# PREVALENCE OF OROPHARYNGEAL SQUAMOUS CELL CARCINOMA AT CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

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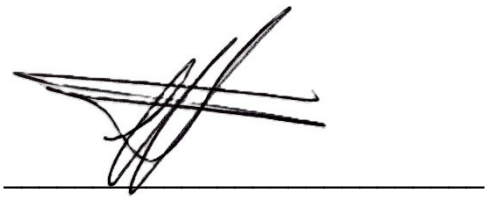
A Research Report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment id the requirements for the degree of Master of Science in Dentistry (Oral Medicine).

Johannesburg, 2024.

## DECLARATION

I, Whitney Gayle Baartman, declare that this Research Report is my own, unaided work. It is being submitted for the Degree of Master of Science in Dentistry (Oral Medicine) at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



**11** day of **November 2024** in **Johannesburg**

To my mother, Jenny, and my husband, Daniel, for your unending love and support always.

## ABSTRACT

**Context:** Limited data regarding prevalence of oropharyngeal squamous cell carcinoma (OPSCC) in South Africa are available.

**Objective:** Examine the prevalence of OPSCC at a Johannesburg Academic Hospital and describe demographics of affected patients, anatomic subsites and associated risk factors.

**Methods:** All laboratory records of OPSCC cases over a 9-year period (July 2013-July 2022) were evaluated. Histological and clinical data were analysed.

**Results:** OPSCC accounted for 32.1% of all head and neck squamous cell carcinomas. 67 men and 29 women were affected (mean age 60.31 years). Most affected subsites included tonsil (n=45) and base of tongue (n=38). Most cases showed keratinisation (n=63) and moderate differentiation (n=62). 16 cases were tested using p16 immunohistochemistry (IHC); 75% (n=12) showed p16-positivity. Five cases underwent HPV-DNA testing, two (40%) tested negative.

**Conclusion:** OPSCC is a substantial burden in this cohort. Given the prognostic implications, routine p16 testing, with HPV-DNA testing if positive, should be implemented.

## ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my supervisors Professor Neil Wood, Dr Sizakele Ngwenya and Dr Tumane Mafojane for their invaluable guidance and unwavering support throughout this study. Your combined expertise and dedication have greatly enhanced the quality of this project. I would also like to extend my heartfelt appreciation to Zvifadzo Matsena-Zingoni for her contribution to the statistical analysis of this research project. Lastly, I would like to thank the National Health Laboratory Service for their assistance in providing access to the data required for this study.

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## NOMENCLATURE

**HNSCC:** Head and Neck Squamous Cell Carcinoma

**HPV:** Human Papillomavirus

**HR-HPV:** High-risk Human Papillomavirus

**IHC:** Immunohistochemistry

**ISH:** In Situ Hybridisation

**OSCC:** Oral Squamous Cell Carcinoma

**OPSCC:** Oropharyngeal Squamous Cell Carcinoma

**SCC:** Squamous Cell Carcinoma

# CHAPTER 1 – INTRODUCTION AND LITERATURE REVIEW

## 1.1 Introduction and Literature Review

Head and Neck Squamous Cell Carcinoma (HNSCC), the seventh most common cancer globally (1,2), refers to a group of malignancies arising from the epithelium of the oral cavity, pharynx and larynx (1,3). Despite a decrease in smoking trends over the last two decades, especially in developed countries, the incidence of HNSCC has risen, with researchers predicting a further increase of 30% each year by 2030, largely due to surges in the number of cases of oropharyngeal squamous cell carcinoma (OPSCC), a sub-category of HNSCC most commonly affecting the deep crypts of the palatine and lingual tonsils as well as the base of the tongue, soft palate, uvula, oropharyngeal walls and epiglottic vallecula (1–9).

Due to differences in the sociodemographic profile, clinical presentation, risk factors, tumour characteristics and prognosis, the 2017 8$^{th}$ edition of the American Joint Committee on Cancer (AJCC) staging system has classified OPSCC into two distinct disease entities. This classification is based on the potential involvement of what has become known as an important risk factor, the Human Papillomavirus (HPV). The 2022 world health organisation (WHO) classification of head and neck tumours makes a clear distinction between HPV-associated and HPV-independent OPSCCs, underscoring the differences in epidemiology, histology, and prognosis. (6,10–15). Patients with HPV-associated OPSCCs are 3-4 years younger than those with HPV-independent OPSCCs. Unlike HPV independent OPSCC which originate from surface epithelium, HPV-associated OPSCCs originate from the crypt epithelium of palatine and lingual tonsils, have a better overall and progression free survival and show a predominantly nonkeratinising morphology (6,10–15).

In a 2020 study on Global Cancer Statistics, OPSCC accounted for approximately 100 000 cases of newly diagnosed cancers (2). It is estimated that the global burden of OPSCC grows by approximately this number each year (16). However, overlapping data from the oral cavity and the oropharynx, owing to their close anatomical relationship,

has previously proven challenging when attempting to establish a precise prevalence of OPSCC (4,5,7,17).

The global prevalence of HPV-associated OPSCC was estimated at 33% in 2021, albeit with substantial geographical variability, ranging from 0% in some regions to as high as 85% in others. (16,18) The increase in cases of OPSCC in recent years, particularly in younger males from high-income countries, has emerged as a significant concern in oncological research. This trend is primarily driven by the rise in HPV-positive OPSCC, which has become more prevalent due to changes in sexual behaviours, such as increased oral sex, and lagging HPV vaccination rates among males. The gender disparity in HPV infection clearance and the generally better prognosis of HPV-positive OPSCC have also contributed to the rising incidence and awareness of this disease in younger males. These factors highlight the need for targeted public health interventions, including increased HPV vaccination coverage and awareness efforts, particularly in high-income countries where this trend is most pronounced (6,18). Over the past four decades, multiple studies have demonstrated an increased incidence of both HPV-associated and HPV-independent OPSCC. However, evidence from high-income countries such as the USA and UK shows the former to be increasing more rapidly (4–6,14,16,19,20), with the incidence in men surpassing that of cervical cancer in women in these regions (6,18,21).

Sub-Saharan Africa, and South Africa in particular, face a significant burden of cervical cancer and other HPV-related malignancies as well as the highest prevalence of HIV globally. (8,21–23) Medical conditions such as HIV impair the cell-mediated immune response, leading to an elevated risk of both acquisition and progression of HPV infections, with affected individuals being significantly more likely to present with multiple HPV infections and with high risk (HR)-HPV types (22,24) including HPV16, a HR-HPV  responsible for approximately 90% of HPV-associated OPSCC (25).

Human papillomavirus (HPV), particularly high-risk (HR-HPV) types such as HPV-16 and HPV-18, plays a pivotal role in the development of  OPSCC. Unlike traditional risk factors such as tobacco and alcohol, HPV-associated OPSCC is primarily driven by the virus's ability to manipulate cellular mechanisms through its oncoproteins, E6 and E7.

These oncoproteins interfere with key tumour suppressor pathways, notably by degrading p53 and inactivating pRB, leading to uncontrolled cell proliferation and evasion of apoptosis. This viral-driven oncogenesis results in distinct molecular and clinical characteristics compared to HPV-independent HNSCC. Notably, HPV-associated OPSCC tends to occur in younger patients with minimal exposure to traditional carcinogens and is often associated with a better prognosis due to its unique tumour biology. The strong causal link between HPV and OPSCC underscores the importance of preventive measures, such as HPV vaccination, and highlights the need for accurate HPV testing in all cases of OPSCC to guide treatment and management strategies.

HR-HPV encodes viral oncoproteins (E5, E6 and E7) which are able to induce oncogenesis in virally infected epithelium (26). While the role of E5 and other oncoproteins is unclear, E6 and E7 are known to target the p53 and retinoblastoma (RB) tumour suppressor pathways, resulting in the degradation and inactivation of p53 and RB proteins, respectively (26–28). The oncoproteins also act on other tumour suppressive and oncogenic pathways including Notch and TGF-β signaling (28). The expression of E6 and E7 is often related to viral genome integration into DNA regions of genomic instability and subsequent disruption of the E2 coding region, the gene responsible for repressing E6 and E7 (26,27). This ultimately leads to the dysregulation of E6 and E7, enabling the virus to establish a persistent infection and continue replicating (26).

E6 Oncoprotein:

The E6 oncoprotein from high-risk HPV types, particularly HPV-16 and HPV-18, plays a crucial role in HPV-driven carcinogenesis by promoting the degradation of the tumour suppressor protein p53. Normally, p53 functions to induce cell cycle arrest or apoptosis in response to DNA damage. However, E6 binds to p53 and, through the recruitment of the cellular ubiquitin ligase E6AP (E6-associated protein), leads to the ubiquitination and subsequent proteasomal degradation of p53. The loss of p53 function prevents the normal DNA damage response through the induction of quiescence, senescence,

apoptosis and the expression of DNA repair genes. This allows cells to accumulate mutations and continue dividing uncontrollably, contributing to malignant transformation.

E7 Oncoprotein:

The E7 oncoprotein from high-risk HPV types also plays a key role in carcinogenesis by inactivating the retinoblastoma protein (pRB). pRB is a tumour suppressor that regulates the cell cycle by controlling the transition from the G1 to the S phase. E7 binds to pRB, leading to its degradation, and disrupts its ability to control the cell cycle. As a result, E2F transcription factors are released from pRB control, leading to the activation of genes required for DNA replication and cell cycle progression. This unchecked cell division further drives the process of carcinogenesis, particularly in the context of HPV-related cancers such as OPSCC (28-30)

In contrast to HPV-associated OPSCC, HPV-independent OPSCC is primarily linked to traditional risk factors including tobacco and alcohol use (6,18,21,32). Persistent exposure to these and other carcinogens, including but not limited to *Toombak*, *Kola nut*, *Miraa/Khat* and *Areca nut* (1,3,11,12,33,34), results in cytological and molecular changes in the epithelium (35), allowing for the accumulation of genetic mutations and epigenetic modifications in the signalling pathways that govern the cell-cycle control system, and ultimately leading to malignant transformation (35,36).

Cigarette smoking not only increases the risk of HPV-independent HNSCC by two to four times in comparison to non-smokers (37), but is also recognised as an independent risk factor for oral HPV infection as well, through decreased clearance of HPV from the oral cavity (1,23,38,39). It has also been shown that the risk of developing HNSCC as well as HPV infection persistence is dose-dependent, resulting in a higher risk for those that smoke more frequently and, more especially, for an increased duration (23,37). In a study of HPV-associated and HPV-independent OPSCC, patients with >10 pack-year smoking history had worse overall survival rates compared to those with ≤10 pack-year history (37). This study was also able to demonstrate that patients who used tobacco products had an increased risk for locoregional recurrence and a 1.3 - 4 times worse overall survival compared to those that never smoked or smoked less frequently (37).

Smoking suppresses the immune response (24) which may contribute to smokers being more likely to have more advanced comorbidities (37). This may also impact treatment-related outcomes resulting in poor wound healing and post-surgical complications as a result, decreased radiation efficacy (37), and ultimately, a higher risk of treatment failure and mortality (40).

Alcohol is also recognised as an independent aetiological factor in the development of HPV-independent OPSCC both directly through its metabolite form, acetaldehyde (a carcinogen), and indirectly through inducing mucosal damage, thus facilitating the absorption of additional toxins, including those present in cigarettes (41). However, when consumed with tobacco products, the two act synergistically in such a way that multiplies the risk of carcinogenesis (1,11–13).

Studies have described males as being typically more likely to take part in risky lifestyle behaviours such as the consumption of tobacco and alcohol products (3,7,42), as well as being more likely to report an increased number of sexual and oral sexual partners in comparison to their female counterparts (6,43), increasing the risk for males overall for both HPV-associated and HPV-independent OPSCC. A South African study exploring oral-genital HPV concordance in couples showed that the majority of couples that displayed oral-genital concordance showed male oral HPV infection corresponding with their partner's vaginal HPV infection, indicating an increased likelihood of transmission occurring vaginal-to-oral rather than penile-to-oral (22), and offering a possible further explanation for why males are more commonly affected than females in HPV-associated OPSCC.

Patients with HPV-associated OPSCC are commonly described as being younger, non-smoking, educated and of a higher socioeconomic status in that these patients are more likely to reside in areas with increased secondary schooling completion rates, have higher median incomes and private insurance when compared with patients with HPV-independent OPSCC. (6,8,44–47)  When considering communities with diverse populations, a study conducted in the USA showed that patients with HPV-associated OPSCC were also significantly more likely to be Caucasian when compared with HPV-independent OPSCC who were more likely to be non-white, possibly due to exposure to

other risk factors likely related to socioeconomic status rather than race (9,47). The rationale for categorizing vulnerable populations by ethnicity by the authors was not specified.

Interestingly, variants of the HPV 16 genotype and antigens have been shown to appear in clusters across various geographic locations (9). Genotypical differences may result in variations pertaining to the risk of contracting an HPV infection, the persistence of the infection and its subsequent development into a carcinoma, and, may therefore account for the differing rates of HPV-associated OPSCC across different population groups (9). This has been demonstrated previously in studies of disparities in the incidence and mortality rates of cervical cancer (9). In addition, individuals of various cultural or ethnic backgrounds may choose to limit their sexual relations to individuals within the same social group, resulting in the circulation of a specific pool of HPV types (9). This could offer an additional explanation for differences seen among various genetic backgrounds (9).

HPV-associated OPSCCs are more likely to present within the oropharynx as a small primary tumour, where HPV-independent OPSCCs are likely to be larger (1,12,14,47), as demonstrated in a 2019 study, where 70.9% of HPV-associated OPSCCs presented as ≤T2 in comparison to 57.0% of HPV-independent OPSCCs (47). In a 2017 study on HPV-associated and HPV-independent OPSCC, affected anatomic subsites of the oropharynx were analysed based on whether the tumour was found to be affecting the tonsils, base of tongue, soft palate or posterior pharyngeal wall (12). In both HPV-associated and HPV-independent OPSCC, the tonsillar complex was found to be most commonly affected (12). The second most commonly affected subsite differed between HPV-associated and HPV-independent OPSCC with the base of tongue second most affected for HPV-associated OPSCC and the soft palate second most affected for HPV-independent OPSCC (12). HPV-associated OPSCC tends to affect the base of tongue and crypts of the lingual and palatine tonsils most frequently, (29,34,38,45,46,48), likely due to the similarities between the tonsillar crypt epithelium and the cervical transformation zone(TZ). (26,49) The TZ and the reticulated epithelium of the palatine tonsils are specialised epithelial sites with distinct characteristics and functions and are

particularly susceptible to HPV infection. The epithelium at both sites is prone to microabrasions and disruptions, which can facilitate HPV entry into basal cells, where the virus begins its lifecycle. In addition, the highly invaginated surface anatomy of the tonsils may facilitate viral access to the basal cells, resulting in viral maintenance and an increased susceptibility to carcinogenic transformation (49).

The crypts are irregularly lined by stratified squamous epithelium and reticulated epithelium composed of epithelial and lymphoid cells (lymphoepithelium). The basement membrane is discontinuous, variably thin or completely absent in areas. The junction between the lymphoid and epithelial components is masked by a dense lymphocytic infiltrate that fragments the epithelial component into nests and cords.

The TZ is lined by metaplastic stratified squamous epithelium and simple cuboidal cells differentiated from the reserve cell, a specialised cell targeted by HPV and derived from the basal cells of the ectocervix or cuboidal cells at the squamocolumnar junction (SCJ). The compromised epithelial barrier, the lack of maturation and increased cell turnover in basal cells at these specialised anatomical sites facilitates HPV access to the basal cells and the subsequent establishment of a persistent infection resulting in an increased the risk of malignant transformation (50, 51).

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Immune Evasion Mechanisms:

Both the cervical transformation zone and the tonsillar crypts have specialized immune environments. The tonsillar crypts, like the cervical transformation zone, are areas where immune surveillance is somewhat reduced, allowing HPV to evade the immune system and establish persistent infections.

While tonsillar and cervical cells are not identical, they share significant structural and functional similarities that make both regions susceptible to HPV infection. Specifically, HPV targets the stratified squamous epithelium found in the transformation zone of the cervix and the crypts of the tonsils. These regions are characterized by areas of high cellular turnover, presence of immature basal cells, and microabrasions, which facilitate viral entry and persistence. Moreover, the unique immune environments of these areas

contribute to the virus's ability to evade immune detection and establish long-term infections, leading to malignancies such as OPSCC and cervical cancer (51).

Establishing infection at this site requires cuts, breaks, and microabrasions to gain the virus access to the basal layer of the epithelium through interaction with receptors like alpha-6 integrins. The cervix with its relatively large area of immature squamous epithelium in the transformation zone is particularly vulnerable to HPV infection as it lacks the typical micro ridges present on mature squamous epithelial cells. Normally, the transitional zone coincides with the external os, but in pregnancy, at puberty, and in some contraceptive users, the position of os and transformation zone may not coincide. This exposes the tissues in the endocervical canal to vagina. This undergoes metaplasia as a protective response and it is during this metaplastic process the epithelium is most vulnerable to viral entry (10).

Despite the likelihood of early lymph node metastasis seen in HPV-associated OPSCC (14,38), patients display a markedly improved prognosis compared to that of HPV-independent OPSCC (33,52). Although the reason behind this is not fully understood, it is believed to be related to an improved response of HPV-associated OPSCC to treatment modalities such as radiation and chemotherapy, likely as a result of fewer genetic alterations seen in HPV-associated tumours compared to those of HPV-independent tumours (14,49,52,53). In addition, the apoptotic response remains intact in HPV-associated OPSCC which may result in increased sensitivity to radiation therapy when compared to HPV-independent OPSCC (14,49). It has been postulated that the improved response to treatment seen in HPV-associated OPSCC may also result from the stimulation of the host’s immune system to viral-specific tumour antigens (49). Furthermore, due to molecular differences seen in HPV-associated OPSCC, tumours seem to show an absence of field cancerisation, a concept described in HPV-independent HNSCC which leads to a higher tendency for local recurrences and secondary tumours (14,49,52,54). Lastly, due to the often younger  age of affected patients in HPV-associated OPSCC, an improved prognosis may be attributable to a more robust immune system overall, as well as fewer comorbidities (49). However, exposure to tobacco and alcohol products, as in HPV-independent OPSCC, has been

found to worsen the prognosis, even in HPV-associated OPSCC cases (1,12,14,23,38,39).

Histological grading:

The prognosis of OPSCC is related in part to the histological and clinical characteristics of the tumour (12). As a result, prognosis can largely be determined through histological grading and clinical staging (52). Keratinisation refers to the process where epithelial cells produce and accumulate keratin, a hallmark of terminal differentiation in keratinocytes (55). Differentiation refers to the degree to which tumour cells resemble their normal counterparts and plays a crucial role in determining the clinical behaviour and prognosis of a tumour. In 1920, Broder introduced a grading system still widely utilised today and advocated by the World Health organisation (WHO) (52). In this system, a SCC is graded as well, moderately or poorly differentiated based on how closely it resembles the normal squamous epithelium from which it arises (52).

Typically, well-differentiated tumours show a close resemblance to normal squamous epithelium, pronounced keratinisation with keratin pearl formation in well-defined tumour islands and cords with a cohesive pattern of invasion (3,52). Moderately differentiated tumours show a greater degree of cellular atypia, a reduced degree of keratinisation with fewer keratin pearls, a less cohesive pattern of invasion infiltrating in small islands or cords of cells (3,52).

Conversely, poorly differentiated tumours often display minimal to no keratinisation, significant departure from normal cellular and structural organization with abundant pleomorphism, prominent atypical mitotic figures and non-cohesive pattern of invasion with small groups of cells and single cell invasion (3,52).

The degree of differentiation has been found to correlate with survival rates. Well, moderately and poorly differentiated SCC correlate with 89%, 68% and 45% five-year disease-specific survival respectively. Histological grading of HPV independent OPSCCs (52), provides valuable prognostic information that guides treatment planning.

Originally it was believed that this morphology represented a poorly differentiated tumour, and as such, a poor prognosis, but it is now understood that these tumours

often arise from tonsillar crypt epithelium, which is non-keratinising, suggesting that OPSCCs are often, in fact, well differentiated which may also contribute to the improved prognosis seen with these tumours (52). In these cases, histologic grading is not recommended as it is not able to accurately predict the prognosis (15,56,57).

Tumour-Node-Metastasis (TNM) Staging System (8th Edition):

The Tumour-Node-Metastasis (TNM) staging system, particularly the 8th edition, has incorporated significant updates to better classify the prognostic factors for HNSCC. One of the key features of the updated TNM system is the inclusion of HPV status as a separate entity, particularly for oropharyngeal cancers. The TNM staging now distinguishes between HPV-associated and HPV-independent OPSCC due to their differing biology and clinical behaviour. HPV-associated OPSCC generally has a better prognosis and is staged differently compared to its HPV-independent counterpart (58,59).

Critical Features for the use of the TNM system include:

- T (Tumour): The T classification considers the size and extent of the primary tumour, with updates focusing on the involvement of surrounding structures.
- N (Node): The N classification considers the extent of regional lymph node involvement, which is a key prognostic factor in head and neck cancers.
- M (Metastasis): The M classification refers to the presence or absence of distant metastasis.
- HPV Status: The 8th edition of the TNM system importantly recognizes the influence of HPV status in oropharyngeal cancers, providing a separate staging system for HPV-associated p16.

Therefore, in order to provide clinicians with a highly specific diagnosis and to assist with the grading of OPSCC (15), it has been recommended that all OPSCC specimens be tested for HPV association as part of each patient's work-up. (60–64) Various techniques are available for the detection of transcriptionally active HPV in OPSCCs, including p16 immunohistochemistry (IHC) (4,32,63), together with HPV-DNA detection using either target amplification by Polymerase Chain Reaction (PCR), or signal amplification using In Situ Hybridisation (ISH), as there is evidence that shows that double positivity for p16 protein and HPV-DNA improves the accuracy of the diagnosis and enhances the prognostic value (3,4,32,40,62,63). p16 IHC has multiple benefits in

that it is easily available, easily reproducible, inexpensive, feasible to use in small samples and easy to interpret (32,40). Although p16 IHC has a high sensitivity the specificity for HPV is relatively lower compared to mRNA ISH, DNA ISH and PCR. Up to 20% of p16-positive OPSCCs may be HPV-negative, potentially due to HPV-independent mechanisms. DNA ISH offers higher specificity and better histological context with the identification of HPV within tumour cells. Confirmatory testing with mRNA ISH, HPV-DNA ISH or PCR is therefore recommended (65).

Testing practices differ by country as guidelines have not been fully established yet (4). Unfortunately, as a result, the literature has shown that different HPV detection techniques lead to significant variation between studies in the prevalence of HPV-associated SCC (13,40,60). A Nigerian study completed in 2016 found that the prevalence of HPV was reported to be higher when PCR methods were used in place of ISH (13), however, Daapah et al. describe ISH as the gold standard to provide evidence of transcriptionally active HPV (32), indicating that a consensus has likely not yet been reached. In addition, in countries such as the USA and UK that show a high prevalence of HPV-associated OPSCCs where almost all (99%) cases that demonstrate the typical morphology for HPV-associated OPSCC are positive for p16 and 100% positive by PCR, p16 is considered an acceptable surrogate marker for HPV (52). In contrast, in countries that show a lower prevalence of HPV-associated OPSCC, p16 positivity may not be a reliable marker for HPV, as seen in the South African study by Dapaah *et al.* (32) in which p16 demonstrated a suboptimal positive predictive value of 36.1%

Furthermore, p16 IHC, an indirect test for transcriptionally active HPV, without a specific confirmatory DNA test may not be the ideal way to approach such tumours specifically in these regions, as a consequence of the demonstrated greater discordance between p16 IHC and specific DNA testing. Particularly concerning is the poorer patient survival in discordant p16+, HPV DNA- tumours when compared to double positive (p16+ / HPV DNA+), as the former show clinical and molecular similarities to HPV-independent OPSCCs and may not benefit from treatment de-escalation protocols (40). A study in India, a country with a low prevalence of HPV associated OPSCC and a high tobacco and alcohol burden suggested that unlike in developed countries with a high prevalence

of HPV associated OPSCC, p16 is inadequate as a surrogate marker in such settings (66).

In the context of HPV-associated OPSCC, accurate HPV testing is crucial for diagnosis, prognosis, and treatment planning. The methods available for detecting HPV in clinical specimens vary in sensitivity, specificity, and their ability to discern between mere viral presence and active infection. HPV DNA ISH, PCR and mRNA in situ hybridization (mRNA ISH), have different roles and implications in the clinical setting.

In the clinical context of OPSCC, PCR testing, while highly sensitive, may lead to false positives due to its inability to differentiate between transcriptionally active and inactive HPV. This limitation underscores the importance of utilizing mRNA ISH as the gold standard for detecting transcriptionally active HPV infection, which is essential for accurate diagnosis and treatment planning in an ideal setting with sufficient funding and resources. Studies, such as those by Schache et al. (2011) and Chernock et al. (2013), demonstrate the superiority of mRNA ISH in providing clinically relevant information by identifying the expression of E6 and E7 oncogenes (67,68).

**PCR Testing for HPV:**

PCR is a molecular technique that amplifies specific DNA sequences, making it highly sensitive for detecting HPV DNA. This sensitivity allows PCR to identify even low levels of HPV DNA in clinical samples. However, this high sensitivity can be a double-edged sword. PCR may detect "latent" HPV infections or the presence of viral DNA without actual oncogenic activity, leading to false positives in the context of OPSCC. Specifically, PCR can detect both transcriptionally inactive and active HPV, which means it might identify HPV DNA in cases where the virus is not contributing to the oncogenic process (69).

- Strengths: High sensitivity, ability to detect a broad range of HPV types.
- Limitations: Cannot distinguish between transcriptionally active and inactive infections, leading to potential false positives. This is particularly problematic in OPSCC, where only transcriptionally active (oncogenic) HPV is clinically relevant.

**mRNA ISH Testing for HPV:**

mRNA ISH is a more specific method that detects the transcriptionally active form of HPV. This technique identifies the expression of E6 and E7 oncogenes at the mRNA level, which are the key drivers of carcinogenesis in HPV-positive OPSCC. By targeting mRNA, this method directly assesses whether the virus is contributing to the oncogenic process, thereby providing a more accurate reflection of clinically significant HPV infection. The advantage of mRNA ISH is its ability to distinguish between mere viral presence and active, oncogene-expressing infections that are driving tumourigenesis (67).

- Strengths: High specificity, detects only transcriptionally active HPV, reducing the risk of false positives. Clinically relevant in determining prognosis and guiding treatment.
- Limitations: While mRNA ISH is more specific, it is technically more demanding and less widely available compared to PCR.

Currently, the guidelines for the therapeutic management of OPSCC do not differ depending on whether the tumour is HPV-associated or HPV-independent (14). However, because of the difference in prognosis seen between these two entities, clinical trials are underway to potentially allow for the de-escalation of treatment for HPV-associated OPSCC and, as a result, improve patient outcomes (14). Treatment currently involves primary surgical removal of the tumour with or without adjuvant radiation or chemoradiation therapy (CRT), or alternatively, definitive CRT, based on the stage of the cancer and according to the pathological findings (14).

In spite of the high prevalence of cervical cancer and HIV in Sub-Saharan Africa and South Africa, the landscape of OPSCC, especially those that are HPV-associated, remains under-researched in these areas, presenting a significant gap in the global epidemiological understanding of OPSCC (6,34). The limited role of HPV in HNSCC within African contexts, as suggested by other studies conducted in Africa, contrasts with the high HPV attribution rates seen in OPSCC cases in developed countries. (8,13,20,23,34). This discrepancy highlights the need for more comprehensive, region-specific studies focusing on HPV's role in OPSCC, particularly given the global increase in HPV-associated OPSCC cases.

Our study is significant as it aims to provide localised data essential for understanding the epidemiology of OPSCC in a South African context, where such data has been

limited. In addition, this research aligns with the global need for comprehensive epidemiological data on OPSCC, particularly in the wake of changing sexual behaviours that have been associated with an increased risk of HPV-associated OPSCC.

The OPSCC research undertaken at CMJAH represents a critical endeavour to fill the existing knowledge gaps in the epidemiology of oropharyngeal cancers in South Africa as well as to contribute to the global corpus of knowledge on OPSCC. By offering insights into the prevalence, demographic distribution, and HPV association of OPSCC, our study holds the potential to inform public health policies, screening protocols, and possibly HPV vaccination strategies in South Africa and other sub-Saharan African countries with similar epidemiological profiles. This may assist in contributing to more effective public health interventions, enhancing OPSCC screening and prevention strategies, and ultimately contributing to the reduction of OPSCC burden in the region and beyond.

## 1.2 Aim

The aim of the study was to determine the prevalence of OPSCC in relation to all confirmed cases of HNSCC identified at the Witwatersrand Oral Health Centre (WOHC) and Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) during July 2013-July 2022, while also considering the demographics of those affected, the most commonly affected anatomic subsites and potential risk factors involved in the development of OPSCC.

# CHAPTER 2 - METHODOLOGY

## 2.1 Methodology

### 2.1.1 Sampling method

A retrospective cross-sectional study utilising purposive sampling was performed. Past patient laboratory records from July 2013 to July 2022 from patients attending either the WOHC or CMJAH were obtained from the National Health Laboratory Services (NHLS)

and evaluated to identify those that demonstrated a histopathological diagnosis of HNSCC.

### 2.1.2 Inclusion and Exclusion Criteria

Patients who had attended either the WOHC or CMJAH during July 2013-July 2022 and received a histopathological diagnosis of HNSCC by the NHLS were included in the study. Single SCC lesions involving the oropharynx but overlapping other sites in close proximity (nasopharynx/oral cavity/laryngopharynx) were excluded from the OPSCC category when calculating prevalence and when further analysing the data due to difficulty in determining the site of primary involvement (32).

### 2.1.3 Data collection and variables

The histological diagnosis and a limited clinical history were obtained from the laboratory records provided by the NHLS. Records of SCC affecting the nasopharynx (NPSCC), the oral cavity including the lip (OSCC) and the laryngopharynx (LSCC) were separated from records of SCC affecting the oropharyngeal subsites (OPSCC). If the SCC occupied a region involving any of the abovementioned anatomical sites in close proximity to the OPSCC site affected, it was recorded as a “combination” case and excluded.

For all included records, variables including sex, age at diagnosis, year of diagnosis as well as any lifestyle habits (smoking, alcohol use or betel quid use) were recorded. For cases identified as OPSCC, variables recorded also included anatomic subsite (tonsils/ tonsillar pillars, uvula/soft palate, base of tongue, walls of oropharynx/oropharynx/vallecula) (4), histological grading and the presence/ absence of keratinisation as described by the pathologist in the report. (52,53) In addition, if mentioned in the records, the testing for HPV involvement, including the type of test utilised was also recorded (32,70).

### 2.1.4 Data Analysis

The prevalence of histologically confirmed cases of OPSCC was analysed by determining the proportion of patients diagnosed with SCC affecting the oropharynx versus patients diagnosed with SCC affecting only the nasopharynx, oral cavity or

laryngopharynx or a combination of these. This value was then displayed as a percentage. The continuous variables including age were assessed for normality using the histogram plot with superimposed normal curve and the box-and whisker plot. Descriptive statistics for categorical variables including gender, year of diagnosis and race were summarised using frequencies and percentages. Similarly, lifestyle habits (smoking, alcohol intake) were summarised using frequencies and percentages.
The anatomic subsite distribution was displayed using a bar graph. Risk factors associated with the diagnosis of OPSCC in patients diagnosed at CMJAH between July 2013 and 2022 were assessed using the Pearson Chi-2 test for categorical variables and the independent T-test for continuous variables. Univariate logistic regression was done to quantify the measure of association between OPSCC and risk factors. Results were presented in tables showing odds ratio, 95% confidence interval and p-values. Significance was set at 5%. The analysis was done using STATA version 17.

### 2.1.5 Ethical considerations

The Human Research Ethics Committee (HREC) of the Faculty of Health Sciences at the University of the Witwatersrand has granted ethical clearance (M220756). Data obtained from the NHLS was anonymised prior to being provided to the researchers.

# CHAPTER 3 – RESULTS

## 3.1 Results

Using the laboratory records, a total of 299 cases of HNSCC were identified between July 2013-July 2022 and included in the study according to the selection criteria. Of the 299 cases, patients diagnosed as having OPSCC amounted to 96, resulting in a prevalence of 32.1%.

When considering all HNSCC cases between July 2013-July 2022, the age of patients ranged from 11-88, skewing the data to the left. When considering the cohort included in the OPSCC category, the age of patients ranged from 35-78 years at diagnosis. The mean age of those diagnosed with OPSCC was 60.31 years. Males accounted for 69,79% (n=67) of cases, although this was not found to be statistically significant. The laboratory records did not disclose the race of patients and therefore, the race of all patients in the study was unknown.

As seen in *table 1*, most participants with OPSCC were diagnosed in 2018 (16.67%; n=16). 2015 and 2020 showed the next highest number of diagnoses made with 13.54% each (n=13). 27.08% (n=26) of the OPSCC participants were confirmed to be smokers while 6.78% (n=8) were confirmed to consume alcohol. In each of the cases of alcohol use, the patient was also found to be a smoker 6.78% (n=8). 1 record specifically mentioned that the patient had no history of engaging in any lifestyle habits while the remaining 71.87% (n=69) of records did not mention it. Most variables were not found to be statistically associated with having OPSCC.

**Table 1**: Factors associated with the diagnosis of OPSCC in patients diagnosed at CMJAH during July 2013-July 2022.

| Variable | OPSCC | | | Univariate logistic regression | |
|---|---|---|---|---|---|
| | No n(%) | Yes n(%) | p-value | OR(95%CI) | P-value |
| Age, mean(SD) | 59.1(11.5) | 60.3(9.1) | 0.3824 | 1.01(0.99 to 1.03) | 0.381 |
| Sex | | | | | |
| Male | 133(66.5) | 67(33.5) | 0.463 | 1 | |
| Female | 70(70.71) | 29(29.29) | | 0.82(0.49 to 1.39) | 0.464 |
| Year of diagnosis | | | | | |
| 2013 | 11 (5.42) | 6(6.25) | | 1 | |
| 2014 | 23(11.33) | 11(11.46) | | 0.88(0.26 to 2.99) | 0.834 |
| 2015 | 22(10.84) | 13(13.54) | | 1.08(0.32 to 3.63) | 0.897 |
| 2016 | 17(8.37) | 8(8.33) | 0.981 | 0.86(0.23 to 3.17) | 0.824 |
| 2017 | 25(12.32) | 12(12.5) | | 0.88(0.26 to 2.95) | 0.836 |
| 2018 | 26(12.81) | 16(16.67) | | 1.12(0.35 to 3.65) | 0.840 |
| 2019 | 27(13.3) | 9(9.38) | | 0.61(0.18 to 2.12) | 0.439 |
| 2020 | 31(15.27) | 13(13.54) | | 0.77(0.23 to 2.52) | 0.664 |
| 2021 | 12(5.91) | 5(5.21) | | 0.76(0.18 to 3.23) | 0.714 |
| 2022 | 9(4.43) | 3(3.13) | | 0.61(0.12 to 3.16) | 0.557 |
| Lifestyle habit | | | | | |
| Not mentioned | 142(66.98) | 70(33.02) | 0.598 | 1 | |
| Yes | 61(70.11) | 26(29.89) | | 0.89(0.5 to 1.49) | 0.598 |
| Smoker | | | | | |
| Not mentioned | 143(67.14) | 70(32.86) | 0.659 | 1 | |
| Yes | 60(69.77) | 26(30.23) | | 0.89(0.51 to 1.52) | 0.659 |
| Consumption of alcohol | | | | | |
| Not mentioned | 194(68.79) | 88(31.21) | 0.174 | 1 | |
| Yes | 9(52.94) | 8(47.06) | | 1.96(0.73 to 5.24) | 0.181 |

*Legend to Table 1:*

*Table 1: Distribution of patients based on OPSCC diagnosis and associated variables. The table presents the number of patients (n) and percentage (%) in each category, with comparisons between those without OPSCC (No OPSCC) and those with OPSCC (Yes OPSCC). The p-values indicate the statistical significance of the differences between the groups, and the Odds Ratios (OR) with 95% Confidence Intervals (CI) provide a measure of association between each variable and the likelihood of having OPSCC.*

***OPSCC*** *(Oropharyngeal Squamous Cell Carcinoma): Indicates whether the patient was diagnosed with OPSCC.*

***Age*** *(mean ± SD): The mean age and standard deviation for patients in each category.*

***Sex****: Comparison between male and female patients.*

***Year of Diagnosis****: Distribution of OPSCC diagnoses by year.*

***Lifestyle Habit****: Whether the patient had a documented lifestyle habit (e.g., smoking or alcohol consumption).*

***Smoker****: Indicates whether the patient is a smoker.*

***Consumption of Alcohol****: Indicates whether the patient consumes alcohol.*

Of the 96 cases of OPSCC, the most commonly affected subsite was the tonsil (46.88%, n=45) followed by the base of the tongue (39.58%, n=38) as demonstrated in *Figure 1*. 26.04% (n=25) of cases involved two or more subsites.

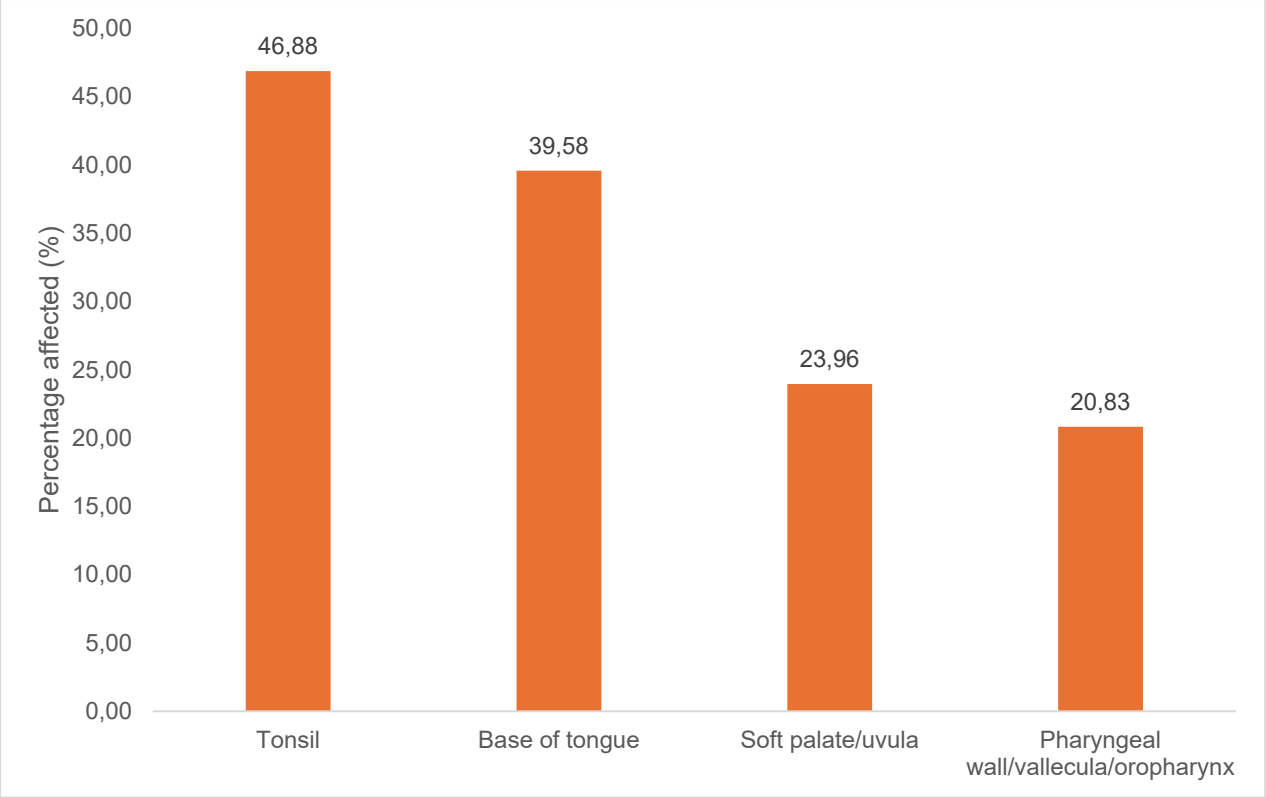


**Figure 1***:* Affected anatomic subsites

Histologically, 65.63% (n=63) of OPSCC cases were keratinising tumours, 17.71% (n=17) were non-keratinising and 16.67% (n=16) were not specified in the record. 1 tumour was described as well differentiated where the majority of the remaining tumours showed moderate differentiation (64.58%, n=62). Poorly differentiated tumours made up 12.50% (n=12), and moderate-poorly differentiated tumours were seen in 5.21% (n=5) of the cases. 11.46% (n=11) of the records did not mention the grade of the tumour and carcinoma in situ was seen in the remaining 5.21% of cases (n=5).

*Figure 2* shows the distribution of histological grading across each OPSCC subsite. When assessing the association of anatomical subsite and histological grading, it was seen that there was an association between the grade and “tonsil” (p-value=0.016), as well as an association between the grade and “base of tongue” (p-value=0.0034).

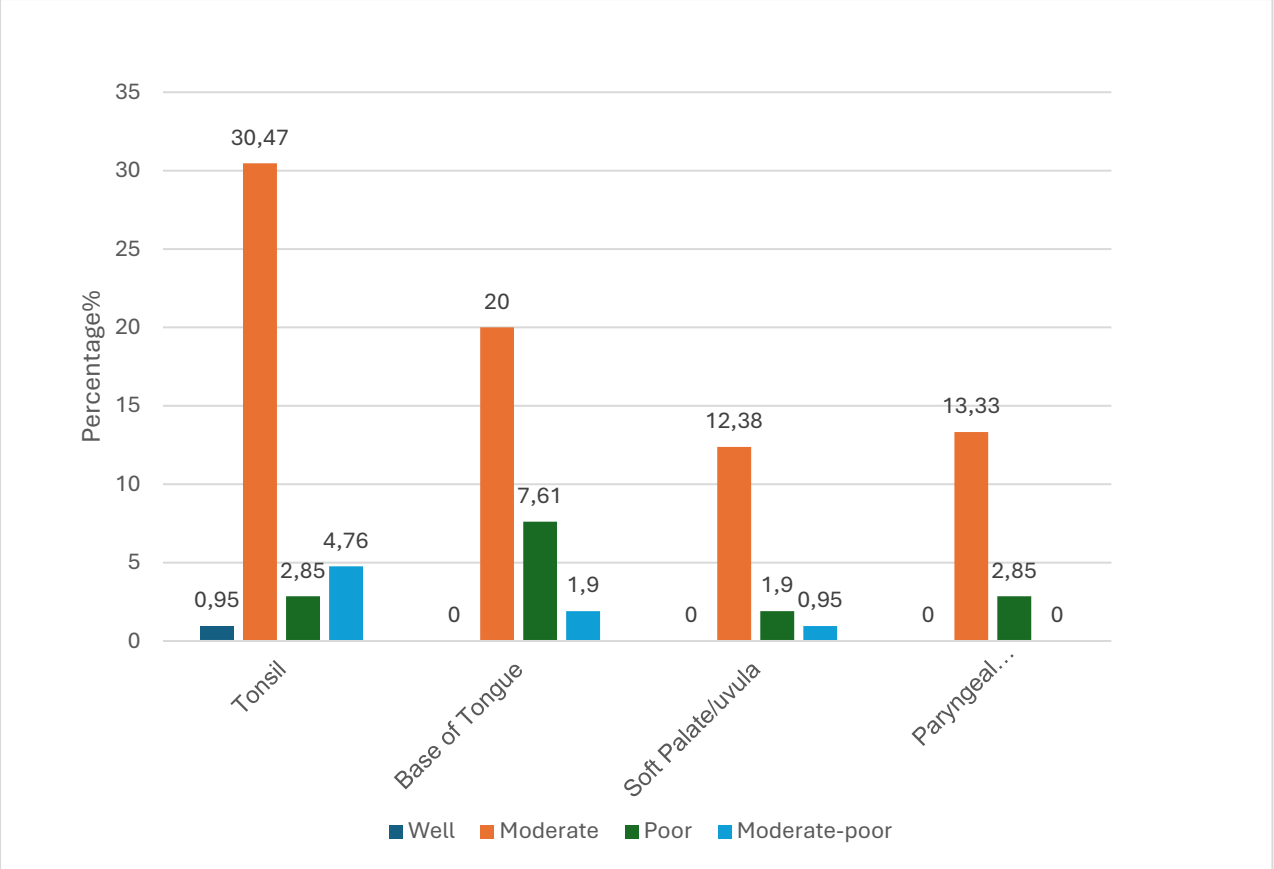


**Figure 2***:* Distribution of subsite and histological grading

Assessing the association of keratinisation and histological grading revealed a statistically significant association (p-value= 0).

*Figure 3* shows the distribution of keratinisation across each OPSCC subsite. Most subsites showed keratinisation of at least 52.17% of cases. Assessing the association between anatomical subsite and keratinisation showed a statistically significant association (p-value<0.001).

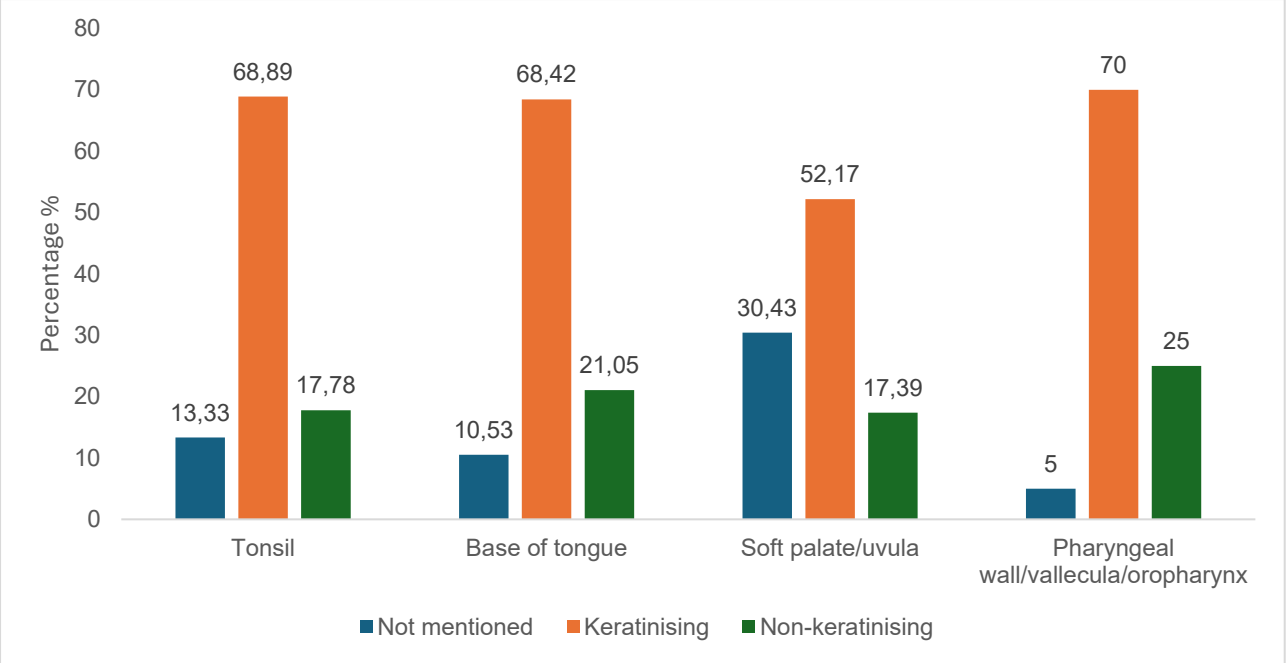


**Figure 3***:* Distribution of subsite and keratinisation

No statistical association was found between the absence or presence of keratinisation and lifestyle habits (smoking/consumption of alcohol/use of betel quid/other).

Of the 96 cases of OPSCC, 16.66% (n=16) were tested using p16 IHC. Of those that were tested using p16 IHC, 75% (n=12) showed p16-positivity and the remaining tumours (25%; n=4) were negative for p16. Five of the cases that showed p16-positivity (31.25%) were then subjected to further testing to determine the presence of HPV DNA using ISH. The results of 2 of these cases were made available to the researchers, both of which were HPV DNA negative.

# CHAPTER 4 – DISCUSSION AND CONCLUSION

## 4.1 Discussion

A surge in the number of cases of OPSCC has become a notable focal point in oncological research, with data from countries such as the USA and UK indicating a sharper incline in the number of HPV-associated OPSCC cases, in particular (4–6,14,16,19,20). Due to the limited research regarding OPSCC in South Africa and across the African continent, particularly in the wake of changing sexual and cultural behaviours that have been associated with an increased risk of OPSCC, the primary objective of our study was to determine the prevalence of OPSCC through assessing NHLS laboratory records at Charlotte Maxeke Johannesburg Academic Hospital and the Wits Oral Health Centre, in order to provide further region-specific data that may offer some clarification regarding whether the lower prevalence of cases of OPSCC observed in previous studies across the continent oppose the rising trend seen in some larger studies conducted in developed countries, such as the USA, UK and Europe (6,16,32,45,71).

### 4.1.1 Prevalence

The study conducted by Carpén *et al.* (12) is one of few studies in the literature that discuss both HPV-associated and HPV-independent OPSCC together, likely as a result of the current increase in HPV-associated OPSCC seen in various countries around the world. (45,71) This has led to multiple studies opting to specifically investigate HPV-associated SCC, including on the African continent such as Cameroon (23), Senegal (34), Central African Republic (CAR) (20), Nigeria (13) and Mozambique (8). These studies in particular all agree that HPV seemingly plays a limited role in the development of HNSCC in Africa. (8,13,20,23,34) What is important to note is that each of these studies utilised a small sample size and considered HNSCC in general, rather than looking at cases affecting the oropharynx specifically. In Nigeria, none of the 17 tumours considered were from the oropharynx (13) whereas in Senegal, Cameroon, and CAR only 4.27%, 8.39% and 14.07% of tumours respectively, involved the oropharynx. (20,23,34) Analysing HNSCC cases in general as opposed to specifically looking at tumours of the oropharynx may lead to a lower number of cases of HPV-associated OPSCC as HPV-related tumours are more commonly associated with the oropharynx in comparison to any other HNSCC

site (60). In addition, each of these studies were published between 2013-2019 which means that data utilised may be somewhat out of date (13,20,23,34).

A 2020 study completed in the Western Cape, South Africa by Daapah *et al.* considered the prevalence of OPSCC with a large sample size of 266 tumours, however, this study, like the others, specifically considered HPV-associated OPSCC. (32) In this study on the prevalence of HPV-associated OPSCC, 14% of cases were p16-positive, but only 5% of the total cases were confirmed as HPV-positive using Polymerase Chain Reaction (PCR) testing leading the authors to believe, as in other African studies, that HPV was not a significant risk factor among this population (32).

Of the 299 cases of HNSCC evaluated in our study, 96 cases of OPSCC were identified and included resulting in a prevalence of 32.1% at CMJAH between July 2013-July 2022, similar to, although lower than the prevalence seen in Mozambique of 43.13% (8). This is a significant finding as it highlights the substantial burden of OPSCC within the broader category of HNSCC and provides a clearer picture of the disease distribution within the patient population at WOHC and CMJAH. While the prevalence of OPSCC in the current cohort is substantial, it aligns with emerging trends in some parts of Africa, albeit slightly lower than those observed in high-income countries such as the USA, UK and Europe (21). This could reflect regional variations in risk factors, including HPV prevalence, lifestyle habits and genetic predispositions.

The burden of OPSCC in South Africa, where it accounts for almost one-third of all HNSCC, is substantial. This figure is significant when compared to both global and regional data, where the proportion of OPSCC among HNSCC cases varies. The growing incidence of HPV-positive OPSCC, driven by changing risk factor profiles and demographic shifts, underscores its importance as a public health concern. The substantial burden of OPSCC in South Africa highlights the need for targeted screening, prevention, and treatment strategies that address both the infectious (HPV) and non-infectious risk factors associated with this disease (32,72).

Justification for Describing OPSCC as a "Substantial" Burden:

> **Global Trends in OPSCC Incidence:** The incidence of OPSCC has been increasing globally, largely driven by the rise in HPV-positive cases. In many high-income countries, the incidence of HPV-positive OPSCC has surpassed

that of cervical cancer, traditionally the most well-known HPV-related malignancy. For instance, in the United States, HPV-positive OPSCC now accounts for more than 50% of all OPSCC cases, reflecting a dramatic shift in the epidemiology of head and neck cancers. This trend is not confined to the developed world; there is emerging evidence from Sub-Saharan Africa and other regions that the burden of HPV-related OPSCC is also growing, although it remains underreported (21, 32, 72).

**Comparative Data from Africa:** In South Africa, recent studies have shown that OPSCC accounts for nearly one-third of all HNSCC. This figure is significant when compared to other African countries, where the proportion of OPSCC among HNSCC cases varies but is generally lower due to differing risk factor profiles and the challenges in diagnosing and reporting HPV-associated cancers. For example, studies from other regions of Africa report variable rates of HPV-positive OPSCC, with some regions showing rates as low as 0% and others as high as 50%. This variability highlights the importance of the data from South Africa, where OPSCC represents a substantial proportion of the HNSCC burden.

**Clinical and Public Health Impact:** The significance of OPSCC being a substantial burden lies not only in its incidence but also in its clinical and public health implications. OPSCC, particularly HPV-positive OPSCC, affects a younger demographic and is associated with different risk factors (e.g., HPV infection rather than smoking or alcohol). This shift necessitates changes in screening, prevention (e.g., vaccination programs), and treatment strategies. Furthermore, HPV-positive OPSCC has a different clinical course and prognosis, often responding better to treatment but also requiring different management approaches compared to HPV-negative cases.

**Substantial in the Context of South Africa:** In South Africa, the proportion of OPSCC cases being nearly one-third of all HNSCCs is particularly notable given the country's dual burden of infectious and non-communicable diseases. The perception of a rising incidence of HPV-positive OPSCC in a region already grappling with high rates of HIV and other infections places an additional strain on healthcare resources. The coexistence of these conditions complicates the clinical management of patients

and underscores the substantial public health challenge posed by OPSCC (21,32,72,73).

### 4.1.2 Age Distribution

OPSCC may occur across varying age groups, however, individuals diagnosed with HPV-independent OPSCC have typically been shown to be older than those with HPV-associated OPSCC (1,12,60), attributed to changing sexual behaviours between generations including an earlier sexual debut, increased number of oral/vaginal sexual partners and/or having a sexual partner with a history of multiple sexual partners (10,13,23,24,44,74,75).

The wide age range (11 to 88 years) and the left skew of the age distribution seen in the 299 cases of HNSCC suggest a broader risk profile that may extend into younger populations than typically expected.  Of interest in this data set was the 11-year-old HIV-positive female who was found to have OSCC affecting the left cheek, palate and alveolar process of the mandible. It has been suggested by the AIDS-cancer registry studies that HIV-positive individuals are at an increased risk for developing both HNSCC and OPSCC in particular, when compared to the general population. (76) In a South African study conducted in 2018, OSCC was found to be the third most commonly diagnosed cancer (19.8%) amongst this cohort, in spite of it not being considered an AIDS-defining cancer (77). This is significant considering the high prevalence of HIV among the South African population (22,77).

In our study, the mean age reported for patients with OPSCC was 60.3 years. Interestingly, the other instances of HNSCC, not including those diagnosed as OPSCC, showed a slightly lower mean age of 59.1 years. While this aligns closely with the literature where the mean or median age for patients with HPV-associated OPSCC ranged from 55,7-63,5 years and the mean or median age for patients with HPV-independent OPSCC was found to range from 59,1–67,1 years (40), the similarity in mean ages across both categories may reflect an upward shift in the median patient age to 55-58 years, leading to a closing age gap between HPV-associated and HPV-independent groups as a result of a recent increase in the prevalence of HPV-associated OPSCC amongst older individuals (15). It may also suggest a common risk factor between the two groups, likely traditional lifestyle related risk factors, including the consumption of tobacco and alcohol.

### 4.1.3 Sex distribution

The higher prevalence of OPSCC among males (69.79%) is consistent with the literature where multiple studies have demonstrated males to be more likely to be affected in both HPV-associated and HPV-independent OPSCC (1,6,9,12,47,60), although this was not found to be statistically significant. The lack of statistical significance in differences between the sexes could be due to sample size limitations or possibly due to the distribution of the OPSCC in the studied population.

### 4.1.4 Racial distribution

Conclusions regarding race could not, and should not be drawn in this context, and additionally, the laboratory records did not disclose information about race.

### 4.1.5 Risk factors and lifestyle habits

It is important to take both an academic and nuanced approach, recognizing the evolving epidemiological landscape of HNSCC, particularly in the context of South Africa.

Multiple factors may play a role in influencing both sexual behaviours and lifestyle habits among individuals including religious and cultural beliefs, social norms, community or media influences as well as socioeconomic status. (9,42,43) While it is not necessarily possible to generalise results from developed countries when looking at a South African cohort, in countries such as the USA, it has been proposed that HPV-associated OPSCC is predominantly a disease of younger males who tend to report multiple oral sex partners where HPV-independent OPSCC is more common amongst minority groups that are of a lower socioeconomic status, often linked to the higher rates of smoking seen amongst these communities (6,9,12,32,47). It has been demonstrated that the highest rates of HPV-independent HNSCC tend to be found among individuals living in poorer communities, in both developed and developing countries (1,7). HPV-independent OPSCC patients typically have a diet low in fresh produce, and are often self-neglecting, tending to present with poor/inadequate oral hygiene (1,7,11,12,17,26,33).

Traditionally, smoking and alcohol consumption have been well-established risk factors for HNSCC. However, recent epidemiological data indicate a significant shift, particularly in high-income countries, where HPV-associated OPSCC cases are increasingly diagnosed in individuals with minimal or no history of these risk factors.

This trend is also emerging in South Africa, albeit to a lesser degree, as the prevalence of HPV-associated OPSCC rises. Studies have shown that HPV-positive OPSCC patients are often younger, healthier, and less likely to smoke or drink compared to their HPV-negative counterparts (39). A study by Chaturvedi et al. (2011) in *Journal of Clinical Oncology* demonstrated that the incidence of HPV-associated OPSCC increased significantly among non-smokers and non-drinkers in the United States (78). This pattern is beginning to be observed in other regions, including parts of Sub-Saharan Africa. D'Souza et al. (2016) highlighted the rise of OPSCC in individuals with low exposure to traditional risk factors, further supporting the argument that HPV-associated OPSCC should be considered a distinct clinical entity (79).

A study completed in Senegal looking at the role of HPV in head and neck cancer within the local population found a low prevalence of HPV-associated HNSCC, together with low smoking rates and low alcohol consumption among their cohort. (34) This led the authors to believe that environmental factors and dietary practices may be more important in the development of HNSCC in this population. In addition to the more "classical" risk factors, multiple studies have demonstrated that social factors including poverty, lack of education, malnutrition, poor general and oral health as well as limited access to healthcare are also likely to contribute to the development of HPV-independent OPSCC (1,7,13,33,34).Furthermore, in developing countries, workplace safety regulations are often not enforced as employment is mainly through the informal sector (34). Without the correct safety equipment, workers are often exposed to hazardous materials at work in various industries resulting in an increased association with HPV-independent HNSCC (34).

An increased exposure to oral HPV through oro-genital practices has been found to greatly increase the risk of developing HPV-associated OPSCC, as HPV is a sexually transmitted virus. (1,6,8,10,22,24,32,74) It is likely, therefore, that the increased incidence of OPSCC seen in the USA, UK and Europe may be following evolving sexual trends as demonstrated in a study of 4 256 participants between the ages of 20 and 69, where 85.4% of males and 83.2% of females had participated in oro-genital practices (8,75). In contrast, the authors of the studies completed in Cameroon, Nigeria and Mozambique believe that the seemingly low prevalence of

HPV-associated OPSCC in Sub-Saharan Africa may be linked to the limited oral sexual practices taking place in these regions (8,13,23).

The discussion about evolving sexual trends contributing to the increased incidence of HPV-associated OPSCC in developed regions, contrasted with the perceived lower prevalence of such practices in sub-Saharan Africa, provides an essential cultural and behavioural lens to OPSCC epidemiology. It suggests that the rising OPSCC rates, even if lower than in developed countries, might still be influenced by gradual changes in sexual behaviours within the African context.

From a sociological perspective, it's essential to recognize that low socioeconomic status does not preclude the possibility of HPV-driven carcinogenesis. In fact, limited access to healthcare and vaccination programs can exacerbate the risk of HPV infection, leading to higher rates of HPV-related malignancies in underserved populations. We cannot draw conclusions on limited and statistically insignificant data for any study, but in particular in this case as the habit was not known in most of the cases.  However, remarking on the observation of perceived low percentages of smokers and alcohol consumers among OPSCC patients should still highlight the current broader epidemiological observations that challenge traditional assumptions about HNSCC risk factors. This maintains to an extent, the importance of recognizing HPV as an independent risk factor for OPSCC in these populations as the research front develops in this area.

A study by Reddy et al. (2021) examined the burden of HPV-related cancers in low-resource settings and found that even in populations with high smoking rates, HPV plays a significant role in the aetiology of OPSCC (80). McKay et al. (2019) in discussed the impact of HPV vaccination disparities in low-income populations, further emphasizing the need to address HPV as a significant risk factor independently of traditional behaviours like smoking and alcohol consumption (81).

Due to gaps in data collection and availability, especially regarding sexual behaviours and lifestyle factors such as tobacco/alcohol consumption, the full spectrum of OPSCC risk factors in this cohort cannot be understood. The low reported percentages of smokers and alcohol consumers among OPSCC patients (27.08% and 6.78%, respectively) are intriguing, especially given the well-established associations between these habits and HNSCC. This likely represents a

potential underreporting as most laboratory records did not provide a clinical history. The lack of statistical association with OPSCC might also reflect sample size issues or the influence of other, unmeasured risk factors, such as HPV infection. Incorporating multivariate analyses in future studies could provide more nuanced insights into the interplay between various risk factors and OPSCC, helping to isolate independent predictors of disease.

The findings from this study, particularly the low reported percentages of smokers and alcohol consumers among OPSCC patients (27.08% and 6.78%, respectively), are noteworthy within the context of evolving global and regional trends in head and neck cancers. Traditionally, tobacco and alcohol use have been established as primary risk factors for HNSCC, including OPSCC. However, the rise of HPV as a significant aetiological factor in OPSCC challenges these assumptions, especially as HPV-associated cases increasingly present in patients with minimal or no history of smoking or alcohol consumption.

This trend is not limited to high-income countries. In South Africa, the dual burden of infectious diseases and non-communicable diseases adds complexity to the clinical landscape. The emergence of HPV-associated OPSCC, particularly among younger patients and those with lower exposure to traditional risk factors, underscores the importance of integrating HPV testing into routine diagnostic protocols for all OPSCC cases (39,82). Furthermore, while the socioeconomic context of South African public hospital patients may suggest a higher prevalence of traditional risk factors, the data from this cohort indicate that HPV-driven carcinogenesis is an increasingly relevant concern.

Thus, the low percentages of smokers and alcohol consumers observed in this study should not be dismissed, as they reflect a broader epidemiological shift that has significant implications for both clinical practice and public health policy. Addressing this shift requires a nuanced understanding of the interplay between HPV and traditional risk factors in OPSCC, particularly in resource-limited settings where access to comprehensive healthcare and vaccination programs may be limited.

### 4.1.6 Year of diagnosis

In this study, most participants with OPSCC were diagnosed in 2015 (13.54%), 2018 (16.67%), and 2020 (13.54%). Across most years from 2013 onwards, the results

demonstrated an increase in the number of diagnoses of OPSCC made, as seen in other countries around the world. (4–6,14,16,19,20) This may be an indication that the incidence of OPSCC was on the rise. In 2021 and 2022 (only considered from January-July), the number of OPSCC diagnoses seemingly declined, coinciding with the COVID-19 pandemic that began in South Africa in March 2020 and continued until approximately June 2022, when all remaining health regulations were lifted (83). According to a 2023 systematic review by Angelini *et al.,* multiple studies conducted at the time of the COVID-19 pandemic confirmed a significant decrease in the number of newly identified cases of cancer, as well as a decrease in cancer screening tests and diagnostic investigations during this time. (84) Considering the retrospective cross-sectional design of our study, longitudinal cohort studies could offer insights into temporal trends, risk factor changes over time, and long-term outcomes of OPSCC patients.

### 4.1.7 The Associations between Histological Features, Anatomic subsites, and Testing

#### *4.1.7.1 Histological Grading and Keratinisation*

The relationship between keratinisation and differentiation in SCC is complex and highly relevant to the tumour's biological behaviour and aggressiveness. Keratinisation, a hallmark of differentiation in keratinocytes, involves the production and accumulation of keratin proteins. In SCC, the expression of keratin proteins can indicate the differentiation state of tumour cells and is associated with the aggressiveness of the carcinoma as it invades deeper structures. The selective loss of expression of keratin polypeptide markers of differentiation in SCC as they invade deeper is associated with more aggressive biologic behaviour. Similarly, the expression of keratinisation markers at deep margins in basal cell carcinomas indicates aggressive growth patterns, suggesting a relationship between keratin expression and tumour behaviour (85).

The differential expression of keratins during the progression of cutaneous SCC highlights the increase in KRT6 and KRT16 along with a decrease in differentiation markers KRT2 and KRT10. KRT13 and KRT19 show significant elevation throughout the stages, suggesting their potential as markers for malignant transformation and invasion capacity in this malignancy. SCCs and adenocarcinomas show a limited

differential expression of keratins, with some overlap in keratin profiles due to the cell lineage and differentiation state of tumour cells.

The relationship between SCC and keratoacanthoma has also been debated, with some considering keratoacanthoma as a benign entity, others viewing it as a subtype of carcinoma, or even a precursor to SCC. The distinction between the two remains clinically relevant, emphasizing the continuum of keratinocyte differentiation and transformation in skin carcinogenesis.

Furthermore, the transition from actinic keratosis to SCC involves genomic progression marked by the acquisition of driver alterations like NOTCH1 and TP53 mutations, followed by additional genomic alterations leading to SCC development. This genomic evolution underscores the molecular underpinnings of keratinocyte transformation from a pre-invasive stage to invasive carcinoma, reflecting changes in keratinisation and differentiation (85).

In our study, 64.58% of tumours showed moderate differentiation and 65.63% of tumours showed keratinisation, revealing a highly significant association between the two. This significant association  suggests that keratinisation, as a morphological characteristic, is closely associated with the cellular differentiation status assessed through histological grading (52). Typically, keratinisation is more pronounced in well-differentiated tumours, which tend to resemble the normal epithelium more closely, whereas poorly differentiated tumours exhibit less keratinisation due to their deviation from normal cellular and tissue architecture (52). This association underscores the biological interplay between keratin production, which is a marker of terminal squamous epithelial cell differentiation, and the overall tumour biology as reflected in the histological grading system. (55)

These findings highlight the importance of considering histological grading and therefore, keratinisation, in the diagnostic and prognostic evaluation of OPSCCs. (3,52,53) The clear association between these two parameters reinforces their role in determining prognoses and in potentially tailoring therapeutic approaches in patients diagnosed with OPSCC.

A significant association was also found between histological grading and the anatomical subsite. This finding suggests that the location of the carcinoma within the oropharyngeal regions significantly influences its histological differentiation, a

critical factor in tumour prognosis that may assist in treatment strategy formulation (14,52,53,86). Such an association underscores the complexity of the biological behaviour of OPSCC and its interaction with the anatomical subsites (87). Studies have shown that HPV-associated OPSCCs, often located in the tonsillar region or base of the tongue, tend to present with better prognosis and response to treatment compared to HPV-independent tumours found in other locations (88,89). Our findings contribute to the growing body of evidence suggesting that the anatomical site is not only a determinant of the cancer's clinical presentation but also a predictor of its molecular characteristics and, by extension, its histological grading (52,87). This association emphasizes the need for anatomical site consideration in research, diagnosis, and therapeutic decision-making processes for OPSCCs.

Keratinisation, the process by which squamous cells become more cornified and accumulate keratin, is a key histological feature that influences the classification and prognosis (52,53,55). As a result, the analysis of keratinisation in relation to habits and anatomical subsites within the context of OPSCC is crucial. Given the varied prognosis and response to treatment seen when comparing classical histomorphological features of HPV-independent OPSCC and HPV-associated OPSCC characterised by keratinisation  and non-keratinisation respectively (12,52), by examining the relationships between keratinisation and these parameters, we aim to uncover patterns that may enhance understanding of tumour biology, underscore the indications for secondary investigative tests following microscopic examination of haematoxylin and eosin stained OPSCC samples and ultimately guide clinical decision-making, and potentially inform targeted intervention strategies.

Keratinisation, while historically significant in the classification and prognosis of SCC, must be considered alongside more contemporary factors, particularly HR-HPV status. The presence of keratinisation in tumours may still influence clinical decision-making, but HR-HPV status has emerged as the primary determinant of prognosis in OPSCC. Therefore, all newly diagnosed cases of OPSCC should be tested for HR-HPV status to guide treatment and management strategies effectively.

Broder’s Grading and Keratinisation:

While Broder’s grading system, which classifies tumours based on their degree of differentiation, has historically been a cornerstone in the prognostication ofSCCs, its

role in OPSCC has evolved. The relevance of this system is acknowledged, particularly in its ability to provide insights into the tumour's cellular characteristics, such as keratinisation. Keratinisation, the presence of keratin within the tumour cells, remains an important histopathological feature that can influence treatment decisions and outcomes in certain cases. Moreover, discussions on keratins and cutaneous cancers, while perhaps more traditionally associated with other forms of SCC, offer valuable insights into the biological behaviour of tumours with varying degrees of keratinisation.

However, as explained in the review of the literature above, it is important to recognize that the focus in OPSCC prognosis has shifted towards more nuanced and predictive systems, such as the Pattern of Invasion (POI) grading system and the TNM staging system, particularly in the context of HR-HPV status.

Modern Prognostic Systems:

Pattern of Invasion (POI) Grading System: This system assesses the morphology of the tumour's invasive front, distinguishing between cohesive and non-cohesive patterns. Non-cohesive or infiltrative patterns are associated with more aggressive tumour behaviour and poorer outcomes, making this system a valuable tool in predicting prognosis.

TNM Staging System (8th Edition): The updated TNM staging system has incorporated HR-HPV status as a critical factor in OPSCC prognosis. This system now differentiates between HPV-positive and HPV-negative OPSCC, with the former generally associated with a better prognosis due to distinct biological behaviour. HPV-positive OPSCC tends to present with smaller primary tumours and early nodal involvement, but it responds better to treatment, underscoring the importance of accurate HPV testing in all new cases.

HR-HPV Status as the Primary Prognostic Factor:

The shift towards recognizing HR-HPV status as the primary prognostic factor in OPSCC reflects the growing body of evidence that HPV-driven cancers behave differently from those associated with traditional risk factors like tobacco and alcohol. HR-HPV status has been shown to be a more reliable predictor of clinical outcomes, with HPV-positive OPSCC patients generally experiencing better survival rates and

responses to treatment compared to their HPV-negative counterparts (BioMed Central). As such, we strongly recommend that all newly diagnosed OPSCC cases undergo testing for HR-HPV status, in accordance with current clinical guidelines. This approach not only aids in prognostication but also guides treatment decisions, particularly in the context of de-escalation strategies for HPV-positive patients.

The analysis of keratinisation in OPSCC relative to lifestyle habits, sex, and anatomical subsites reveals significant insights into the intricate interplay between histopathological characteristics and external factors influencing tumour behaviour and disease progression. We found no significant association between keratinisation and lifestyle habits and sex. The lack of association with habits and gender might indicate that keratinisation as a histopathological characteristic is more closely influenced by tumour biology and the microenvironment than by external behavioural factors.

*4.1.7.2 Anatomic subsites*

When considering commonly affected subsites of HPV-associated OPSCC in their sample, Dapaah *et al.* demonstrated that the tonsil was the most common tumour site (56%), followed by the base of the tongue (22%) (32). This was the case in our study as well, where tonsil involvement was most common (30.43%) followed by the base of the tongue (15.1%), the predominance in our study is consistent with known OPSCC patterns. (12,3) It was further demonstrated in our study that 26.04% of cases involved two or more subsites of the oropharynx.

No statistical significance was found when considering the relationship between the combined lifestyle habits or sex. However, the relationship between the combined anatomical subsites and keratinisation status revealed a statistically significant association, echoing the findings previously discussed regarding keratinisation and anatomical subsites in OPSCC involving a single site, thus reinforcing the importance of considering anatomical specificity in the histopathological evaluation of SCCs (87).

While the study's findings suggest that the anatomical distribution of OPSCC may not be strongly associated with lifestyle habits, such as tobacco and alcohol consumption, it is important to recognize that these traditional risk factors still play a significant role in the aetiology of HNSCCs. The current data set may be limited, and

further research is needed to clarify these associations, particularly in populations with varying exposure to these risk factors.

The absence of significant associations between lifestyle habits, such as tobacco and alcohol consumption, and sex with the anatomical distribution of SCCs in the head and neck region raises further questions from a pathological perspective. It suggests that the aetiological factors influencing the development and localization of SCCs extend beyond traditional lifestyle risk factors. This observation aligns with the increasing understanding that histopathological characteristics, notably keratinisation, play a pivotal role in reflecting the intrinsic tumour biology and its state of differentiation. This intrinsic biological behaviour of tumours, as evidenced by the expression of keratin proteins, is critical in determining tumour aggressiveness and its potential for deeper invasion. Thus, the genetic makeup of the tumour and viral infections, particularly with HPV, may have a more pronounced impact on the site-specific development of SCCs in the head and neck than previously understood.

Furthermore, the complexity of head and neck SCC aetiology is underscored by the differential expression of keratins during the carcinogenesis process, which reflects changes in tumour differentiation and aggressiveness. The progression from actinic keratosis to invasive SCC involves a series of genomic alterations, including changes in keratin expression, which are crucial for the transformation process. This evolution suggests a nuanced interplay between genetic predispositions, environmental exposures, and viral oncogenesis in the pathogenesis of these cancers.

Understanding the molecular and cellular underpinnings of SCC, including the role of keratinisation and differentiation, is crucial for accurate diagnosis, prognostication, and treatment planning. It highlights the necessity for a multifaceted approach to SCC research and clinical management that incorporates molecular genetics, tumour pathology, and epidemiological factors to fully appreciate the disease's heterogeneity. As such, while lifestyle factors like smoking and alcohol remain important in the broader epidemiology of head and neck cancers, the specific contributions of tumour biology, genetic predispositions, and HPV status demand focused investigation to unravel the complex aetiology of SCCs at various anatomical sites within the head and neck region.

The lack of significant associations with lifestyle habits and sex suggests that the influence of these factors on the anatomical distribution of carcinomas might be less pronounced than the histopathological characteristics, such as keratinisation, that directly reflect tumour biology and differentiation. (12,52) This further suggests that, within this dataset, the location of SCCs in the head and neck is not significantly influenced by the patients' sex or tobacco and/or alcohol consumption. While this might seem contrary to expectations, given the well-documented role of these factors in the aetiology of HNSCC (6,18,21,32), it could suggest that the development of SCCs at specific anatomical sites is more strongly governed by other factors, such as genetic predispositions or viral infections such as HPV, rather than these external risk factors alone. This is not a conclusion though. Literature supports this by highlighting the complex aetiology of head and neck SCCs, where HPV status, rather than smoking or alcohol, is a stronger predictor of OPSCC location The complex interplay between anatomical site, keratinisation, and the aetiological factors of HNSCCs is further highlighted by these findings. While external factors including lifestyle habits and sex are crucial in the broader epidemiology of these cancers, the specific interactions between tumour biology and site emphasize the need for nuanced, site-specific approaches in research and clinical management (87).

The application of keratinisation as a criterion for tumour grading and differentiation is nuanced, particularly when comparing HPV-positive and HPV-negative SCCs in the head and neck region. HPV-positive SCCs, which are often linked to a better prognosis and distinct molecular profiles, typically exhibit non-keratinizing histological features, regardless of their well-differentiated status. This non-keratinizing phenotype reflects the unique pathogenesis and biological behaviour of HPV-positive tumours, distinguishing them from their HPV-negative counterparts. In contrast, HPV-negative SCCs, which are more frequently associated with traditional risk factors such as tobacco and alcohol use, often demonstrate varying degrees of keratinisation, correlating with their level of differentiation. This distinction underscores the importance of considering HPV status in the evaluation of head and neck SCCs. The presence or absence of keratinisation, while informative, cannot be uniformly applied across all SCCs without considering the tumour's HPV status. Hence, while keratinisation remains a valuable marker of differentiation and grading

in SCC, its significance must be contextualized within the broader framework of HPV-related tumour biology to accurately assess and manage these malignancies.

#### *4.1.7.3 HPV testing and p16 positivity*

Guidelines released from the College of American Pathologists suggest that all patients with newly diagnosed OPSCC undergo routine HPV testing using p16 IHC, as prognosis has been found to be largely dependent on HPV status (64). The limited testing (n=16; 16.66%) for HPV involvement using p16 IHC in our study of cases tested is a notable gap, given the critical role of HPV in OPSCC pathogenesis demonstrated across multiple studies. (6,16,32,45,71) The high p16-positivity rate (75%) among those tested aligns with the implemented practice of testing OPSCC that show typical histomorphological features of HPV-associated OPSCC. Unfortunately, the low number of cases tested together with the low number of subsequent ISH testing for those cases that were positive for p16 limits the conclusions that can be drawn about the true HPV status of the cohort. The variability in HPV testing methods (p16 vs. ISH vs. PCR) and the low testing rates in our study underscore a critical gap in standardising and implementing HPV testing protocols (21,32,64). This gap impacts the ability to accurately assess HPV's role in OPSCC in this cohort and could influence treatment planning and prognosis assessment. Future studies should aim for consistency in HPV detection techniques and strive for broader implementation of testing in suspected OPSCC cases (32,64). There is a clear need for consensus and standardization in HPV testing protocols for OPSCC, ideally incorporating confirmatory DNA tests alongside p16 IHC to accurately determine HPV involvement (13,40,60).

In the investigation of OPSCCs, the inclusion of HPV status emerges as a pivotal element, reflecting the virus's significant role in the aetiology, prognosis, and therapeutic responsiveness of these malignancies (6,10–15). The examination of HPV's impact through p16 IHC and PCR/ISH testing enables a nuanced exploration of the oncogenic potential of HPV in head and neck cancers (4,32,63). Our study's focus on HPV data underlines the evolving paradigm in head and neck oncology, where HPV-associated cancers are recognised for their distinct clinical behaviour and superior outcomes compared to their HPV-independent counterparts. (6,10–15)

Despite the relatively small sample size of HPV-tested cases within this dataset, the inclusion of HPV status is invaluable. It facilitates critical insights into the biological diversity of SCCs, highlighting the prognostic and therapeutic implications of HPV-related oncogenesis. (6,10–15) The differentiation between HPV-associated and HPV-independent OPSCC, through p16 IHC and ISH testing, enriches our understanding of tumour heterogeneity, providing a robust framework for personalised medicine.

P16 immunohistochemistry has been widely used as a surrogate marker for HPV positivity in HNSCC, particularly in OPSCC. The expression of p16, a cyclin-dependent kinase inhibitor, is significantly upregulated in HPV-positive tumours due to the viral oncogenes E6 and E7 leading to cell cycle deregulation. High p16 expression is correlated with HPV-driven carcinogenesis, making it a useful proxy for HPV infection in OPSCC.

However, while p16 is a valuable surrogate marker, it is important to recognize its limitations. P16 overexpression can occur in the absence of HPV infection, and not all HPV-positive tumours will overexpress p16 to the same degree. This is particularly relevant in non-oropharyngeal head and neck cancers, where the correlation between p16 expression and HPV positivity is less consistent than in OPSCC. Consequently, relying solely on p16 for determining HPV-driven oncogenesis may lead to misclassification in a subset of cases.

The use of p16 as a surrogate marker for HPV positivity is supported in clinical guidelines for the management of OPSCC due to the strong correlation between p16 overexpression and HPV-driven oncogenesis in this specific site. However, for a more definitive assessment of HPV status, additional molecular tests such as HPV DNA in situ hybridization (ISH) or PCR are recommended, especially when p16 results are equivocal or in non-oropharyngeal sites. Combining p16 immunohistochemistry with direct detection of HPV DNA provides a more accurate determination of HPV's aetiological role in tumour development and ensures appropriate classification for prognosis and therapeutic decision-making (85).

The high p16-positivity rate (75%) among those tested is indicative of the implemented practice of screening for OPSCC that show typical histomorphological features of HPV-associated carcinogenesis. However, it is important to recognize

that p16 positivity, while strongly correlated with HPV-driven oncogenesis, does not always equate to the presence of transcriptionally active HPV. As such, HPV DNA in situ hybridization (ISH) should be utilized in conjunction with p16 IHC to confirm HPV status, particularly in cases where the clinical or pathological findings are ambiguous. This approach aligns with current clinical guidelines and ensures accurate diagnosis and management of HPV-associated OPSCC.

HPV DNA ISH is considered the gold standard for confirming the presence of transcriptionally active HPV in OPSCC. Unlike p16 IHC, which serves as a surrogate marker, HPV DNA ISH directly detects the presence of HPV DNA within the tumour cells. This method is highly specific and can confirm whether the tumour is indeed driven by HPV (90,91).

The evolving landscape of HNSCC underscores the pivotal role of HPV status in shaping the clinical management and prognostic outlook of these tumours. HPV-positive HNSCCs, particularly oropharyngeal SCCs, represent a distinct clinical entity characterized by a non-keratinizing morphology, favourable response to treatment, and improved survival outcomes. This contrasts sharply with HPV-negative tumours, which typically exhibit keratinizing features and are associated with traditional risk factors such as tobacco and alcohol use, presenting a more challenging prognosis. The dichotomy in clinical behaviour between HPV-positive and HPV-negative tumours necessitates a nuanced approach to diagnosis, grading, and treatment. Molecular diagnostics, including p16 immunohistochemistry and HPV DNA testing, have become indispensable tools in the pathologist's arsenal, enabling the accurate stratification of tumours based on HPV status. This molecular stratification not only informs tailored therapeutic strategies, potentially sparing HPV-positive patients from the morbidity of aggressive treatments, but also guides surveillance and prognostic assessments. As the field moves towards a more personalized medicine paradigm, integrating HPV status into the multidisciplinary care of HNSCC patients represents a critical step in optimizing outcomes and advancing our understanding of this heterogeneous disease. The clear distinction between HPV-positive and HPV-negative HNSCCs, in terms of pathogenesis, morphology, and clinical course, highlights the importance of comprehensive tumour profiling in achieving precise diagnoses and implementing evidence-based treatment plans tailored to the unique characteristics of each tumour. Thus, the viability of the HPV data, even within a limited sample, offers a substantial contribution to the

comprehensive assessment of OPSCCs, reinforcing the necessity of integrating HPV status into clinical and research endeavours in oropharyngeal oncology.

## 4.2 Limitations of study

A lack of information in the laboratory records regarding race resulted in an incomplete epidemiological set for our study. In addition, using laboratory records meant that limited information was made available regarding risk factors. It was not possible to comment extensively on sexual or lifestyle behaviours as only a few of the laboratory records contained information on risk factors, but the study explored this within the limited data available. This is likely as a result of an inadequate clinical history being provided in pathology request forms leading to a lack of reflection of relevant information in the laboratory records. As laboratory records were anonymised, clinical records (that would likely allow for a more comprehensive assessment of lifestyle and environmental risk factors) were not retrievable, limiting the possibility of multivariate analyses which would assist in better understanding the contribution of these variables to OPSCC development. Because testing for the presence of p16 and HPV DNA is not routinely done at the NHLS, our study could not comment on the prevalence of HPV-associated/HPV-independent OPSCC, specifically. Our study was conducted at a single institution and completed using purposive sampling, which may result in potential selection bias. As a result of these factors, it would be unwise to generalise the findings. The wide confidence intervals suggest the need for a larger sample size in order to provide more precise estimates.

## 4.3 Conclusion

Rising cases of OPSCC in countries including the USA and UK has emerged as a significant focus in oncological research. To assist in addressing the limited data available regarding OPSCC in South Africa and across the African continent, our study assessed OPSCC prevalence at Wits Oral Health Centre and Charlotte Maxeke Johannesburg Academic Hospital. The findings offer insights into the regional distribution and characteristics of OPSCC, enhancing our understanding of this carcinoma.

The prevalence of OPSCC in the studied cohort, although substantial, aligns with emerging global trends, albeit lower than in some high-income countries. The

predominance among males is consistent with the literature while racial distribution remains inconclusive due to constraints in data availability.

While our study sheds light on the interplay between various risk factors and OPSCC, it is important to acknowledge the limitations in the available data, particularly the lack of records on race and the limited information regarding sexual behaviour and lifestyle factors. These gaps highlight the need for more comprehensive data collection in future studies to fully understand the complex etiological landscape of OPSCC in this population.

The limitations noted in our study, such as the lack of data on race and incomplete information on risk factors, reflect broader challenges in data collection within the healthcare setting. These constraints may impact the generalizability of our findings, particularly regarding demographic trends and risk factor profiles. Nevertheless, the insights gained from this study contribute to the growing body of knowledge on OPSCC in South Africa and underscore the importance of improving data collection practices in future research.

While tobacco and alcohol consumption are well known risk factors in the development of OPSCC, evolving sexual trends are likely to lead to a higher prevalence of OPSCC among various communities. The associations seen between histological grading and keratinisation, histological grading and anatomical subsites, keratinisation and anatomical subsites as well as the high number of p16-positivity among tested samples underscores the importance of routine testing using p16 IHC and either PCR/ISH to determine the presence of HPV DNA due to the improved prognosis observed in HPV-associated OPSCC when compared to HPV-independent OPSCC.

Despite limitations such as incomplete epidemiological data, a limited/incomplete clinical history provided in laboratory request forms and thus a smaller sample size, when considering risk factors/lifestyle habits and specimens tested for HPV, the findings provide a foundation for future research endeavors. They highlight the necessity of standardized testing protocols and larger-scale studies to deepen our understanding of OPSCC and improve clinical management strategies tailored to the African context.

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# 6. Appendices

## 6.1 Appendix A - Ethics Clearance Certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R49 Dr WG Baartman

**HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)**
**CLEARANCE CERTIFICATE NO. M220756**

| | |
|---|---|
| **NAME:** (Principal Investigator) | Dr WG Baartman |
| **DEPARTMENT:** | School of Oral Health Sciences<br>Department of Oral Medicine and Periodontology<br>Dental School<br>University |
| **PROJECT TITLE:** | *Prevalence of oropharyngeal squamous cell carcinoma at Charlotte Maxeke Johannesburg Academic Hospital* |
| **DATE CONSIDERED:** | 2022/07/29 |
| **DECISION:** | Approved unconditionally |
| **CONDITIONS:** | |
| **NOTE:** | If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za> |
| **SUPERVISOR:** | Drs T Mafojane and M Turton |
| **APPROVED BY:** | Dr CB Penny, Chairperson, HREC (Medical) |
| **DATE OF APPROVAL:** | 2022/12/20 |

**This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.**

**DECLARATION OF INVESTIGATORS**

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **July** and therefore reports and re-certification will be due in the month of **July** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator

10-01-2023
Date

6.2 **Appendix B –** Plagiarism Report

ORIGINALITY REPORT

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|---|---|---|---|
| SIMILARITY INDEX | INTERNET SOURCES | PUBLICATIONS | STUDENT PAPERS |

PRIMARY SOURCES

| | | |
|---|---|---|
| 1 | Submitted to University of the Western Cape<br>Student Paper | 1% |
| 2 | meridian.allenpress.com<br>Internet Source | 1% |
| 3 | Stephanie Y. Chen, Sean Massa, Angela L. Mazul, Dorina Kallogjeri et al. "The association of smoking and outcomes in HPV-positive oropharyngeal cancer: A systematic review", American Journal of Otolaryngology, 2020<br>Publication | 1% |
| 4 | Human Papillomavirus (HPV)-Associated Oropharyngeal Cancer, 2015.<br>Publication | 1% |

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