do cancro do esófago – Realizada a definição, selecção e envio ao laboratório de 50 blocos em parafina para a realização d Estudo anátomo-patológico e molecular do cancro do esófago os testes histológicos e imuno-histoquímicos pretendidos

Participação em Congressos e Cursos que abordavam o cancro do esófago

1. Cancro de esófago -1º Curso Teórico-Prático com dissecção em cadáver e intervenção cirúrgica em directo
   21 e 22 de fevereiro de 2018
   Hospitais da Universidade de Coimbra
   Coimbra- Portugal

2. Encontros da primavera 2018
   20 a 22 Abril 2017
   Évora - Portugal

3. 14˚Congresso Nacional do Cancro Digestivo
   9 a 10 de Novembro 2018
   Hotel São Rafael Atlântico
   Algarve-Portugal

4. 15˚Congresso Nacional de Oncologia
   22 a 24 de Novembro 2018

Hotel Vila Galé
Coimbra-Portugal

5. Estágio sobre oncologia cirúrgica no cancro do esófago e outros
   29 de Outubro a 29 de Novembro 2018
   Instituto Português de Oncologia
   Porto- Portugal

Jotamo Come

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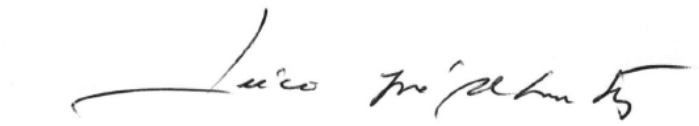
INFORMAÇÃO

O relatório do 2º ano de Doutoramento do aluno Jotamo **Come**, analisa as actividades realizadas e que foram reflectidas nos posters, comunicações e artigo que realizou durante 2018.

O trabalho desenvolvido, a seriedade e qualidade permite considerar que o percurso doutoral do aluno tem sido o adequado e o esperado.
Os dados destes dois anos de trabalho antecipam que caso mantenha as mesmas características de disciplina e trabalho o programa doutoral terminará com êxito e no prazo estabelecido.

Porto 26 de Noembro de 2018



Lúcio Lara Santos MD, PhD
(Prof. Afiliado do ICBAS)

3◦ Ciclo de Doutoramentos – Ciências Médicas

Tema : Cancro do esófago em Moçambique. Caracterização da doença para a definição de um programa de ação proficiente

Actividades realizadas no ano lectivo 2018/19

A - Comunicações realizadas

"Clinical and Pathologic Profiles of Esophageal Cancer in Mozambique: A Study of Consecutive Patients Admitted to Maputo Central Hospital"

"25th International Conference on Cancer Research and Oncology"(Cancer 2019) Rome, Italy on May 20-21 with the theme "Recent Trends in Cancer Research and Oncology"

Cancro da mama em Moçambique. Caracterização da doença. IV Congresso da AORTIC, 5 a 08 Novembro 2019. Cidade de Maputo - Moçambique

Abordagem Cirúrgica de Cancro no geral e especial no Esófago. I Curso Básico de Oncologia Cirúrgica em Moçambique
Faculdade de Medicina da Universidade Eduardo Mondlane, 11 a 13 de Fevereiro 2019. Maputo, Moçambique

B - Artigos elaborados e em curso

1. Artigo de revisão sistemática sobre factores de risco o cancro do esófago em Africa – Realizada a selecção dos artigos para referenciação na descrição do artigo numa base ampla de mais de 506 pesquisados. Em

curso nova actualização dos artigos por ter havido publicações mais actualizadas.

2. Estudo anátomo-patológico e molecular do cancro do esófago – Em fase de finalização testes moleculares em laboratórios internacionais de referência

3. Esophageal cancer in Mozambique: should mycotoxins be a concern? Jotamo Come, Edgar Cambaza, Rita Ferreira, José Manuel Correia da Costa, Carla Carrilho, Lúcio Lara Santos *Pan African Medical Journal 33, 187, 11/07/2019*

4. Factores de rísco do cancro do esófago em Moçambique. Em fase de elaboração do artigo final para publicação

C - Congressos e outras actividades na abordagem do cancro do esófago

1. 15˚Congresso Nacional de Oncologia
   24 de Novembro 2018
   Hotel Vila Galé
   Coimbra-Portugal

2. Elaboração de uma proposta para criação de uma unidade de oncologia cirúrgica no Hospital referência, Maputo, Moçambique. Documentos em anexo.

D - ANEXOS

a) Artigo publicado sobre Micotoxinas e cancro do esófago em Moçambique
b) Proposta de criação de uma unidade de cirurgia oncológica no hospital local
c) Abordagem na esofagectomia (pathway)/Hospital Central do Maputo (HCM)

d) Abordagem na ressecção colorectal/HCM
e) Abordagem na mastectomia/HCM
f) Proposta de uniformização do tratamento e seguimento de pacientes com cancro do esófago
g) Posters e diplomas de participação

Jotamo Come, Novembro 2019

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INFORMAÇÃO

O relatório do 3º ano de Doutoramento do aluno **Jotamo Come**, analisa as actividades realizadas e que foram reflectidas nos posters, comunicações e artigo que realizou durante 2019.

O trabalho desenvolvido, a seriedade e qualidade permite considerar que o percurso doutoral do aluno tem sido o adequado e o esperado.

Os dados destes três anos de trabalho antecipam que caso mantenha as mesmas características de disciplina e trabalho o programa doutoral terminará com êxito e no prazo estabelecido.

Porto 18 de Novembro de 2019

Lúcio Lara Santos MD, PhD

(Prof. Afiliado do ICBAS)

3º Ciclo – Doutoramento em Ciências Médicas

Doctoral Programme in Medical Sciences

EDIÇÃO 2016/2017 EDITION

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INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR
UNIVERSIDADE DO PORTO

Formulário de Proposta de Tese / Thesis Proposal Form

Using this Form, you can submit a project proposal to the Doctoral Programme when applying and before commencing the research project. You should consult and obtain the agreement of your supervisor(s) prior to submitting the proposal. Note that the Form is expandable, but please use only the amount of space that is strictly necessary, and do not use a text font other than Arial (9 point). The proposal will be reviewed by members of the Scientific Committee, and may be rejected or accepted (eventually in a provisional way). Please, submit the Form (in PDF format) with your application.

PROJECT INFORMATION (answer in ENGLISH or in PORTUGUESE)

Candidate Name: Jotamo Come

Phone: jotacome@gmail.com

Email:

Note: Please attach PDF copy of the CV (as in FCT-SIG database - http://www.fct.mctes.pt/fctsig/cv/# or in Europass CV)

PROPOSED SCIENTIFIC SUPERVISORS (maximum 3)

General Supervisor and Mozambique supervisor: Carla Carrilho

Highest Degree: MD,PhD,

Institution: Universidade Eduardo Mundelane

Category / Position: Professor

Phone:

Email:

Note: Please attach PDF copy of the CV (as in FCT-SIG database - http://www.fct.mctes.pt/fctsig/cv/# or in Europass CV)

Portugal Supervisor : Lúcio Lara Santos

Highest Degree: MD, MSc e PhD

Institution: ICBAS e Instituto Português de Oncologia Porto

Category / Position: Professor auxiliar convidado

Phone:916331754

Email: llarasantos@gmail.com

Note: Please, attach PDF copy of the CV (as in FCT-SIG database - http://www.fct.mctes.pt/fctsig/cv/# or in Europass CV)

Co-supervisor (Portugal) : Jorge Santos - Santo António

Highest Degree: MD, PhD

Institution: ICBAS

Category / Position: Professor Auxiliar Convidado

Phone:919981890

Email:

Note: Please attach PDF copy of the CV (as in FCT-SIG database - http://www.fct.mctes.pt/fctsig/cv/# or in Europass CV)

Host Institutions, Depts, Labs, Services, Units, for conducting the work related with this project proposal:

1: Hospital Central de Maputo, Moçambiqe
2: Instituto Português de oncologia, Porto, Portugal
3: Centro Hospitalar do Porto, Portugal

Title of Proposal:

Cancro do esófago em Moçambique. Caracterização da doença para a definição de um programa de ação proficiente.

Abstract:

A melhoria das condições de vida, o aumento da esperança de vida ao nascer e a mudança de estilos de vida têm criado condições para que doenças crónicas, entre as quais as oncológicas, se tornem prevalentes. O cancro do esófago é uma neoplasia maligna muito frequente em África, fundamentalmente na costa do índico e não são claramente conhecidos os aspetos epidemiológicos.

Em Moçambique o cancro do esófago é uma neoplasia maligna prevalente, sendo o quarto tumor mais frequente em ambos os géneros e está associado a elevada taxa de mortalidade.
Sendo um problema de saúde pública torna-se necessário caracterizar os fatores de risco, o perfil da doença par melhorar a sua prevenção e tratamento. Assim pretende-se desenvolver um projeto de investigação que permita a definição de um programa de ação no sentido do controlo desta neoplasia maligna.

State of the art :

As taxas de incidência do carcinoma epidermoide do esófago no centro, leste e sul do continente africano são elevadas, sendo responsáveis pela perda de qualidade de vida destes doentes e elevada mortalidade (*1-10)*

.

Os motivos subjacentes à elevada frequência de casos desta neoplasia maligna nesta região africana é desconhecida. São necessários estudos epidemiológicos bem desenhados no sentido de se esclarecer os fatores de risco envolvidos. Um estudo de caso controlo e a caracterização molecular desta neoplasia em Moçambique poderá ajudar a definir eventuais fatores de risco.

O diagnóstico do cancro do esófago é realizado tardiamente o que motiva elevadas taxas de mortalidade. Os dados do registo oncológico de base populacional de Maputo revelaram que o cancro do esófago é o quarto tumor mais frequente em ambos os géneros e o tumor mais frequente do tubo digestivo. Por este motivo esta neoplasia maligna é uma prioridade (11).

Em Moçambique, a disfagia tem sido uma causa importante de internamento. Muitos destes doentes, são admitidos com um quadro de desnutrição e de desidratação pela dificuldade na ingestão de alimentos e líquidos. A cirurgia é na sua maioria de cariz paliativa incluindo a realização de gastrostomia de alimentação. Muitos doentes desenvolvem complicações associadas a este procedimento. A paliação endoscópica é necessária.

A reorganização do sector oncológico de Moçambique está em curso há uma clara melhoria das instituições, existe um excelente serviço de anatomia-patológica há a incorporação novos recursos terapêuticos, tem havido um incremento no conhecimento, tem-se definindo indicadores, bem como mecanismos de supervisão e de auditoria, cremos, assim que é o momento para definir um programa de ação de combate ao cancro do esófago em Moçambique

Brevemente o país poderá contar recursos de radioterapia. Tornar-se á necessário enquadrar esta modalidade terapêutica no protocolo terapêutico do cancro do esófago.

É necessário agregar o conhecimento produzido sobre o cancro de esófago em África nesse sentido desenvolveremos uma revisão sistemática sobre o assunto.

Com o objetivo de criar este programa global de combate a esta doença decidiu-se recorrendo aos registos do Hospital Central de Maputo (HCM) estudar o cancro do esófago no HCM, o seu perfil clínico, patológico e molecular e com base nesses dados definir um plano de ação que possa contribuir para a melhoria do tratamento destes doentes.

Temos a intenção de realizar num estudo caso no sentido conhecer os fatores de risco definindo a população em risco e desenhando um programa de diagnóstico precoce.

Objectives:

1- Conhecer o perfil clinico, patológico e molecular do cancro do esófago em Moçambique
2- Estudar os fatores de risco associados a esta neoplasia maligna
3- Definir o programa de ação de combate ao cancro de esófago em Moçambique

Detailed Description – Material and Methods:

Realizar a revisão sistemática sobre cancro do esófago na África subsaariana;

Proceder ao levantamento exaustivo de todas os dados clínicos e patológicos de uma de cancros do esófago admitidos e tratados no Hospital Central de Maputo;

Avaliar os tipos histológicos mais prevalentes e as alterações moleculares mais frequentes;

Realizar um estudo caso controlo

Timeline (Summarise your work plan during time and milestones for completion of your project. Graph can be inserted):

Ano	**Actividade**	**Responsável**	**Local**
2016	Preparação dos recursos para a concretização do projecto de doutoramento e deum estudo caso-controlo Estudo clinico e patológico de 235 casos de cancro do esófago admitidos e tratados e no HCM	Dr. Jotamo Come Dr. Jotamo Come Prof. Dra. Carla Carrilho Prof. Dr. Lúcio Lara Santos	Maputo, Moçambique Publicação do estudo retrospetivo clínico-patológico
2017 (ano Probatório)	Revisão sistemática sofre o cancro do esófago em África Estudo anatomo-patológico e molecular do cancro do esófago (amostra de moçambique)	Dr. Jotamo Come Prof. Dra. Carla Carrilho Prof. Dr. Lúcio Lara Santos Prof. Dra Carla Carrilho Prof. Dr. Lúcio Lara Santos	Maputo, Moçambique Publicação da revisão sistemática Publicação dos tipos histológicos mais frequentes e alterações moleculares prevalentes
2018	Definição do programa de ação de combate ao cancro do esófago em Moçambique	Dr. Jotamo Come Prof. Dra. Carla Carrilho Prof. Dr. Lúcio Lara Santos Prof. Dr. Jorge Santos	Publicar os resultados do estudo caso-controlo e programa de ação
2018	Elaboração da tese	Dr. Jotamo Come Prof. Dra. Carla Carrilho Prof. Dr. Lúcio Lara Santos Prof. Dr. Jorge Santos	

References:

1 - M.L. Cheng et al. / Cancer Epidemiology 39 (2015) 143–149;

2 - Sewram V et al. Cancer Epidemiology 41 (2016) 113–121;

3 - Schaafsma T, Wakefield J, Hanisch R(3), Bray F, Schüz J et al.Africa's Oesophageal Cancer Corridor: Geographic Variations in Incidence Correlate with Certain Micronutrient Deficiencies. PLoS One. 8;10(10):e0140107. eCollection 2015.

4 - Gupta B, Kumar N. Worldwide incidence, mortality and time trends for cancer of the oesophagus. Eur J Cancer Prev. 2016.

5 - Mwachiro MM, Burgert SL, Lando J, Chepkwony R et al. Esophageal Squamous Dysplasia is Common in Asymptomatic Kenyans: A Prospective, Community-Based, Cross-Sectional Study. Am J Gastroenterol. 2016;111(4):500-7.

6 - Sewram V, Sitas F, O'Connell D, Myers J. Tobacco and alcohol as risk factors for oesophageal cancer in a high incidence area in South Africa. Cancer Epidemiol. 2016;41:113-21.

7 - Mlombe YB, Rosenberg NE, Wolf LL, Dzamalala CP et al. Environmental risk factors for oesophageal cancer in Malawi: A case-control study. Malawi Med J. 2015 Sep;27(3):88-92.

8 - Pillay V, Isaacson C, Mothobi P, Hale M et al.Carcinogenic nitrosamines in traditional beer as the cause of oesophageal squamous cell carcinoma in black South Africans.S Afr Med J. 2015 Sep 21;105(8):656-8.

9 - Kayamba V, Sinkala E, Mwanamakondo S, Soko R, Kawimbe B et al. Trends in upper gastrointestinal diagnosis over four decades in Lusaka, Zambia: a retrospective analysis of endoscopic findings. BMC Gastroenterol. 2015 Oct 6;15:127.

10 - Matejcic M(1), Iqbal Parker M. Gene-environment interactions in esophageal cancer. Crit Rev Clin Lab Sci. 2015;52(5):211-31.

11 – Lorenzoni C et al. Trends in cancer incidence in Maputo, Mozambique 1991-2008. PLOS ONE | DOI:10.1371/journal.pone.0130469 June 25, 2015

Candidate's Signature: ..... **Date:**

___/___/2014

Supervisors' Comments on the Project Proposal:

Is this proposal acceptable to you and do you agree to supervise this student? Yes

Do you grant to have or will have access to the requirements for the project? Yes

Did you check with the institute / department / laboratory heads that you can host the student? Yes

Comments about costs (e.g., how will the costs be met; if there are grant, project, other, then detail which one(s)):

Os custos serão suportados pelo Centro nacional de Oncologia em Angola.

Supervisor's Signature: ..... **Date:**
____/____/2014

Co-supervisor's Signature: ..... **Date:**
____/____/2014

Co-supervisor's Signature: ..... **Date:**
____/____/2014

TO BE FILLED BY THE DIRECTION OF THE DOCTORAL PROGRAMME

Decision of the Scientific Commission on the proposal:

1. **Accepted as it is [ ] Date: ____/____/2014**
2. **Accepted (conditionally) [ ] Date: ____/____/2014**
3. **Rejected [ ] Date: ____/____/2014**

The Director of the Programme: .....

The Other Members of the Scientific Commission:

.....

.....

.....

Boletim de Inscrição no Ensino Superior

Ano Letivo 2019/2020

INSCRIÇÃO EFETUADA COM SUCESSO

Identificação do estudante		**Curso/ciclo de estudos em que se inscreve**	
Nº interno:	201608911	**Designação:**	Doutoramento em Ciências Médicas
Nome:	Jotamo Jose Come	**Ano curricular:**	4º
Doc. ident.:	12AC16832 (Passaporte)	**Regime:**	Tempo integral
Nacionalidade:	moçambicana	**Estatuto:**	Estudante internacional
Endereço:	AV. Paulo Samuel Kankomba 1568, Maputo Moçambique		
Telefone:	258823014024		

Unidades curriculares em que se inscreve

Nº	Ano	Período	Unidade curricular	Código	Créditos
1	4º		Tese de Doutoramento	CM02	

Inscreve-se a um total de 0.0 créditos de um máximo permitido de 60.0 créditos em primeiras inscrições em unidades curriculares e 240.0 créditos no total da inscrição.

Inscrição em ano letivo realizada em 2019-11-15.

Código de validação: eUœ`□ïÇ]}¯à“Š|\

Emitido em 2019-11-15.