HEAD AND NECK CANCERS AMONGST HIV- POSITIVE PATIENTS: A 5 YEAR RETROSPECTIVE STUDY

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Science in Dentistry

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DECLARATION

I hereby declare that this research report is my own unaided work, except where due acknowledgement for assistance received has been made. It is being submitted for the degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been presented before for any degree or examination at this or any other University.

…….

Nompumelelo B. Zwane

Signed this …… day of …… 2017

DEDICATION

To

My loving and supportive husband, Mgcini Thwala

My mother, Margaret Hadebe

My children, Manga, Nkazi and Andiswa

ABSTRACT

Oral manifestations of Human Immunodeficiency Virus (HIV) are common, although since the introduction of HAART, a decline in opportunistic infections has been observed. This decline is more evident in resource-rich than poorly resourced countries. Aids-Defining-Cancer (ADC) cases have also declined in resource-rich countries; however there has been a sharp increase in Non-Aids-Defining-Cancer (NADC) cases.

Aim: The study was to profile and characterize head and neck cancers (HNCs) among HIV-positive cases diagnosed in the Department of Oral Pathology, WOHC, from 2009 – 2013.

Methods: This was a records-based retrospective descriptive study with an analytic component. Archived records from the Department of Oral Pathology were reviewed. HIV serology results as well as CD4+T-cell counts and the viral load were verified from NHLS's archived records.

Results: A total of 1605 cases of HNC were recorded, of which 389 (24.2%) were HIV-positive. The mean age of the patient sample was 51.7 ± 16.4 years. Of the 389 confirmed HIV-positive cases, 52.3% were females and 47.7% were males. Univariate multinomial regression analysis of the morphological type of cancer showed that the likelihood of patients with HIV infection to be diagnosed with KS and NHL, was significantly greater at 35.7% and 34.2% respectively with a p value = 0.00, compared to any other cancer type. Oral squamous cell carcinoma was not found to be an ADC.

Conclusion: Within the period 2003 – 2009 ADCs still frequently occurred amongst HIV-positive people in South Africa, despite HAART being made accessible at state health institutions since 2004.

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LIST OF ACRONYMS AND ABBREVIATIONS

HIV – Human Immunodeficiency Virus

AIDS – Autoimmune Deficiency Syndrome

HNC – Head and Neck Cancer

ADC – AIDS- Defining- Cancer

NADC – Non-AIDS- Defining- Cancer

KS – Kaposi Sarcoma

NHL – Non-Hodgkin's Lymphoma

HL – Hodgkin's Lymphoma

OSCC – Oral Squamous Cell Carcinoma

ICC – Invasive Cervical Cancer

SGT – Salivary Gland tumour

HPV – Human Papilloma Virus

EBV – Epstein – Barr Virus

ARV – Antiretroviral Therapy

HAART – Highly Active Anti-Retroviral Therapy

ECC – European Community Clearinghouse Classification

WOHC – Wits Oral Health Centre

NHLS – National Health Laboratory Services

NGO – Non-Governmental Organization

S. A – South Africa

US – United States

UNAIDS – United Nations Programme on HIV/AIDS

ANC – Ante-natal classes

STI – Sexually Transmitted Infection

OR – Odds Ratio

CI – Confidence Index

SIR – Standardised Index Ratio

WHO – World Health Organization

HVTN – HIV Vaccine Trials Network

NIH – National Institute of Health

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

The human immunodeficiency virus (HIV) is a RNA retrovirus belonging to the lentivirus subfamily. It is the virus that causes HIV infection. The virus first isolated by Barre *et al.* in Paris and Popovic *et al.* in 1983 in the USA, still remains as a huge threat to humanity to date. Since its discovery, HIV has spread at an alarming rate, more so in the resource-poor countries compared to the resource-rich countries. Two forms of the virus have been isolated, HIV-1 and HIV-2 (Barre´-Sinoussi *et al,* 1983). The two strains are related but genetically different. HIV-1 infection was mainly endemic in Central as well as East Africa and is now considered as pandemic in this region and has also spread to the Western countries. HIV-2 is mostly found in West Africa and leads to a 'milder' infection than the HIV-1 strain. HIV is acquired mainly via sexual routes, unsterilized drug injections among drug users, infected blood transfusions or during organ transplant procedures and mother-to-child transmission during birth or breastfeeding. HIV has an affinity for cells presenting receptors for T lymphocytes (CD4+T-cells) of the immune system in the body and will attach itself to these receptors, thus damaging the immune system and ultimately causing disease in an infected individual (Scully, 2013). The most advanced stage of HIV infection is acquired immuno-deficiency syndrome (AIDS). AIDS is diagnosed when an individual(s) have a CD4+ count of <200 cells/mm³ and they begin experiencing opportunistic infections because of the suppressed immune system (CDC, 1993).

In South Africa the estimated number of people living with HIV was estimated at 6. 4 million during 2013 with 500 000 of them being new infections (UNAIDS; HIV and AIDS estimates, 2014). In 2015 this figure had increased to 7 million, with prevalence rate of 19.2% in adults aged 15 – 49 years (UNAIDS, 2015). The epidemic is particularly more severe in Sub-Saharan Africa and Asia. Women between the ages of 15 and over, make up 4 million (57%)

of the estimated number of people infected with HIV in South Africa (UNAIDS, 2015). AIDS still remains the number one cause of deaths in Africa and the second among young people globally (UNAIDS, 2015). The number of people who died from HIV-related illnesses in South Africa during 2015 was 162,445, which make up almost a third (30, 5%) of all deaths in the country (UNAIDS, 2015). This is despite an aggressive campaign by government institutions to educate the public about this deadly virus and highlighting the preventative measures that can be adopted to stop the spread and number of new infections. Free testing has and is still offered at state health institutions nationally as well as the roll-out of highly active anti-retroviral therapy (HAART) since April 2004 to people infected with the virus with CD4+cell counts <200 cells/mm^3, and more recently to all that test positive. Despite all of these measures, more than half (51%) of the people infected with HIV in South Africa do not know of their HIV status (SA stats, 2015). It has been suggested that the main reason behind the unsuccessful prevention and diagnosis of HIV is probably the stigma and discrimination towards people living with HIV by the society, more so among the uneducated and those from low socio-economic backgrounds (Simbayi *et al,* 2007; Airhihenbuwa *et al,* 2009).

Oral lesions are a common finding in people infected with HIV (Narani *et al,* 2001). They are often the presenting feature, and may predict a deteriorating immune system and a poor prognosis for the individual (Narani *et al,* 2001). They also predict HIV infection (Shangase *et al,* 2004) and progression of HIV disease to AIDS (Coogan *et al,* 2005). Cancer is another significant cause of morbidity and mortality in people infected with HIV, and is one of the recognised manifestations of the infection and progression of the individual to AIDS stage (Newcomb-Fernandez, 2003). The two strains of HIV (HIV-1 and HIV-2) are not directly oncogenic. Robin Weiss (2001) stated that these viruses "can indirectly cause AIDS-defining cancer (ADCs) by allowing the DNA viruses underlying these tumours to exert their

oncogenic effects in the immunosuppressed host". This means that on their own the viruses are not oncogenic, but need the presence of other multiple factors in the particular immunosuppressed host to result in cancer (Qadir *et al*, 2016). The incidence of cancer in people infected with HIV is therefore highly increased when compared with the general population (D'Souza *et al*, 2014).

There are established cancers that affect people infected with HIV and these are referred to as AIDS-defining cancers (ADCs) by the Centres for Disease Control (CDC), and they are Kaposi sarcoma (KS), Non-Hodgkin's Lymphoma (NHL) and invasive cervical cancer (ICC). There are however, other types of cancer that appear among HIV-infected people more than the general population and they are referred to as non-AIDS-defining cancers (NADCs), and they are anal, lung, liver , kidney and testicular cancers as well as Hodgkin's lymphoma (HL) and some head and neck cancers (Barbaro *et al*, 2007; Silverberg *et al*, 2009; Deeken *et al*, 2012). These are cancers that were previously not associated with HIV infection. However, studies conducted mainly in resource-rich countries have demonstrated an increased incidence of NADCs among HIV-infected individuals (Engels *et al*, 2008).

Head and neck cancers (HNC) make up 5 – 50% of all cancers diagnosed worldwide (Garfinkel L, 1995). Gilyoma *et al*, (2015) stated that 650,000 new cases of HNC are currently being diagnosed worldwide with an increasing incidence in resource-poor countries. The management of these tumours is complex and requires a multidisciplinary approach. (Licitra *et al*, 2009). HNCs are an important cause of morbidity and mortality as they affect organs that are responsible for vital functions in the body e.g. oral cavity, pharynx, larynx, nose and lymph nodes (Amusa *et al*, 2004; Fan CY, 2004; Gilyoma *et al*, 2015). The reasons cited for this increased morbidity and mortality are the varied nature of histological patterns seen in this anatomical region, and individuals presenting themselves to health facilities for treatment once the cancer is already at an advanced stage making successful treatment of

these tumours difficult (Nwawolo *et al*, 2001, Amusa *et al*, 2004, Licitra *et al*, 2009). It is therefore important for dental professionals to recognize and diagnose these lesions early in order to improve the treatment outcomes.

In the head and neck region ADCs include Kaposi sarcoma (KS) and non-Hodgkin's lymphoma (NHL). In the European Community Clearinghouse (ECC) Classification on Oral Problems Related to HIV Infection (1993), Kaposi sarcoma and non-Hodgkin's lymphoma fall into a group of neoplastic lesions strongly associated with HIV including oral squamous cell carcinoma (OSCC). In 2009, Shiboski *et al* updated the existing classification and organized case definitions according to their aetiology with OSCC, NHL and KS falling under neoplasms associated with HIV. Whether KS is just a benign angioproliferative disorder or a true malignancy owing to the oncogenic nature of the causative factor, the human herpes virus-8 (HHV-8), is still not clearly understood and remains a contentious topic (Feller *et al*, 2007). For the purposes of this current study, KS will be regarded as a cancer.

Even though OSCC appears under lesions that are strongly associated with HIV infection, it is not regarded as an ADC. This is because the risk factors for the development of OSCC in HIV- positive people are the same as those for the general population (tobacco, alcohol and human papilloma virus (HPV) infection), and is therefore regarded as a NADC. Recently HIV- positive people have been found to have an increased prevalence of oral human papilloma virus (HPV) infection (about 2-fold) and HNC (about 2.3-fold) (Beachler *et al*, 2012). Unsafe sexual behaviour is thought to be the risk factor for this sharp increase (Pytynia *et al*, 2014, D'Souza *et al*, 2014). McLemore *et al* (2010) reported laryngeal cancer as the most common malignancy followed by oropharyngeal cancer in the USA particularly in HIV-positive individuals. HPV-16 positive oropharyngeal cancer cases are so much on the rise in the USA that they have been referred to as epidemic, especially among middle aged white males who are non-smokers but have multiple sexual partners (Pytynia *et al*, 2014,

D'Souza *et al,* 2014). The reasons for the development of OSCC in HIV-positive people remains unclear but several mechanisms are thought to be contributing factors. The immunocompromised state of HIV- positive patients is one of the mechanisms thought to assist in the faster progression of HPV (an oncogenic virus) infection to carcinogenesis because of an imbalance between cellular proliferation and differentiation that occurs (Pytynia *et al,* 2014, D'Souza *et al*, 2014). D'Souza *et al,* (2014) stated that in HIV- positive people it is not clear which one of the reasons mentioned increase the risk of cancer more than the other.

A decline in opportunistic infections including oral lesions and ADCs has been observed in resource-rich countries since the introduction of antiretroviral therapy (Engels EA, 2009, Cobucci *et al,* 2014). The same statement however, cannot be said for poorly resourced countries where the incidence of ADCs is still high (Stein *et al*, 2008, Sasco *et al*, 2010, Mbulaiteye *et al*, 2011, Casper, 2011). Previously NADCs were not associated with HIV infection. However, studies conducted mainly in resource-rich countries have demonstrated an increased incidence of NADCs among HIV-infected individuals (Engels *et al*, 2008). In the pre- HAART era, NADCs accounted for only 38% of all cancers compared with 58% of all cancers post-HAART era in the United States (Deeken *et al,* 2012). HAART has resulted in HIV- infected people living longer than before, leading to a growing cancer burden (Engels *et al*, 2008, Engsig *et al*, 2011, Deeken *et al*, 2012). A higher prevalence of virus-related infections, smoking or alcohol intake as well as risky sexual behavior among HIV infected people also elevates the risk for NADCs more than for the general population (40-60% vs. 17%) (Engels *et al*, 2008, Baker *et al,* 2008, Silverberg *et al,* 2011). Studies conducted here in sub-Saharan-Africa on HNCs among HIV- positive people are few, making it difficult for one to draw the same conclusions about ADCs and NADCs as has been done in Europe and America. Casper (2011) reported that the impact of HIV in Africa is slightly

different than that observed in the Western countries, owing to the fact that most infections seen in these resource-poor settings are due to oncogenic viruses and environmental exposure to carcinogens in the general population as well as delayed access to HAART for HIV-infected individuals. Mbulaiteye *et al*, (2011) also stated that the HIV impact in this region may be different compared with that experienced in the Western countries because infections with oncogenic viruses account for most of the cancers observed in sub-Saharan Africa.

Limited data have been collected since the introduction of HAART in resource-poor countries to determine the impact it has had on ADCs (Parkin *et al,* 2010). Data from Uganda cancer registry showed that there has been an increase in the number of ADCs seen in this region instead of a decrease (Parkin *et al,* 2010). Sitas *et al*, (2008) also reported high numbers of KS and NHL in HIV- infected people in a study conducted in Johannesburg, South Africa. Another study conducted in Uganda found that ADCs had an increased risk of still developing in HIV-infected patients, with few cases of NADCs recorded in this region (Mbulaiteye *et al*, 2006). Nwaorgu *et al,* (2007) reported the larynx as the most common anatomical site for HNC diagnosis (31, 3%), followed by the salivary glands in a study conducted in sub-Saharan Africa. Amusa *et al*, (2005) reported different results in a study she conducted in Nigeria where the oral cavity was the most common site for HNCs followed by the neck, thyroid and oesophagus. The least affected sites in Amusa's study were the ears and paranasal sinuses. Stein *et al* (2008) reported similar results in South Africa as other studies conducted in Africa. He found a 50-fold higher risk for developing KS and HNL (ADCs) in HIV- positive people. The risk for NADCs, like Hodgkin's lymphoma (HL) and anogenital cancer, was 1.5 – 2.5 times higher. HAART roll out to the public by government institutions in South Africa was initiated only in April 2004 (Simelela *et al*, 2014). Before the roll-out in South Africa, only people who could afford to purchase antiretroviral medication from private institutions were on HAART. Between 1997 and 2004, death rates increased >5-fold

for young women and doubled for middle-aged males in South Africa (Stein *et al,* 2008). HAART therefore, clearly plays an important role in the types of cancers and incidence rates observed among HIV-positive patients between resource-rich and resource-poor countries. Sitas *et al,* (2000) and Stein *et al,* (2008) noted this fact as one possible reason for the differences observed too.

Successful treatment of the virus has remained elusive with no effective control or prevention of new infections, resulting in an increased number of deaths among HIV- infected individuals as a result of opportunistic infections. Recent progress in the development of an effective HIV vaccine however, provides new hope for future eradication and prevention of new infections (Stephenson *et al,* 2016).

The aim of this study therefore, was to identify and characterise the type and number of HNCs diagnosed histologically in HIV-positive patients in the Department of Oral Pathology, Wits Oral health Centre (WOHC), from 2009 – 2013. This was a period after the roll-out of HAART to people infected with HIV and CD4+T-cell counts of <200 cells/mm^3 by government institutions in SA. We hope that the results will assist in determining whether there was a significant change in the type of HNC cases diagnosed among HIV-infected individuals. This will also confirm whether these HNCs are ADCs or NADCs.

CHAPTER 2: AIM AND OBJECTIVES

The aim of this study therefore, was to identify and characterise the type and number of HNCs diagnosed histologically in HIV-positive patients in the Department of Oral Pathology, Wits Oral health Centre (WOHC), from 2009 – 2013.

The objectives of the study were to:

1. Identify head and neck cancers (HNCs) occurring among HIV-positive patients diagnosed in the Department of Oral Pathology from 2009 to 2013

2. Determine the most common HNCs occurring among HIV-positive patients

3. Determine the site predilection of HNCs among HIV-positive patients

4. Assess the association between the presence of the HNCs and the immune status of the HIV-positive patient (CD4+T-cell count and the level of infection (viral load))

CHAPTER 3: METHODOLOGY

3.1 Study design

This was a records-based retrospective descriptive study with an analytical component, conducted at the Oral Pathology Department, WOHC.

3.2 Study population and sample

The study population consisted of all histologically diagnosed cases of HNC in the Oral Pathology Department's archives from January 2009 – December 2013. A total sample size of 1605 HNC cases was recorded. Confirmation of HIV seropositivity for the subjects was obtained from records of the National Health Laboratory Services (NHLS) of South Africa.

3.3 Data and Statistical analysis

Data analysis was done using the IBM SPSS 23.0. Descriptive statistics of measurement of central tendencies was used to determine the frequency, mean and standard deviation. Inferential statistics of binary and multinomial logistic regression were used to determine the association between the independent variables (age, gender and HIV status) and the dependent variable (cancer diagnosis or type). Since the study sample size was already determined from the retrospective nature of the study, power calculations were done to minimize type 2 errors when making comparisons. Statistical significance was inferred at $p < 0.05$ for all analyses.

3.3.1 Independent variables

The age, gender and HIV status of all the cases recorded were noted as independent variables

3.3.2 Dependent variable

The histological diagnosis of head and neck cancer or type of head and neck cancer in HIV-positive cases, was noted as a dependent variable

3.3.3 Analysis by objective

Table 1: Data analysis by specific objectives

Objectives	**Data analysis**
Identify head and neck cancers occurring among HIV-positive cases presenting at WOHC, from 2009 to 2013 - Identify the diagnosed head and neck cancer - Identify the site of head and neck cancer	Central tendencies of frequency were used to identify the diagnosis and the site of head and neck cancers.
Determine the association between the presence of the HNCs and the immune status	Univariate and multivariate multinomial regression was used to determine the association between the diagnosis of head and neck cancer

3.4 Ethical considerations

Permission to undertake the current study was granted by the University of the Witwatersrand's Human Research Ethics Committee – (Medical - Ethics clearance certificate number: M140655, see Appendix B). Permission to access histological patient records was sought and granted by the Department of Oral Pathology, WOHC, (see Appendix C). The NHLS also granted permission to access and confirm the blood results for all the cases identified and included in the study (see Appendix D and E). All cases recorded were allocated a reference number for protection of their identity.

CHAPTER 4: RESULTS

4.1: Demographic characteristics of the cohort

A total of 1605 cases of head and neck cancer were recorded at the Oral pathology department, WOHC, between 2009 and 2013. The demographic characteristics of the cases are outlined in Table 2 below.

Only 389 (24.2%) of the cases were confirmed HIV- positive. Of the remaining 1216 cases, 229 (14.3%) were confirmed HIV- negative and 987 (61.5%) were unknown/unconfirmed cases. The mean age of all the subjects recorded was 51.7 ± 16.4 years. For the confirmed HIV- positive cases, the mean age was 51.7 ± 16.4 years with 46.1% of them in the 36 – 55 years age group. For confirmed HIV- negative patients, the mean age was 52.1±16.9 and for unconfirmed/unknown group the mean age was 51.6±16.1. More females (52.3%) than males (47.7%) were HIV- positive. HIV- infection was noted more among black patients (91.5%). Only 5.9% and 1.0% of Coloured and Caucasians patients respectively were confirmed HIV-positive cases. The ethnicity of 1.5% confirmed HIV-positive cases remained unknown.

Alcohol use and smoking were mainly unknown in this cohort, 97.6% for alcohol and 79.4% for smoking respectively. Among the HIV-positive cases, only 5.4% were known smokers and only 0.8% had a confirmed history of alcohol use. Some of the HIV-positive people were already receiving HAART (19.0%) with many unconfirmed cases (80.5%) because of missing information. Only 31.9% (< 200 cells/mm^3) and 20.1% (> 200 cells/mm^3) HIV-positive cases had confirmed CD4+T-cell count results available, even after an extensive search from the NHLS archived records. Detectable RNA viral load was confirmed in 27.2% of the HIV-positive subjects with 71.5% remaining unknown. Only 1.3% of HIV-infected subjects had undetectable RNA viral load after being tested.

Table 2: Demographic characteristics of patients with head and neck cancer between 2009 and 2013 at WOHC

Variables	**HIV+ (n=389)**	**HIV- (n=229)**	**Unknown (n=987)**	**p-value**	**Total (N=1605)**
Mean age ± SD*	38.4 ± 11.0	55.4 ± 15.0	56.1 ± 15.6	0.00	51.7 ± 16.4
Age group* n (%)				0.99	
≤ 35 years	71 (24.1)	45 (15.3)	179 (60.6)		295 (18.6)
36-45 years	63 (24.1)	33 (12.6)	165 (63.3)		261 (16.5)
46-55 years	82 (25)	47 (14.3)	199 (60.7)		328 (20.7)
56-65 years	88 (22.6)	56 (14.4)	245 (63)		389 (24.6)
≥ 66 years	77 (24.8)	46 (14.7)	188 (60.5)		311 (19.6)
Gender* n (%)				0.00	
Male	185 (47.7)	81 (35.4)	344 (34.9)		628 (39.2)
Female	203 (52.3)	148 (64.6)	643 (65.1)		976 (60.8)
Ethnicity* n (%)				0.00	
African	356 (91.5)	140 (61.1)	635 (64.5)		1131 (70.6)
Caucasian	4 (1.0)	3 (1.3)	34 (3.5)		41 (2.6)
Indian	-	6 (2.6)	20 (2.0)		26 (1.6)
Mixed	23 (5.9)	74 (32.3)	273 (27.7)		370 (23.1)
Unknown	6 (1.5)	6 (2.6)	23 (2.3)		35 (2.2)
Alcohol, n (%)				0.00	
Yes	3 (0.8)	6 (2.6)	23 (2.3)		32 (2.0)
No	2 (0.5)	-	5 (0.5)		7 (0.4)
Unknown	384 (98.7)	223 (97.4)	959 (97.2)		1566 (97.6)
Smoking, n (%)				0.00	
Yes	21 (5.4)	57 (24.9)	215 (21.8)		293 (18.3)
No	12 (3.1)	5 (2.2)	20 (2.0)		37 (2.3)
Unknown	356 (91.3)	167 (72.9)	752 (76.2)		1275 (79.4)

HAART*				n/a	
Yes	74 (19.0)	-	1 (0.1)		n/a
No	2 (0.5)	-	-		n/a
Unknown	313 (80.5)	8 (3.5)	980 (99.7)		n/a
N/A	-	221 (96.5)	2 (0.2)		
CD 4 count				n/a	
<200	124 (31.9)	n/a	n/a		n/a
>200	78 (20.1)	n/a	n/a		n/a
Viral load				n/a	
Present	106 (27.2)	n/a	n/a		n/a
Not detected	5 (1.3)	n/a	n/a		n/a
Unknown	278 (71.5)	n/a	n/a		n/a

*Missing data

4.1.1: Distribution of head and neck cancer by gender

As illustrated in Figure 1 below, more females (60.8%) than males (39.2%) were diagnosed with head and neck cancer between 2009 and 2013.

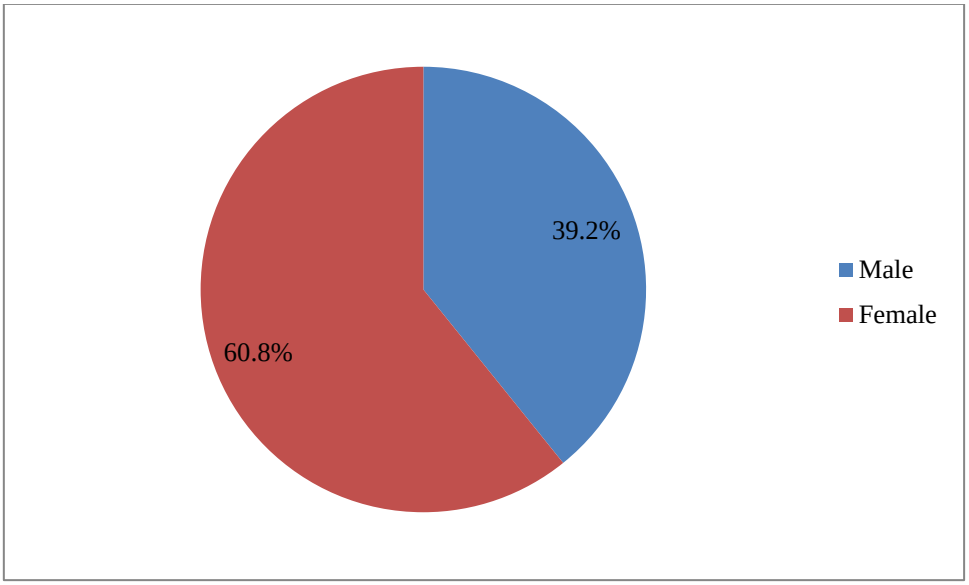


Figure 1: Gender distribution of all patients diagnosed with HNC

When the patients diagnosed with HNC were grouped according to their HIV status, more females (52.3%) were confirmed as HIV-positive whilst most males (64.6%) were HIV-negative in this cohort as shown in Figure 2 below.

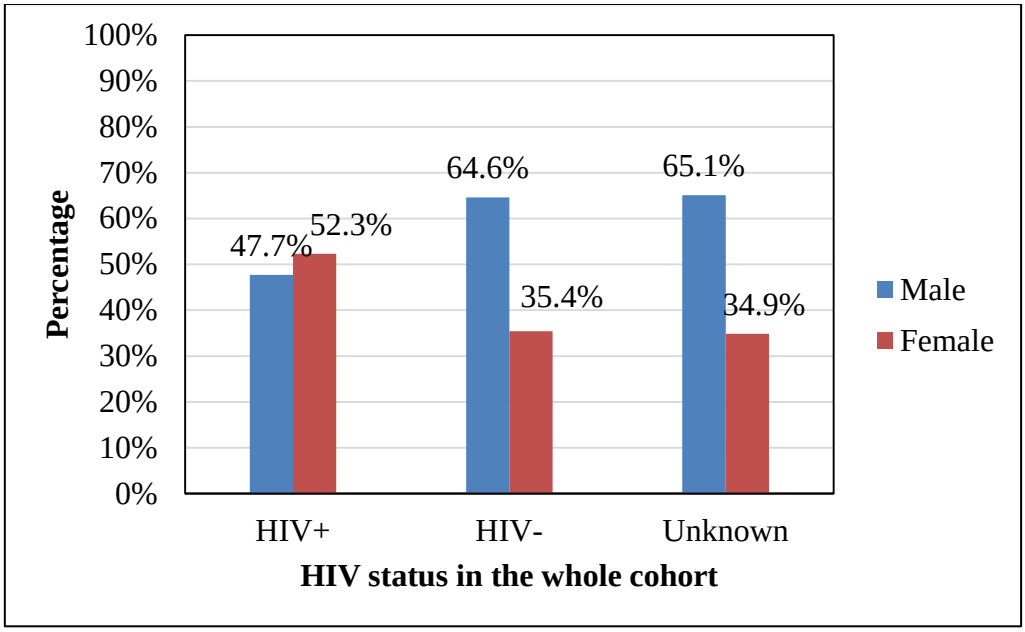
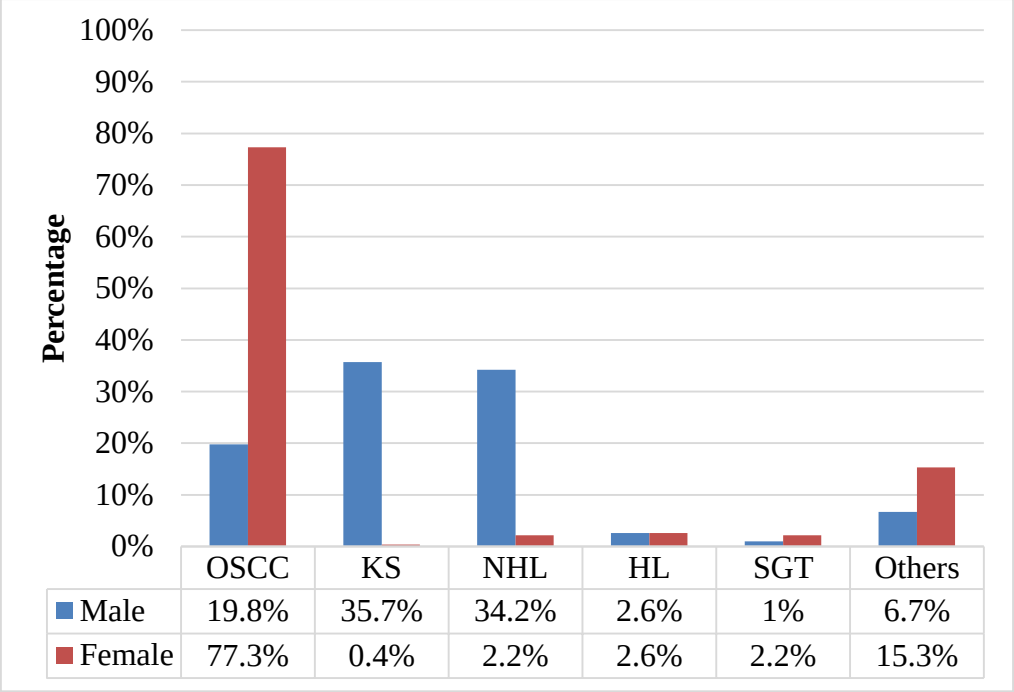


Figure 2: Gender distribution of cases with HNC in relation to their HIV status

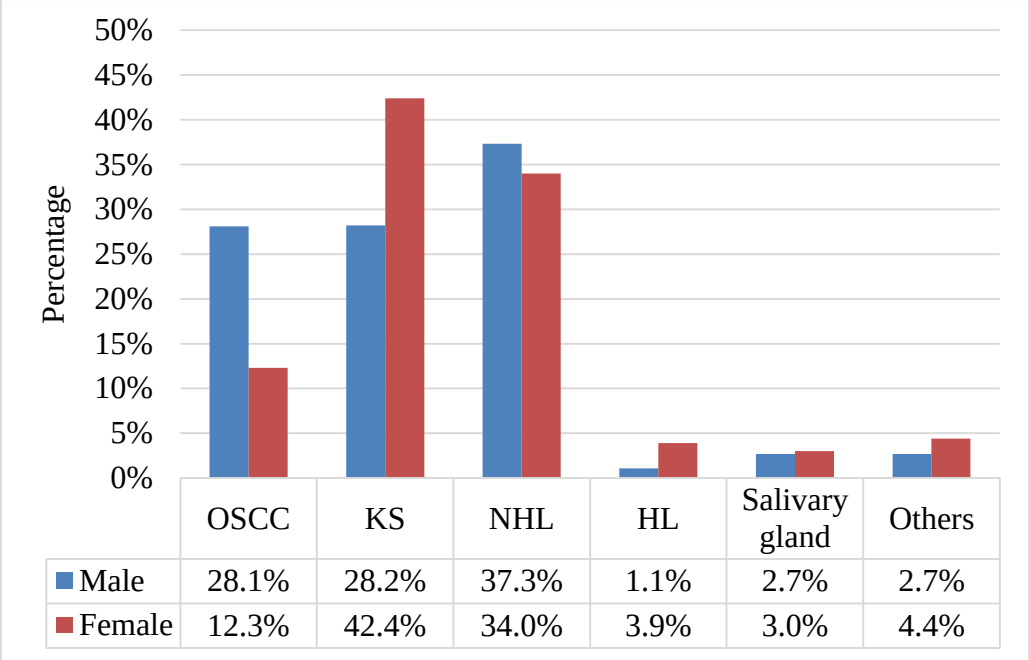
OSCC was diagnosed in (77.3%) of females and (19.8%) of males. Among patients with KS diagnosis, (35.7%) were males and (0.4%) were females. NHL diagnosis was in (34.2%) males, and (2.2%) female patients, HL in (2.6%) males and (2.6%) females, SGTs in (1.0%) males and (2.2%) females and other types of HNCs in (6.7%) males and only (15.3%) female patients as Figure 3 depicts below.



	OSCC	KS	NHL	HL	SGT	Others
Male	19.8%	35.7%	34.2%	2.6%	1%	6.7%
Female	77.3%	0.4%	2.2%	2.6%	2.2%	15.3%

Figure 3: Categories of HNC in relation to the gender of all patients included in this cohort

Figure 4 below illustrates the HNC diagnosis in relation to the gender of HIV-infected patients. Kaposi sarcoma was diagnosed in (42.4%) of female patients and (28.2%) of male patients, NHL (34.0%) female patients and (37.3%) male patients and OSCC (12.3%) female patients and (28.1%) male patients.



	OSCC	KS	NHL	HL	Salivary gland	Others
Male	28.1%	28.2%	37.3%	1.1%	2.7%	2.7%
Female	12.3%	42.4%	34.0%	3.9%	3.0%	4.4%

Figure 4: Categories of HNC related to the gender of HIV- positive cases

4.1.2: Distribution of head and neck cancer diagnosis by age

The largest number of the participants (45.3%) in the study was aged between 46 and 65 years, with the least number (16.5%) aged between 36 – 45 years as depicted in Figure 5. The results also indicated that 18.6% of the patients diagnosed with HNC were ≤ 35 years and 19.6% were ≥ 66 years.

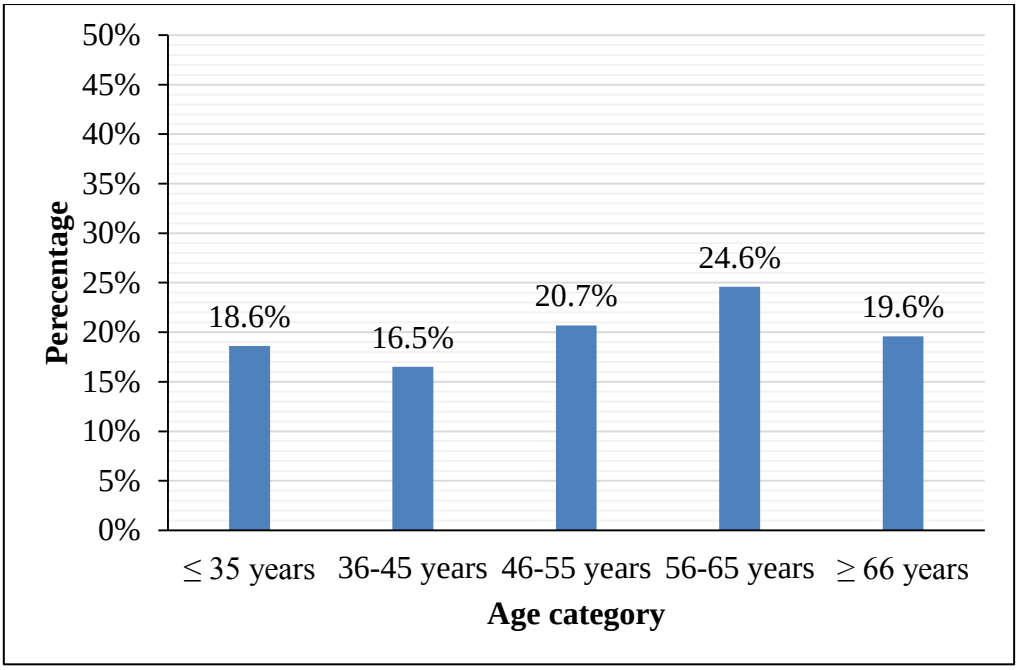
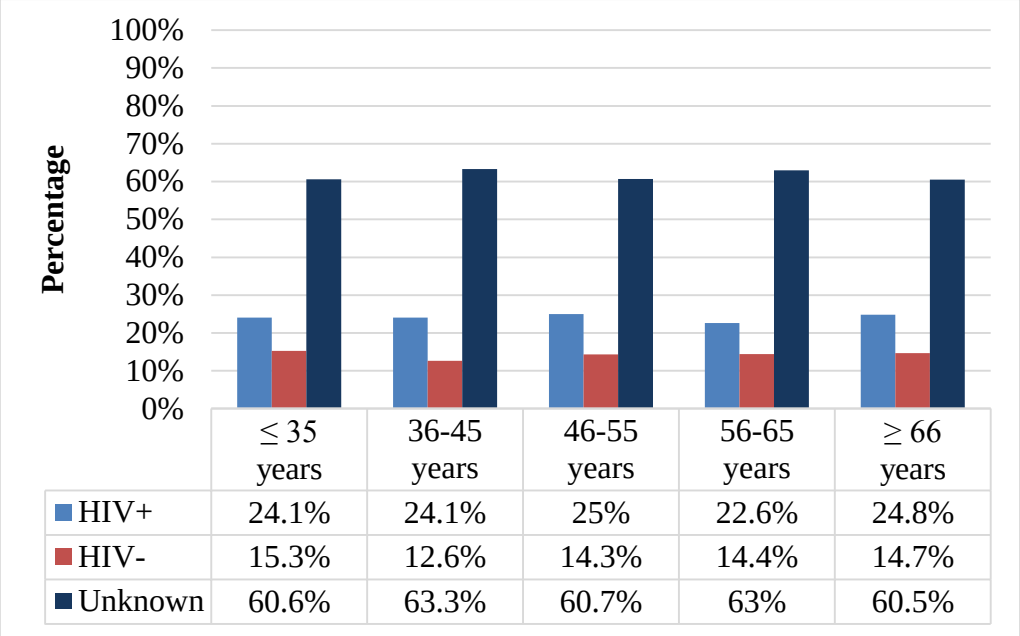


Figure 5: Age distribution of patients diagnosed with HNC

Figure 6 demonstrates that the age groups of patients affected with HIV, those without HIV-infection and the unknown category. In the age group of ≤ 35 years, 24.1% of the patients were HIV- positive, 15.3% HIV- negative and 60.6% of cases having an unknown status. From 36 – 45 years of age, 24.1% were HIV- positive, 12.6% HIV- negative and 63.3% unknown. For the age group of 46 - 55 years, 25% were HIV- positive, 14.3% HIV- negative and 60.7% unknown. Above 55 years of age to 65 years 22.6% of the patients were HIV-positive, 12.4% HIV-negative and 63% unknown. For those ≥ 66 years old, 24.8% were HIV-positive, 14.7% were HIV- negative and 60.5% were unknown cases. The highest number of HIV-positive cases was in the 46 – 55 year age group.



	≤ 35 years	36-45 years	46-55 years	56-65 years	≥ 66 years
HIV+	24.1%	24.1%	25%	22.6%	24.8%
HIV-	15.3%	12.6%	14.3%	14.4%	14.7%
Unknown	60.6%	63.3%	60.7%	63%	60.5%

Figure 6: Age distribution of the patients in relation to their HIV status

Of the 139 diagnosed cases of KS, more than half (78) of them were in the age group of ≤ 35 years, with 45 of the cases in the 36 – 45 years age group, 13 in the 46 – 55 years age group and only 3 cases in the group 56 – 65 years of age. No KS was diagnosed in the age group ≥ 66 years. NHL was the second highest HNC diagnosed among HIV-positive cases, with 60 of them seen in the ≤ 35 years age group, 58 in the 36 – 45 years age group, 13 in the 46 – 55 years age group, 6 in the 56 – 65 years age group and only one case in the age group ≥ 66 years. OSCC had 29 cases in the age group 46 – 55 years, 23 in the 36 – 45 years age group, 15 in the 56 – 65 years and 8 in the ≤ 35 years age group. Only 2 cases were in the age group ≥ 66 years as illustrated in Figure 7 below.

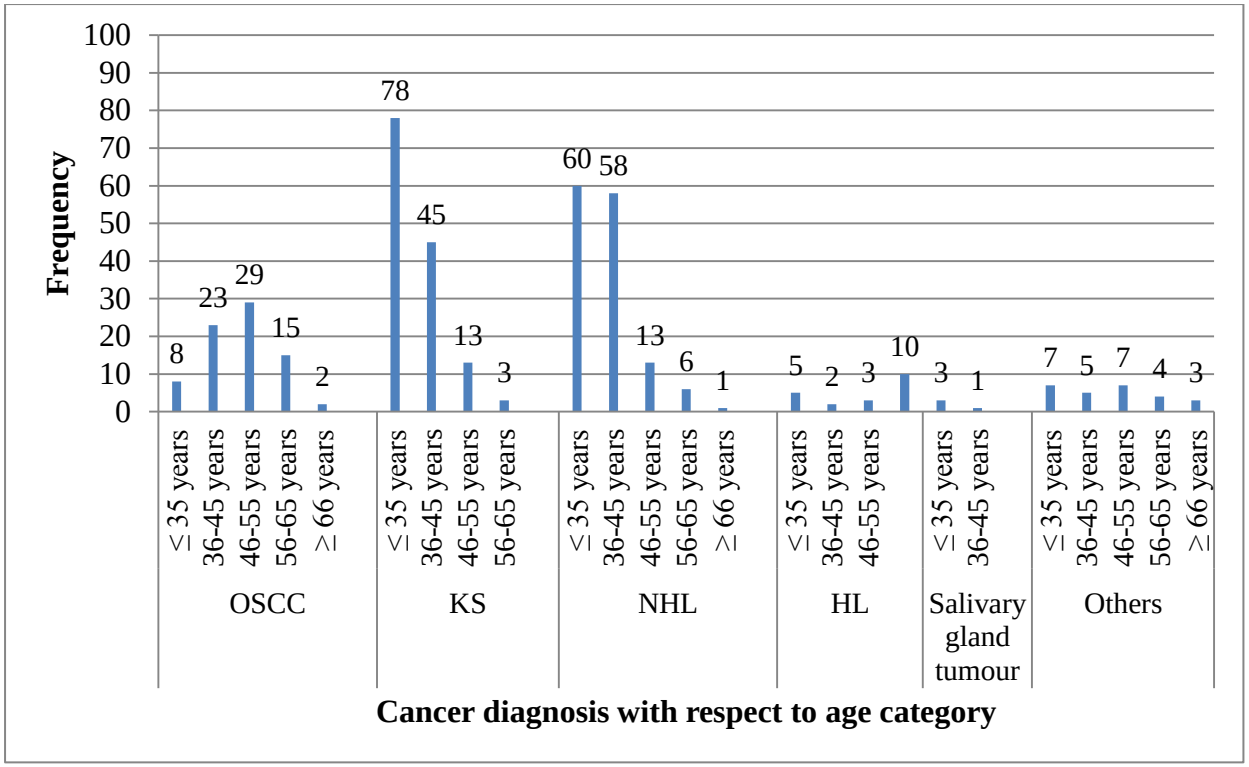


Figure 7: HNC diagnosis in HIV-positive patients with respect to age

4.2: Types of head and neck cancers (HNC) occurring among HIV-infected patients

The most common HNC diagnosed amongst HIV- positive people in this study was KS (35, 7%) and NHL (34, 2%), followed by OSCC (19, 8%) as shown in Table 3. KS was also diagnosed in 0.4% of the HIV- negative cases, NHL in 2.2% and OSCC in 77.3%. The unknown category had 5.8% of KS cases, 7.9% NHL and 62.3% OSCC cases. HL was diagnosed in 2.6% of HIV- positive cases, 2.6% HIV- negative and 2.2% unknown cases. Salivary gland tumours (SGT) were diagnosed in 1.0% of HIV- positive cases, 2.2% HIV- negative and 2.0% unknown cases. An illustration to compare the distribution of the three most common head and neck cancer diagnosed in relation to HIV status is shown in Figure 8.

Table 3: Head and neck cancer diagnosis in relation to HIV status

	HIV +		HIV -		Unknown	
HNC diagnosis	**n**	**%**	**n**	**%**	**n**	**%**
OSCC	77	19.8	177	77.3	616	62.3
KS	139	35.7	1	0.4	57	5.8
NHL	133	34.2	5	2.2	78	7.9
HL	10	2.6	6	2.6	22	2.2
SGT	4	1.0	5	2.2	20	2.0
Others	26	6.7	35	15.3	195	19.7
TOTAL	**389**		**229**		**988**	

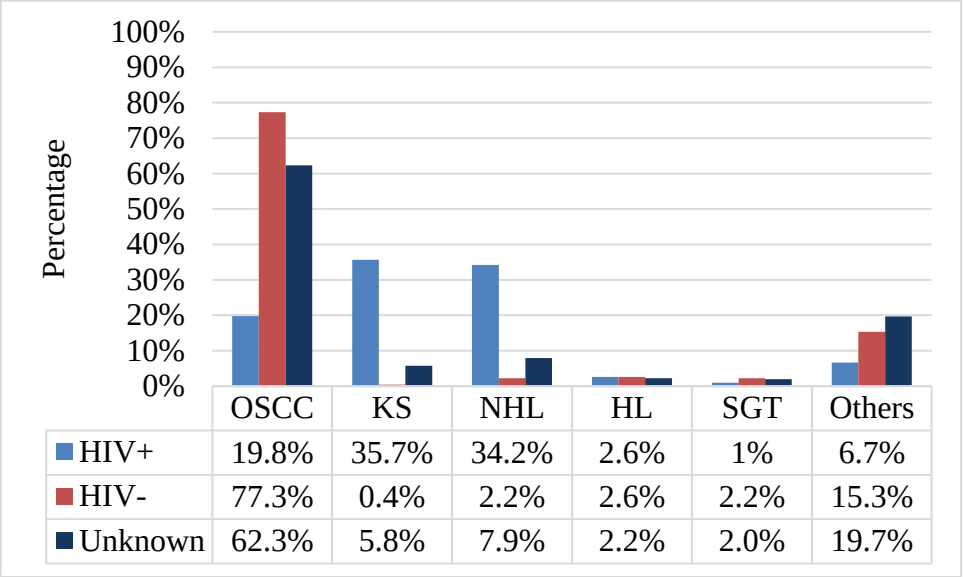


Figure 8: Categories related to HIV status

4.2.1: Head and neck cancer diagnosis and anatomical site predilection in all recorded patients

The oral cavity was the most common anatomical site for head and neck cancer diagnosis at (16.4%) followed by the larynx (15.3%), the tongue (11.3%) and jaws (11.2%). The palate (10.3%), neck (9.9%) and the nasal cavity (7.5%) were not far behind as shown in Figure 9 below followed by the lips (5.3%), the pharynx (4.6%), salivary glands (2.3%), ears (2.1%), the forehead (0.1%). Some of the HNCs diagnosed were affecting multiple sites in some individuals (2.6%). In 0.8% of the cases the anatomical site was not specified.

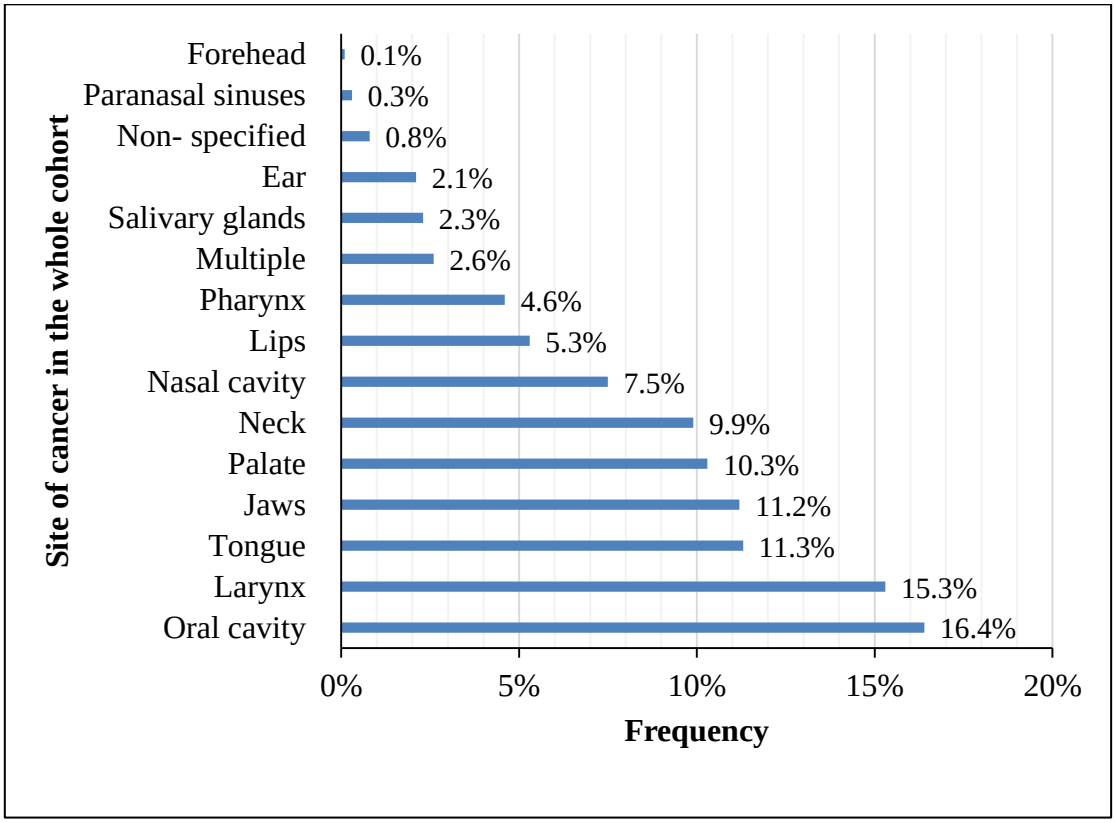


Figure 9: Anatomical distribution of HNC diagnosed in this cohort

Figure 10 below depicts the anatomical site predilection only in patients infected with HIV. The larynx was the main anatomical site that the HNCs were diagnosed in at 19.2%, followed by the palate at 16.5%, tongue 15.7%, oral cavity 14.7%, neck 14.1%, jaws 6.6%, pharynx 6.2%, lips 2.8%, nasal cavity 2.1%, salivary glands 1.3%, ear 0.5% and 0.3% of multiple sites.

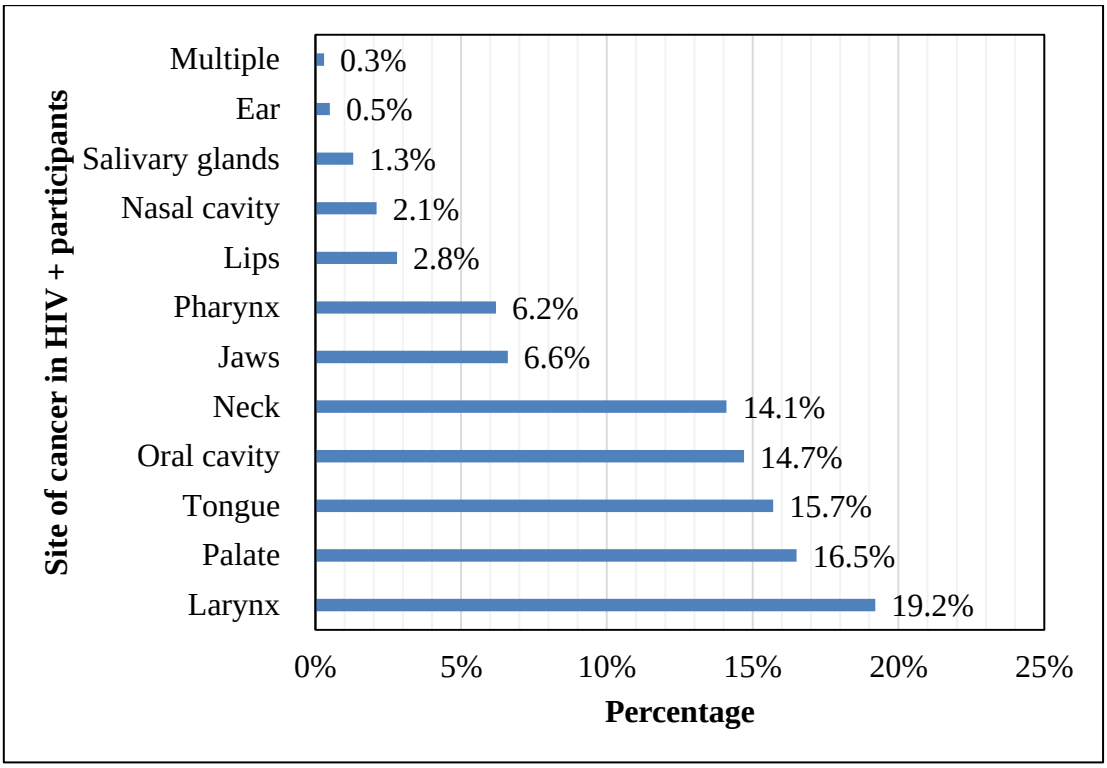


Figure 10: Anatomical distribution of HNC among HIV-positive cases

4.3: Head and neck cancer diagnosis and HAART/ARV therapy in HIV infected patients

Some of the cases diagnosed with HNC were already receiving highly active antiretroviral therapy. Thirty-three cases that were HIV-positive with KS were already receiving HAART and 25 cases with NHL. Of the 77 patients with OSCC, 8 were already on HAART. For all the other types of HNCs, 4 of the patients were on HAART and 4 with HL, as illustrated in Figure 11.

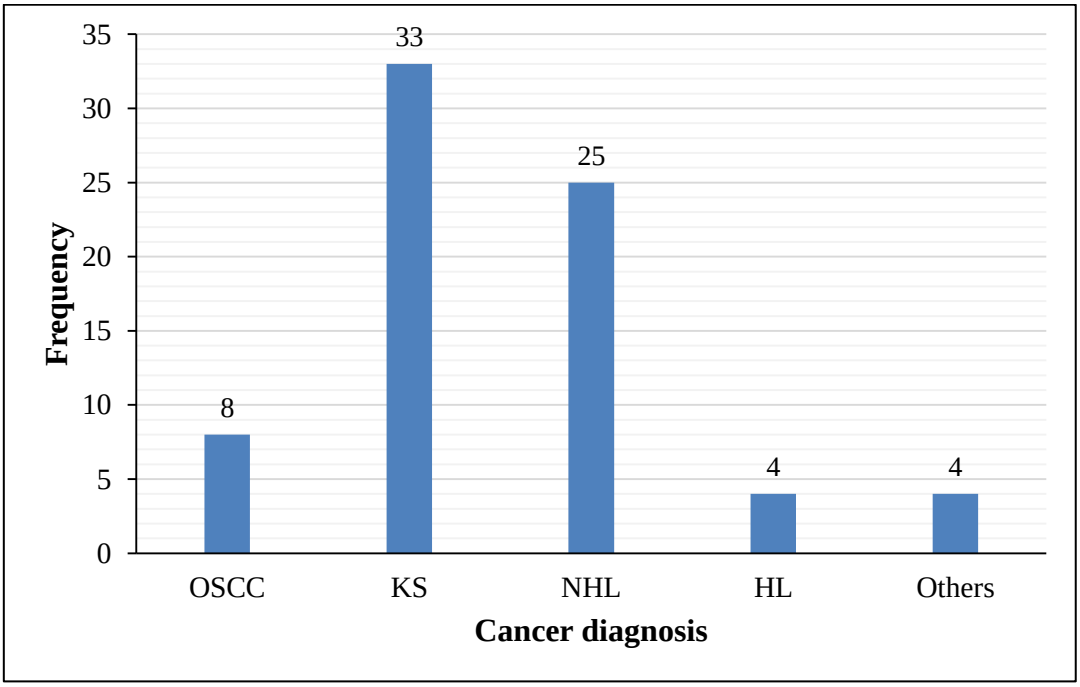


Figure 11: Categories of HNC in relation to HAART/ART use
(N = 74)

4.4: Association of head and neck cancer diagnosis with the HIV status of the patients

The association between cancer diagnosis and HIV status was analysed using multinomial logistic regression. Multivariate regression was done and adjusted for age and gender. Univariate multinomial regression analysis of the morphological type of cancer showed that patients infected with HIV were significantly more likely, 187.1 and 35.8 times, to be diagnosed with KS and NHL respectively, than patients without HIV infection as shown in Table 4 below. OSCC, HL and salivary gland tumours showed no significant difference in patients with or without HIV.

Table 4: Association between cancer diagnosis and HIV infection

	Univariate			**Multivariate**		
Diagnosis	**OR**	**95%CI**	**p**	**OR**	**95%CI**	**p**
OSCC	0.59	0.33-1.04	0.07	0.76	0.42-1.38	0.37
KS	187.12	24.54-1426.71	0.00	130.2	16.6-1023.6	0.00
NHL	35.81	12.82-100	0.00	25.0	8.9-71.2	0.00
HL	2.24	0.72-6.96	0.16	1.94	0.56-6.7	0.29
Salivary gland tumour	1.08	0.26-4.41	0.92	0.69	0.16-2.92	0.62

Multivariate regression analysis was adjusted for age and gender
Reference category was HIV- negative cases

4.5 Association between head and neck cancer diagnosis and CD4+T-cell count

Also, multinomial regression analysis of cancer diagnosis showed a significant association with CD4+T-cell count in KS and NHL. The odds of a diagnosis of KS and NHL in HIV-positive patients with CD4+T-cell count greater than 200 was increased by 0.19 and 0.25 respectively when compared with people with CD4+T-cell count less than 200 as shown in Table 5 (p=0.05).

Table 5: Association between cancer diagnosis and CD4+ count

	Univariate			**Multivariate**		
Diagnosis	**OR**	**95%CI**	**p**	**OR**	**95%CI**	**p**
OSCC	1.58	0.63-3.92	0.33	1.55	0.59-4.04	0.37
KS	0.19	0.08-0.49	0.00	0.15	0.07-0.48	0.00
NHL	0.25	0.10-0.60	0.00	0.27	0.11-0.69	0.01
Others	-	-	-	0.78	0.42-1.38	0.36

Multivariate regression analysis was adjusted for age and gender

Reference category CD4+T-cell count of less than 200

CHAPTER 5: DISCUSSION

This study was aimed at determining the types of head and neck cancer seen in patients infected with the human immunodeficiency virus. The demographic profile of the HIV-positive patients was determined and analysed as well as the confirmatory HIV blood test results, CD4+ T-cell count as well as the RNA viral load from the NHLS records.

5.1: Demographic profile

A total of 1605 cases of head and neck cancer were recorded at the WOHC between 2009 and 2013. Only (389 = 24.2%) of the total sampled was confirmed HIV- positive cases. The rest were either HIV- negative (14.3%) or unconfirmed (61.5%) cases, even after an extensive search from the NHLS database. HIV- infection was mainly found among Black South African patients (91.5%). The reasons for the excess number of cases with an unknown HIV status are multifactorial; one of them being the lack of proper medical details of patients provided by the clinicians when requesting for histological analysis of lesions. Some people also would rather go to the private sector or a public health facility outside of their respective provinces so as to remain anonymous and avoid stigmatization by the community (Snow *et al*, 2010). Another possible reason is that older men and women are less likely to go to clinics and get tested for HIV- infection and will only do so after they become symptomatic and have received a referral from a health professional (Sengayi *et al*, 2015). Socio-economic factors also play a big role as the level of education and proper knowledge about HIV-infection are important factors in so far as whether an individual is more likely to come forth and get tested or not (Vankatesh *et al*, 2011). Young men and women who completed high school or tertiary education usually seek HIV testing voluntarily because they had received

information about the virus before (Tabana *et al*, 2012). McPhail *et al*, (2009) also counted urban residence as an independent correlate for HIV testing among sexually experienced women.

More females (52.3%) were confirmed to be infected with HIV than males (47.7%) in this study. This high prevalence observed in women was the same as that reported in a couple of other cohort studies conducted here in South Africa by Sitas *et al*, (2000), Stein *et al*, (2008), Snow *et al*, (2010), Tabana *et al*, (2012) and Sengayi *et al*, (2015). One of the reasons for this has been said to be possible because women generally have a higher uptake of the free HIV counselling and testing (HCT) offered at state facilities, especially those of child-bearing age (15 – 49 years) (Tabana *et al*, 2012). Males on the other hand usually have very limited health care visits and therefore are not tested for HIV- infection as often as their female counterparts (MacPhail *et al*, 2009; Snow *et al*, 2010; Sengayi *et al*, 2015). McPhail *et al*, (2009) also suggests that men are more likely to present themselves for HIV testing only after developing symptoms associated with the disease.

The age group of subjects diagnosed with HIV- infection was mainly young adults, mainly between the ages 36 – 55 or younger than 35 years. In many other studies conducted here in South Africa, the age range is the same as the one observed in this study. Many reasons have been cited for this, for example; "a higher level of education, residing in an urban area vs. rural area and the fact that most females at this age range are in committed relationships and at child-bearing age and are frequently attending ante-natal clinics (ANC) and therefore are getting screened for HIV- infection regularly" (Peltzer *et al*, 2009; MacPhail *et al*, 2009). Poverty also plays an important role especially when it comes to young women contracting the disease because of sexual interaction with older men in exchange for monetary gain and also the fact that women are biologically more susceptible to HIV- infection (Pettifor *et al*, 2007; Williams *et al*, 2000).

Alcohol use and smoking were mostly unknown in this cohort, 97.6% and 79.4% respectively. This was mainly because of the missing information regarding smoking or alcohol consumption for the subjects on the histological request forms provided by respective clinicians and since this was a retrospective study, verification of the missing information was not possible. It is however a well-known fact that tobacco smoking and alcohol consumption is rife among HIV- infected individuals, because of the high levels of stress and depression experienced by these individuals after they find out about their status (Grulich AE, 2009). Kalichman *et al*, (2007) reported that alcohol ingestion was associated with a high risk of contracting HIV or sexually transmitted infections (STI) in South Africa because of the resultant risky sexual behaviour by these individuals. Men were found to drink more than their female partners leading to an increased risk for the women to contract sexually transmitted infections including HIV because of the risky behaviour (Kalichman *et al*, 2007).

5.2: Head and neck cancer diagnosis in relation to the HIV status of patients

More females were diagnosed with HNC in this cohort at 52.3% than males at 47.7%. The mean age of the subjects was 51.7 ± 16.4 years. Most of them were between ages 46 – ≥ 66. When the subjects diagnosed with HNC were grouped according to their HIV status; females showed a higher prevalence of HNC in the HIV- positive group than males. The HIV-negative and unknown groups showed a higher prevalence of HNC among males.

Kaposi sarcoma (35.7%), non-Hodgkin's lymphoma (34.2%) and oral squamous cell carcinoma (19.8%) were the most diagnosed cancers among HIV-positive cases in this cohort. The results of this current study are similar to results of other studies carried out previously in Sub-Saharan Africa, where elevated risks for KS and NHL were determined among individuals diagnosed with HIV (Sitas *et al*, 2000; Stein *et al*, 2000; Newton *et al*,

2000; Mbulaiteye *et al*, 2006). These types of cancers are often associated with co-infections of oncogenic viruses; human herpes virus (HHV-8) for KS and Epstein-Barr virus (EBV) for NHL (Baker *et al*, 2008). NADC's are mostly associated with lifestyle factors like smoking, alcohol consumption and related viral infections in HIV-infected people for example; human papilloma virus (HPV) for oropharyngeal squamous cell carcinoma and smoking habit/alcohol consumption for oral squamous cell carcinoma (Mbulaiteye *et al*, 2003).

5.2.1: Kaposi sarcoma (KS)

Kaposi sarcoma was common in sub-Saharan Africa and South Africa even before HIV was discovered, and to a lesser extent was also seen in the Mediterranean countries like Italy, Greece and the Middle East (Sitas *et al*, 2000). The incidence in South Africa increased almost threefold between 1988 and 1996 and continued to rise as the HIV epidemic was growing (Sitas *et al*, 2000). Nowadays, KS is strongly associated with HIV (Sasco *et al*, 2010). Studies conducted in South Africa and Rwanda found a definitive association between HIV infection and KS with Odds Ratios (ORs) ranging from 21.9 to 47.1 (Mbulaiteye *et al*, 2006). KS is one of the AIDS-defining-cancer, according to the 1993 Centers for Disease Control classification (CDCC).

In the current study KS was found more in females than males (42.4%: 28.2%), (OR = 130.2; 95% CI 16.6 – 1023.6). These results differ somewhat with results found in other studies in SA and Zimbabwe where the incidence of KS was higher among males than females (Sitas *et al*, 2000; Sasco *et al*, 2010). In a Ugandan record linkage study the incidence for KS among HIV infected individuals was found to be the same for both males and females (Mbulaiteye *et al*, 2006). The reasons for the difference in the incidence of KS and the gender of infected individuals is not fully understood as yet, especially because the prevalence of the causative

factor HHV-8, is the same in both males and females (Sitas *et al*, 2000). In sub-Saharan Africa, HHV-8 sero-prevalence is very high among the population and its prevalence increases the older the infected individual becomes, suggesting that infection with the virus in most of these individuals is not really a new one or sexually transmitted as is the case in the United States (Sitas *et al*, 2000). Mbulaiteye (2003) stated how this virus (HHV-8) is readily detected in saliva more than in genital secretions of infected people in Africa and suggested horizontal transmission of the virus through saliva between families. Anti-HHV-8 antibodies were also detected in infants of mothers confirmed to be HHV-8 seropositive in a study conducted in rural South Africa suggesting vertical transmission from mother to child as another reason for the high prevalence of the virus in Africa (Wilkinson *et al*, 1999). The low prevalence of HHV- 8 infection observed in white males with higher levels of education when compared to a high one observed among black males with lower levels of education in South Africa, also implicates poverty related factors as another reason for the high prevalence of the virus (Sitas *et al*, 2000). However, this is in complete contrast to the findings in Uganda where infection with HHV-8 is associated with better education and wealth among black males (Wabinga *et al*, 1993). He also found a low incidence (<7%) of KS among those also infected with HIV in the same study.

Kaposi sarcoma is strongly linked with immunodeficiency, the lower the CD4+ cell count, the higher the risk of developing KS, although the risk is still raised even in well preserved cell counts (Grulich AE, 2009). HHV-8 alone does not cause KS as immunosuppression in the particular individual is also necessary for carcinogenesis to occur (Mbulaiteye *et al*, 2008). Results from the current study showed that the odds (OR) of an HIV- positive individual being diagnosed with KS were increased by 0.19 with a CD4+ T-cell counts of > 200 cells/mm^3 compared to those with lower counts. A study done by Mosam *et al*, (2008) in KZN SA, supports this as their results showed that 21% of the patients diagnosed with KS

had CD4+ T-cell levels of >350 cells/mm³. The reason behind this is not clearly understood, but unlike in the USA where KS is associated with a declining CD4+ cell count, in Africa this is not necessarily true. Mosam *et al*, (2008) suggested that maybe a severe immune suppression is not really required for the development of KS in African populations infected with HIV because of the age at which the infection with HHV8 occurs. In 2007, Maurer *et al*. reported on nine HIV- positive cases in San Fransisco, who were on HAART with CD4+T-cell counts of >300 cells/mm³ that had persistent KS. The drug regimen they were taking consisted of a protease inhibitor, known to have antitumorigenic as well as antiangiogenic effects yet KS wasn't resolving. The high number of aging HIV- positive patient also infected with HHV-8 was suggested as another possible reason for this enigma in their cohort. In Sub-Saharan Africa HAART roll-out did not happen immediately after the discovery of HIV and AIDS and hence people infected with the virus did not have the 'opportunity' of developing KS as they did not live long enough (Mbulaiteye *et al*, 2006). Mbulaiteye *et al* (2003) also proposed that the contradicting evidence in regard to HHV-8 seropositivity and the incidence of KS that is experienced in Africa is possibly because of differences in the distribution of unknown infective cofactors or inadequate assay performances conducted.

After the introduction of HAART in resource-rich countries, KS cases declined immediately (Mbulaiteye *et al*, 2003). In South Africa, the prevalence of KS is still high even after the introduction of HAART in 2004 to HIV- positive people by the government as demonstrated in a few studies like those of Sitas *et al* in 2000 and Sasco *et al* in 2010. The results of the present study also support this as KS was the highest diagnosed cancers (35.7%) among HIV-infected people. The possible explanation for this increased incidence might be the fact that HIV infection is still a highly stigmatised disease in South Africa as well as other countries in Africa and patients are too scared to present themselves to clinics to receive HAART as they do not want people to know of their positive status or the type (protease inhibitor or

nucleoside analogue reverse transcriptase) of HAART regimen they are receiving. Another explanation might be due to immune reconstitution inflammatory syndrome (IRIS) following HAART as natural killer and cytotoxic T-lymphocyte activity increases (Bower *et al*, 2005). Only 33 out of 139 patients diagnosed with KS in this study were already on HAART as illustrated in Figure 11, we could not conclude whether this was IRIS- related KS or not as we failed to determine the exact time frame between the diagnosis of KS, CD4 T-cell count, viral load levels and the introduction of HAART in an individual. The type of HAART regimen the subjects were receiving could also not be determined.

5.2.2: Non-Hodgkin's Lymphoma (NHL)

NHL is the second most common cancer associated with HIV infection (ECC, 1999). The results of the current study also show that NHL was the second highest cancer diagnosed among HIV-infected cases (34.2%). Risk factors for development of NHL are poorly understood but absolute risk develops with age and it's higher in men and white people (Mbulaiteye *et al*, 2003). In people living with HIV the risk of developing NHL is even greater compared to that of the general public and it increases with the duration of infection (Mbulaiteye *et al*, 2003). NHL is generally associated with both congenital and medically related immunosuppression and hence the association with HIV (L. Dal Maso *et al*, 2001, Bower *et a*l, 2005). In a study conducted by Bower *et al*, (2005) three factors were identified that were significantly associated with the development of NHL namely; the age of the individual, CD4+ T-cell count and no prior received HAART, supporting the association of NHL with a suppressed immune system.

Similar findings have been confirmed in several other studies conducted in SA and sub-Saharan Africa before. Studies conducted by Sitas *et al*, (1997) and (2000) in Johannesburg,

South Africa found a strong association between HIV and NHL (OR = 5.0, 95%, CI = 2.7-9.5). Lymphoma cases (37%) studied in the Tygerberg area of the Western Cape, SA from 2002-2009 were reported to be related to HIV- infection, which was a significantly high increase of 6% in 2002 (Abayomi *et al,* 2011). Stein *et al* (2008) reported a six-fold higher risk of NHL in HIV infected individuals in Johannesburg, South Africa. Another increased risk for the development of NHL among HIV infected individuals was reported in a study conducted in central Africa (Newton *et al,* 2000). In Uganda, NHL standardized incident ratios (SIR) were increased 6.7 times (95% CI, 1.8-17) compared to the general population, in a HIV/AIDS-cancer match study (Mbulaiteye *et al,* 2006).

The risk for NHL in people infected with HIV was relatively low in Kampala, Uganda, which is a resource-poor area compared to the high risk demonstrated in resource-rich countries (Parkin *et al,* 2000). Engels *et al* (2006) reported the risk for NHL among people with AIDS in the US decreased by 57.5% (SIR, 53.2 during the period 1990-95 and 22.6 during 1996-2002; $P<0.0001$) compared to that of the general population. Mbulaiteye *et al* (2011) stated that the reasons for this difference in risk between Africa and the Western countries could be artefactual due to under-diagnosis, mortality or environmental factors experienced in resource-limited settings. Patients with severe immunosuppression usually die from other infectious diseases (tuberculosis and malaria) in Africa before they develop NHL, making this another possible reason for the low prevalence (Parkin *et al,* 2000; L. Dal Maso *et al,* 2001). EBV is usually acquired early in childhood in Africa resulting in a declined lymphoma risk following immunosuppression and reactivation of the virus in HIV-infected individuals later on in life, which is another possible reason behind the differences experienced between resource-rich and poor countries (L. Dal Maso *et al,* 2001).

NHL was diagnosed more in males than females in this study (37.3%:34.0%). In Western Europe NHL was reported to be highest among males, homosexual and bisexual men (L. Dal

Maso *et al*, 2001). It was also seen more in the ≤ 35 and 36 - 45 years age groups. Tanon *et al*, (2012) reported from a study conducted in West Africa that NHL was diagnosed at the median age of 41.5 years in HIV-positive cases; this is within the same age range as found in this study.

Of the 133 cases diagnosed with NHL, 25 were already on HAART. The OR for NHL diagnosis in a HIV-infected subject with a higher CD4+T-cell count (>200 cells) was increased by 0.25 in the current study. The reason behind the failure of HAART in decreasing the incidence of NHL in an African setting is not clearly understood. Casper (2011) conducted a review on the increasing burden of cancer in resource-poor countries and suggested; 1) the lack of sufficient HAART coverage in the population at risk for cancer, 2) the type of HAART regimen provided or available in resource-poor countries and 3) the higher prevalence of oncogenic viruses in these settings, as possible reasons for the reduced impact that HAART has had in decreasing rates of NHL and KS in Africa.

5.2.3: Oral Squamous Cell carcinoma (OSCC)

The EC-Clearinghouse classification in 1993 classified OSCC as one of the neoplasms strongly associated with HIV. Shiboski *et al* (2009) also included OSCC in their case definitions as one of the neoplasms strongly associated with HIV. The risk factors for developing OSCC among HIV infected individuals and the general population is the same though and hence OSCC was never considered an ADC because of this reason (D'Souza *et al*, 2014). OSCC was the third most common cancer diagnosed among HIV- positive people at 19.8% in this study, but there was no significant correlation between its diagnosis and CD4+T- cell count levels. It was diagnosed more among males (28.1%) than females (12.3%) and in older age groups of ≥45 years and above.

Pro-oncogenic virus-related and tobacco or alcohol-related cancers are common among HIV infected individuals because of the impaired immune surveillance as CD4+T- cell count becomes depleted (Baker *et al,* 2008, Silverberg *et al,* 2011, Franzetti *et al,* 2013). Recently an increase in HPV-related oropharyngeal cancers has been noted in AIDS cancer registry studies as well as cohort studies (Frisch *et al,* 2000, Clifford *et al,* 2005, Chartuvedi *et al,* 2008 and 2009). HPV-16, the oncogenic type of HPV family, is highly prevalent in HIV infected people at 2 – 7% (Beachler *et al,* 2012). HPV persistence is also increased among immunosuppressed individuals (D'Souza *et al,* 2014). There is also increased usage of tobacco and alcohol among HIV infected people because of the high stress levels these individuals are subjected to and also sexual risk factors making them more prone to develop oropharyngeal cancer (Silverberg *et al,* 2011, D'Souza *et al,* 2014). The fact that HIV infected people are now also living longer because of HAART implies that the risk for cancer development increases even more. Which one of these factors explains the risk increase exactly more than the other is still unclear (D'Souza *et al,* 2014).

In the current study it is not clear how many of the OSCC cases were HPV-related and whether all had a history of tobacco or alcohol usage due to missing information in the archives. Only 5.4% of the HIV-infected individuals had a confirmed history of smoking and only 0.8% of alcohol ingestion before diagnosis with HNC. Ninety-one point three percent and 98.7% of HIV- infected people had an unknown history of tobacco or alcohol usage respectively making it difficult to draw conclusions on how much of the OSCC cases were associated with the habits. Gilyoma *et al* (2015) reported a 76.6% association between HNC, HIV infection and a history of smoking and alcohol consumption in his cohort. HPV testing for oropharyngeal cancers is not done routinely in our hospital because of the cost implications, similar to Gilyoma *et al,* 2015 study and therefore, it is not reported on in our study.

5.3: Head and neck cancer diagnosis in relation to anatomical site predilection

The most involved site for HNC in HIV- positive individuals was the larynx (19.2%) followed by the palate (16.5%) and the tongue (15.7%). The oral cavity (14.7%) and the neck (14.1%) were not far behind. Only (5.4%) of the HIV- infected individuals had a confirmed history of smoking and only (0.8%) of alcohol ingestion before diagnosis with HNC. Thirty-seven percent of the cancers observed in Bugando medical centre, Tanzania, occurred in the oral cavity followed by the pharynx (16.2%) and the larynx (13.9%) in HIV- infected people between January 2009 and December 2013 (Gilyoma *et al,* 2015). The oral cavity was the most involved site in Gilyoma's study which differs slightly with our study, where the larynx was the most affected site. Amusa *et al,* (2004) also reported the oral cavity (36.8%) as the most common site for HNC in a study conducted in Nigeria. A study done in Sub-Saharan Africa in 2007 on the prevalence of HNC among HIV seropositive people found laryngeal cancers as the most common HNCs (31.3%), with (15.0%) of patients having a history of smoking and alcohol ingestion (Nwaorgu *et al,* 2007). SGTs were the most common cancers associated with HIV infection (40%) in their study, whereas in the current study only 1% of SGTs were found among HIV-positive patients. This is contrary to a study done by Tanon *et al* (2012) in West Africa where the oral cavity, pharynx and larynx only represented 9.7% of HNCs in HIV- infected people. Sitas *et al* (2000) in a study conducted in South Africa only found 5.4% of laryngeal cancers among HIV infected individuals. In Kenya, the larynx was reported as the most common site for HNC followed by the tongue (Onyango et al, 2006), similar to the findings of the current study.

There is a definite difference in the anatomical sites that exists from all studies that have been conducted in different regions, but the reasons for this are not clearly understood.

Geographical location as well as socio-cultural practices that exist among people from these regions might be some of the reasons for these differences (Gilyoma *et al,* 2015).

5.4: Head and neck cancer and HAART/ARV therapy

KS and NHL are known as ADCs and the risk and severity of the two cancers increases greatly as the CD4 cell count decreases and HIV infection progresses in an individual infected with the virus (Mbulaiteye *et al,* 2003). Silverberg *et al* (2015) reported HIV RNA levels to be closely associated with an increased risk of KS and NHL in a study conducted in California, but not for other type of cancers. It still remains unclear exactly how depressed immunity increases the risk for cancer development in HIV infected people (Barbaro *et al,* 2007). Besides immunosuppression, several other mechanisms are thought to be contributing to cancer development e.g. infection by oncogenic viruses and imbalance between cellular proliferation and differentiation (Barbaro *et al,* 2007). In the Western countries, the introduction of HAART resulted in significant decline in KS rates and a modest decline with NHL rates but not with other types of cancer (Int. Coll. Cancer and HIV, 2000). In resource-limited countries, KS and NHL rates are still very high even after the introduction of HAART (Mbulaiteye *et al,* 2006, Stein *et al,* 2008, Casper, 2011).

In this study KS and NHL were the two most common cancers to be diagnosed in people that have HIV infection (OR = 187.1, 95% CI: 24.5 – 1426.7; OR = 35.8, 95% CI: 12.8 – 100 respectively). The odds of a diagnosis of KS and NHL in HIV infected subjects with CD4 cell count > 200 cells/mm^3 was increased by 0.19 and 0.25 respectively when compared with people with CD4+T-cell count < 200 cells/mm^3. Higher levels of CD4 T-cell count have been reported previously in African studies as well (Morgan *et al,* 2000, Mosam *et al,* 2005 and

2008). This then supports the idea that immunosuppression in an African setting is not a prerequisite for the development of KS (Mosam *et al,* 2005).

5.5: Limitations of the study

- Some of the cases (61.4%) with a histologic diagnosis of head and neck cancer at the Oral Pathology Department, WOHC, did not have confirmed positive serologic results for HIV infection both on the departmental records and the NHLS archives and this made it difficult to draw conclusive correlations between the type of HNC diagnosed and the HIV status of the cases.
- Whether there was a time lag between the histologic diagnoses of the HNC and the tests/results for CD4+ T cell count and the RNA viral load or not remains unknown. This could not be verified due to a lack of information and proper record keeping and linkage from both of the utilised archives (Oral pathology and NHLS) in the study. Therefore, the correlations/associations between the time of HNC diagnoses and the patient's immune status and the level of infection might not be a true reflection.

5.6: Conclusion

The aim of the study was to identify and characterise the types of histologically diagnosed HNCs occurring among HIV-infected people. The results of the study indicated Kaposi sarcoma and Non-Hodgkin's lymphoma, both confirmed AIDS-defining cancers (ADCs), as the two most commonly occurring HNCs among HIV-infected cases. The third most common cancer seen among the HIV infected cases was oral squamous cell carcinoma, a non-AIDS defining cancer (NADC). Only 2.6% of the HIV-infected cases were diagnosed with Hodgkin's lymphoma and only 1.0% with salivary gland tumours, both NADCs. From this it is clear that the incidence of KS and NHL among HIV-positive people is still high, despite the roll-out of HAART in April 2004 by the South African government to people with CD4+T-cell counts of < 200 cells/mm^3.

A correlation was found between HIV infection, a CD4+ T-cell count of >200 cells/mm^3 and development of KS and NHL (OR of 0.19 and 0.25 respectively). Thirty-three of the HIV-positive patients diagnosed with KS and 25 diagnosed with NHL were already receiving HAART. Whether this could be attributed to the effects of a possible immune reconstitution inflammatory syndrome (IRIS) or HAART failure is unclear. The type of HAART regimen and the level of infection (CD4+T-cell count and RNA viral load count) of the patients at the time when they started with HAART could not be verified leading to inconclusive results. More studies need to be conducted here in SA to determine why KS and NHL incidence is not decreasing in our country despite HAART being freely available to HIV- positive people from government institutions. Better surveillance systems as well as population-based cancer registries are vital for this to happen and should be integrated with established HIV programs in South Africa and sub-Saharan Africa as a whole. This will assist tremendously with future studies in this particular field and will result in proper coordinated approach and improve the

quality of services aimed at controlling and reducing the ever increasing number of cancer cases and resultant deaths among HIV-infected people.

Sub-Saharan Africa still has the highest number of people infected with HIV and South Africa still experiences high numbers of new infections yearly, especially among female teenagers. This is affecting the economic growth of the country negatively because of high morbidity and mortality rates among the working class resulting in skill shortages and job losses. This also places a huge burden on the health systems of the country, and hence the need for improved disease control and management thereof.

In June of 2015 a decision was announced during the 7th South African AIDS conference held in the city of Durban, to start HAART immediately in people diagnosed with HIV infection, irrespective of the CD4+T-cell count. This new policy was implemented in September 2016 (UNAIDS, 2016). This is aimed at improving the quality of life for HIV-infected people and hopefully decrease the morbidity and mortality rates that the country is experiencing. Prospective studies should be conducted to determine whether this new initiative will reduce the high incidence rates of ADCs as well as NADCs among HIV-positive people in the country and whether the quality of life is improved as expected or not.

A number of research studies have been and are currently being conducted worldwide to try and develop a vaccine against the human immunodeficiency virus (HIV), to assist in curbing the number of new infections still occurring to this day. An HIV vaccine trial study was announced to be initiated in South Africa and is to be funded by the National Institute of Allergy and Infectious Diseases (NIAID) and its partners. This clinical trial (HVTN 702) will be conducted by the HIV Vaccine Trials Network (HVTN). This is to determine if the vaccine is effective in preventing HIV infection among South African adults or not and whether it is safe and tolerated by the participants involved in the study. The results are

expected in 2020. If successful, this will decrease the burden of HIV disease in populations with high rates of HIV infections like ours in South Africa (NIH news, 2016). This vaccine study will thus be beneficial for future prospective studies to assess the impact of its use in decreasing the number of new HIV infections and the manifestations thereof.

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APPENDICES

Appendix A

Number	Site of involvement	Diagnosis including the date	Age	Gender	Ethnicity	HIV status	CD4+Tcell count + date	Viral load + date	Histological grading of tumour	Smoking and Alcohol
1										
2.										
3										
4										
5										
6										
7										
8										
9										
10										
11										

Appendix B

R14/49 Dr Nompumelelo B Zwane

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M140655

NAME: (Principal Investigator)	Dr Nompumelelo B Zwane
DEPARTMENT:	Oral Medicine and Periodontology Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	Head and Neck Cancers among HIV Positive Patients: 5 Year Retrospective Study (2009-2013)
DATE CONSIDERED:	27/06/2014
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof SL Shangase
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	30/06/2014

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

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 resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature

Date 15/07/2014

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix C

Department of Oral Pathology
School of Oral Health Sciences
Faculty of Health Sciences
3E22, 3rd floor, Wits Medical School
7 York Road, PARKTOWN, 2193
Private Bag 3, Wits 2050, South Africa
Tel: 0117172139/97
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Email: HOD: Sizakele.Ngwenya@wits.ac.za
Secretary: Phindile.Mashinini@wits.ac.za

4 June 2014

Human Research Ethics Committee (Medical)
Research Office
Faculty of Health Sciences

Applications for HREC (Medical) Ethics Clearance

Dear Sir/Madam

I, **Sizakele Ngwenya** in my capacity as **Head of the Department of Oral Pathology** grant **Dr NB Zwane** access to the department's archived material to conduct research in partial fulfilment towards the MSc(Dent) Periodontology degree, for her study entitled: **Head and Neck cancers amongst HIV positive patients: a 5 year retrospective study** .

Yours Sincerely,

Dr SP NGWENYA
HOD: ORAL PATHOLOGY

Appendix D

NATIONAL HEALTH LABORATORY SERVICE

School of Pathology

University of the Witwatersrand, Johannesburg and National Health Laboratory Service

Tel: +27 11 489 8413 **Fax:** 27 86 602 6416 **Address:** 7 York Road, Parktown

5 June 2014

Professor Cleaton-Jones
Chair: Wits HREC
Wits University

Dear Professor Cleaton-Jones

MSc research study : Dr Zwane

PROTOCOL : Head and Neck cancers amongst HIV positive patients: a 5 year retrospective study.

This is to confirm that Dr Zwane has my permission to access patient data in the relevant NHLS Laboratory division to perform the above named study for her MSc research. This study should be done in consultation and collaboration with the relevant divisional head in the NHLS. Please do not hesitate to contact me if you need further information in this regard.

Yours sincerely

Professor J Mahlangu

Head: School of Pathology, Faculty of Health Sciences

University of the Witwatersrand and National Health Laboratory Service

Pathology, the only true pathway to medical understanding

Appendix E

NATIONAL HEALTH LABORATORY SERVICE

Academic Affairs and Research
Modderfontein Road, Sandringham, 2031
Tel: +27 (0)11 386 6142
Fax: +27 (0)11 386 6296
Email: babatyi.kgokong@nhls.ac.za
Web: www.nhls.ac.za

17 December 2015

Applicant: Dr Nompumelelo B Zwane
Institution: University of the Witwatersrand
Department: Oral Medicine & Periodontology
Email: Nompumelelo.zwane@wits.ac.za
Cell: 011 488 4139

Re: Approval to access National Health Laboratory Service (NHLS) Data

Your application to undertake a research project of "**Head and Neck cancers amongst HIV positive patients**" using data from the NHLS database has been reviewed. This letter serves to advise that the application has been approved and the required data will be made available to you to conduct the proposed study as outlined in the submitted application.

Please note that the approval is granted on your compliance with the NHLS conditions of service and that the study can only be undertaken provided that the following conditions have been met.

- Processes are discussed with the relevant NHLS departments and are agreed upon.
- Confidentiality is maintained at participant and institutional level and there is no disclosure of personal information or confidential information as described by the NHLS policy.
- A final report of the research study and any published paper resulting from this study are submitted and addressed to the NHLS Academic Affairs and Research office and the NHLS has been acknowledged appropriately.

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research. Any data related queries may be directed to Sue Candy, manager NHLS Corporate Data Warehouse, Tel: (011) 386 6036. Email: sue.candy@nhls.ac.za.

Yours sincerely,

Dr Babatyi Malope-Kgokong
National Manager: Academic Affairs and Research