AN AUDIT OF ORAL LESIONS ASSOCIATED WITH HPV INFECTION IN THE ORAL PATHOLOGY DEPARTMENT, WITS ORAL HEALTH CENTRE

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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

Johannesburg, 13th March 2020

DECLARATION

I, Sagwati Golele, declare that this research report is my own, unaided work. It is being submitted for the Degree of Master Science in Dentistry (MSc Dent) in Oral Medicine and Periodontology at the University of Witwatersrand, Johannesburg. Neither the whole work nor any part of it has been submitted for any degree or examination in this or any other University.

SGolele

Signature of candidate

13th day of March 2020 in Parktown, Johannesburg

DEDICATION

I dedicate this work to my family and those close to me who encouraged me to push through until the end, thank you for all your prayers. I dedicate all my work to God.

ABSTRACT

Human papillomavirus (HPV) is a DNA tumour virus that has been increasingly seen in both healthy and immunocompromised individuals. This virus belongs to the papillomaviridae family that infects epithelial cells exclusively. The clinical presentation and microscopic features of HPV-associated oral lesions vary depending on the specific genotype and the anatomical site involved.

Aim: The study aimed to report on the oral lesions associated with HPV infection, histologically diagnosed in the Department of Oral Pathology at Wits Oral Health Centre (WOHC).

Methods: The study population comprised of 128 patient records of HPV associated oral lesions that were histologically diagnosed in the Oral Pathology Department. A descriptive analysis of the data was carried out.

Results: The results showed a strong association of HPV-associated oral lesions with age and HIV-status than with gender. The lesions were of a low risk HPV subtypes and focal epithelial hyperplasia was the most commonly occurring lesion.

Conclusion: In light of the results from this study, it was evident that there was a strong association between occurrence of HPV-associated oral lesions with age and HIV-status of patient. In a country where HIV prevalence is alarmingly high, studies looking at association of HPV occurrence, HIV infection and demographics of the population needed to be investigated.

ACKNOWLEDGEMENTS

I would like to acknowledge Prof Sindisiwe Shangase and Dr. Nompumelelo Zwane my supervisors for the support, input and guidance during the process of conducting this study.

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LIST OF ACRONYMS AND ABBREVIATIONS

ART	Antiretroviral Therapy
FEH	Focal epithelial hyperplasia
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HNSCC	Head and neck squamous cell carcinoma
HR-HPV	High risk HPV
LR-HPV	Low risk HPV
HPV-OL	HPV-associated oral lesions
RRP	Recurrent Respiratory Papillomatosis
WHO	World Health Organization
WOHC	Wits Oral Health Centre

1. CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

1.1. Background

Human papillomavirus (HPV) is a DNA virus that is increasingly seen in both healthy and immunocompromised individuals. This virus belongs to the papillomaviridae family and infects epithelial cells exclusively. (Feller *et al,* 2009). HPV infection is highly transmissible and oral HPV infection can be acquired in various ways including oro-genital contact, mouth-to-mouth, autoinoculation or in infants by mother-to-child transmission. (Longworth *et al,* 2004). The clinical presentation and microscopic features of HPV-associated oral lesions vary with anatomical site and the specific genotype involved. Numerous variables contribute to the clinical manifestations and microscopic features, and these include host immune response, viral genetic variables, phenotype of infected epithelial cell against the backdrop of different lifestyles and environments. (Martin *et al,* 2005). The oral cavity is a good indicator for progression of HIV disease since oral lesions like hairy leukoplakia and oral candidiasis might be the first presentation amongst the HIV positive patients (Shiboski *et al,* 2009). A decline in opportunistic infections including oral lesions has however been observed in resource-rich countries since the introduction of antiretroviral therapy (Engels EA, 2009, Cobucci *et al,* 2014). The incidence of HPV oral warts has not declined despite the use of active antiretroviral therapy (HAART) (Cameron *et al,* 2008), suggesting that some oral conditions may occur as part of the immune reconstitution inflammatory syndrome (IRIS) following antiretroviral therapy (ART) (Shiboski *et al,* 2009). Sero-positive patients frequently have subclinical oral HPV infection, or overt HPV-related lesions in the form of either squamous papilloma, or common wart or condyloma acuminatum; collectively known as oral warts (Shetty *et al,* 2005). HPV is aetiologic for the development of cervical, anogenital and oropharyngeal cancer, with an increasing incidence of HPV-associated oropharyngeal cancers

amongst individuals with and without HIV infection (Muller *et al,* 2012). The various genotypes within HPV are grouped according to their predilection to affect certain areas, such as cutaneous HPV genotypes that infect the hands and feet resulting in common and plantar warts, and HPV genotypes that typically affect the ano-genital and oral mucosa resulting in benign condylomas, papilloma's or malignant diseases (Cameron *et al,* 2008). The distinction between benign and malignant HPV infection has prompted a classification that is more specific to the encountered HPV subtypes, into low and high risk (Muller *et al,* 2015) (Table 1.1).

In the oral cavity HPV subtypes commonly encountered are of the low risk category. An association between age and HPV infection has been ascertained, where the virus is more likely to occur in patients less than 50 years of age (Muller *et al,* 2015).

Table 1:1: HPV genotypes and their associated diseases

Diseases and anatomical sites	HPV genotype	References
1. Benign oral lesions		
1.1. Oral squamous cell papilloma	HPV types 6 and 11	[16, 17]
1.2. Veruca vulgaris (common wart)	HPV types 1, 2, 4, 7 and 57	[16, 17]
1.3. Condyloma acuminatum	HPV types 2, 6, 11 (and less frequently HPV types 16, 18, 31, 33 and 35	[2, 16, 17, 18]
1.4. Focal epithelial hyperplasia (Heck's disease)	HPV types 13 and 32	[2, 16, 17, 18]
2. Potentially malignant oral lesions		
2.1. Leukoplakia	HPV types 16 and 18	[27, 28, 29]
	HPV types 6 and 11	[25, 28]
2.2. Erythroplakia	HPV types 6, 11, 18, 31 and 33	[25, 28]
3. Oral and oropharyngeal squamous cell carcinoma	HPV types 16 and 18	[14, 15, 16, 17, 18]
4. Recurrent respiratory papillomatosis	HPV types 6 and 11	[2, 16, 17, 18]
5. Anogenital		
5.1. Condyloma acuminate	HPV types 6 and 11	[2, 13]
5.2. Intraepithelial neoplasia		
5.2.1. Low-grade	HPV types 6 and 11 (less frequently HPV types 16, 18, 31, 33, 35)	[2, 13]
5.2.2. High-grade	HPV types 16 and 18 (less frequently HPV types 6, 11, 31, 35)	[2, 13]
5.3. Squamous cell carcinoma	HPV types 16 and 18 (less frequently 31, 33, 35, 39, 45, 51, 52, 58)	[2, 6, 13]
6. Cutaneous		
6.1. Common warts	HPV types 1 and 2	[2]
6.2. Flat warts	HPV types 3 and 10	[2]

Some HPV-associated oral lesions occurring in adults with or without HIV infection have been associated with risky sexual behaviours like lifetime sexual partners, sexual orientation, oral sex practices and unprotected sexual practices. A study explored the prevalence of oral HPV infection associated with oral sexual behaviour and ascertained that there were strong associations between risky sexual behaviour and HPV 16 related oropharyngeal squamous cell carcinomas (D'Souza *et al*, 2009).

Oral HPV related lesions present and manifest in various forms intra-orally; and include squamous papilloma, verruca vulgaris, condyloma acuminatum and focal epithelial hyperplasia. These lesions can be categorised into two groups based on their biologic behaviour. The first group includes the above-mentioned oral lesions which commonly contain HPV DNA and exhibit characteristic HPV-induced cytopathic effects, with the exception of squamous papilloma which rarely exhibits these viral-associated changes (Garlick *et al,* 1991). The second group includes potentially malignant and malignant lesions such as squamous cell carcinoma and leukoplakia however the aetiological role of HPV is still not clear (Garlick *et al,* 1991).

The squamous cell papilloma usually presents as a solitary lesion with numerous finger-like projections of either parakeratinized or orthrokeratinized squamous epithelium surrounding a connective tissue central core. (Garlick *et al,* 1991). If excessive keratinization is present, the lesion appears white in colour and if there is less keratinization it appears raspberry and/ or pink in colour. (Prabhu *et al,* 2013). It affects both males and females equally and commonly occurs on the tongue, soft palate and the lips, although there may be other sites that are involved. It is an exophytic papillary lesion that is painless, soft and usually pedunculated (Neville and Damm, 2009). These lesions are usually 1cm in size and may be clinically indistinguishable from verruca vulgaris (Eversole *et al,* 1998). Surgical excision is the treatment of choice, with no recurrence. The HPV types that are implicated in squamous papilloma are 6 and 11. (Prabhu *et al*, 2013).

Similarly, Verruca vulgaris (VV) is a benign proliferation of the stratified squamous epithelial cells, also known as the common wart. It appears as a painless, papular or nodular projection that is either sessile or pedunculated with a white, pink or yellow colour. It is contagious and can spread to other sites of the same individual via auto-inoculation or to other individuals through physical contact with the infected person. Verruca vulgaris as a skin lesion is commonly found on the hands, feet, eyelids, and mucocutaneous surfaces of the genito-anal area (Scully *et al,* 1985); whilst intra-orally they have a predilection for the vermillion border of the lips, labial mucosa or anterior aspect of the tongue. Unlike the squamous papilloma, this lesion enlarges rapidly and then plateaus (Neville and Damm, 2009). These lesions commonly occur in children and examination of other sites should be conducted as oral lesions are usually due to autoinoculation. Recurrence of VV is uncommon and treatment is by surgical excision. HPV subtypes associated with squamous papilloma are 2 and 4 (Prabhu *et al*, 2013).

Condyloma acuminatum, commonly known as the venereal wart is a proliferated stratified epithelium of the genitalia, larynx, perianal region and mouth. This particular HPV associated lesion is considered to be a sexually transmitted infection (STI), appearing at the site of sexual contact or trauma, it is also often seen in HIV positive individuals (Reznik D.A, 2006). Presence of this lesion in children can also be due to sexual abuse. (Davis *et al,* 1989). These lesions are usually sessile, well-circumscribed, pink, exophytic masses with blunt projections on the surfaces (Neville and Damm, 2009). Mild to moderate dysplasia has been seen in this type of lesion (Garlick *et al*, 1991). HPV types 6, 11, and 16 are found in condyloma lesions (Eversole L.R, 2000).

Focal epithelial hyperplasia (FEH), also known as Heck's disease, is a localized proliferation of oral squamous epithelium. It is primarily a childhood condition but occasionally affects young and middle-aged adults. FEH appears as multiple soft, non-tender, flattened or rounded papules, which are usually clustered, same colour as the normal mucosa, but may also be scattered, pale or rarely white in colour. Occasionally the lesions may show a slight papillary surface change (Neville and

Damm, 2009). FEH is classified as one of the oral lesions less commonly associated with HIV-infection (EC-Clearinghouse and WHO on oral manifestations of HIV, 1993). Any part of the mucosa may be affected; however, the most commonly affected sites are the lower labial mucosa, buccal mucosa, labial commissures, upper labial mucosa, tongue, gingivae, alveolar mucosa and palatal mucosa (Jaramillo *et al*, 1991). The implicated HPV types in FEH are 13 and 32.

There are three major causes that have been implicated in head and neck squamous cell carcinomas (HNSCC), namely: tobacco, alcohol and HPV infection. (D'Souza *et al,* 2007). A study on oral oncology conducted in North America investigated the incidence and risk factors of HPV- related HNSCC in sero-positive individuals (Chartuvedi *et al,* 2007, 2011). The study showed a decline in HNSCC over the past couple of decades due to the reduction in tobacco use amongst individuals however; the incidence of HPV infection seemed to increase over the same period (Chartuvedi *et al*, 2007 & 2011).

Whilst HPV infection can be sexually transmitted, it can also be transmitted non-sexually through perinatal vertical transmission, physical contact, iatrogenic infection and autoinoculation.

A form of HPV known as recurrent respiratory papillomatosis (RRP) is transmitted from mother to foetus via vertical transmission if the birth canal is infected (Szydlowski. *et al,* 2014).

HPV infections may recur following treatment, however some spontaneously resolve without intervention. There are numerous ways to treat oral lesions associated with HPV infection including surgical excision by means of a scalpel or laser. Results of a study that investigated healing and relapse of HPV-associated oral lesions after the use of diode laser, reported that 80% of infections were transient, asymptomatic and resolved spontaneously, which supported the hypothesis that in the natural history of the condition, equilibrium is established between virus and host (Angiero *et al*, 2015). Further, a majority of patients treated using lasers reported no pain 24 hours' post-surgery. Squamous papilloma was the most commonly seen in the study. (Angiero *et*

al, 2015). The natural history of oral HPV is not well documented and the data around it is limited (Beachler *et al,* 2013). Other studies do however show that infections tend to resolve within two years.

Whilst a few studies on HPV have been carried out in South Africa, none have reported on the prevalence of HPV-associated oral lesions in a South African setting.

1.2. Aim and objectives

The aim of this study was to report on the oral lesions associated with HPV infection histologically diagnosed in the Department of Oral Pathology at Wits Oral Health Centre (WOHC). The objectives of this study were:

i. To present the demographic characteristics of the patients with oral HPV lesions
ii. To determine the frequency of occurrence of oral HPV-associated lesions at the Wits Oral Health Centre (WOHC)
iii. To determine the types of HPV-associated oral lesions histologically diagnosed at WOHC-Oral Pathology department
iv. To determine whether a relationship exists between HPV-associated oral lesions and HIV infection
v. To determine the relationship between types of oral HPV-associated lesions and patient demographics

2. CHAPTER TWO: METHODOLOGY

2.1. Study design

This was a records-based retrospective study.

2.2. Study sample and population

The sample size consisted of all the patient records in the Oral Pathology department with a histological diagnosis of HPV associated oral lesions from a period of 2007-2017

2.2.1. Inclusion criteria

- Histologically diagnosed oral lesions associated with HPV infection

2.2.2. Exclusion criteria

- Oral lesions not associated with HPV infection
- Extra- oral lesions associated with HPV infection
- Oral lesions beyond the soft palate

2.3. Study instrument

A data collection sheet was designed and used to collect all the relevant information from the patient records such as; age, gender, HIV- status etc. (Appendix A)

2.4. Procedure

Data was collected from the WOHC Oral Pathology patient records database dating from 2007 to 2017. The search was limited to histologically diagnosed HPV associated oral lesions, inclusive of squamous papilloma, verruca vulgaris, focal epithelial hyperplasia and condyloma acuminatum. The case definitions for these lesions were based on histological diagnostic criteria according to Neville and Damm, 2009 as follows:

- **Squamous papilloma**
    - Proliferation of keratinized stratified squamous epithelium arrayed in fingerlike projections with fibro-vascular connective tissue cores
    - keratin layer thickened in lesions with a whiter clinical appearance
- **Verruca vulgaris**
    - Proliferation of hyperkeratotic stratified squamous epithelium arranged into fingerlike or pointed projections with connective tissue cores.
    - Elongated rete ridges converging towards the center of lesion (“cupping” effect)
    - Hypergranulosis exhibiting coarse, clumped keratohyaline granules
    - Koilocytes in the superficial spinous layer
    - Eosinophilic intranuclear viral inclusions within cells of granular layer
- **Focal epithelial hyperplasia (Heck’s disease)**
    - Acanthosis of the oral epithelium (hallmark of FEH)
    - Widened, often confluent sometimes club shaped rete ridges
    - Superficial keratinocytes show a koilocytic change
    - Virus-like particles within both the cytoplasm and the nuclei of cells within the prickle cell layer
- **Condyloma acuminatum**
    - Benign proliferation of acanthotic stratified squamous epithelium with mildly keratotic papillary surface projections
    - Mature and differentiated covering epithelium

- Prickle cells demonstrate pyknotic, crinkled nuclei surrounded by clear zones (Koilocytes)

From these records, the following data were extracted and recorded in a structured data collection sheet: age, gender, race, HIV-status, site affected, size and colour of lesion and HAART treatment. Additional information was sought from records in the NHLS and Oral Medicine files where necessary.

2.5. Data management and analysis

The data was cleaned and transported to an Excel spreadsheet. The statistical tests used to analyse the data according to the specific objectives as presented in Table 2. All statistical tests were done using the IBM SPSS statistics 25.

Table 2:1: Data analysis per specific objective

Objective	**Variables**	**Statistical analysis**
To present the demographic characteristics of the patients	Continuous variable: Age Categorical variable: Gender	Descriptive statistics of mean and standard deviation was used to summarize the age of the patients. Frequency and percentage was used to summarize the gender of the patients.
To determine the frequency of HPV-associated oral lesions at the WOHC	Categorical variable: Prevalence of HPV-associated oral lesions	Frequency and percentage was used to summarize the data.

To determine the types of oral lesions associated with HPV infection seen at WOHC	Categorical variable: Types of HPV-associated oral lesions	Frequency and percentage was used to summarize the data.
To determine whether a relationship exists between HPV infection and the patient's HIV status	Categorical variable: Presence of oral lesions associated with HPV infection and HIV	Chi-Square test was used to determine the association between categorical variables of HPV and HIV.
To determine the relationship between HPV and patient demographics	Categorical variable: HPV, Gender Continuous variable: Age	Chi-Square test was used to determine the association between categorical variables of HPV and gender Student T-test was used to determine the significant difference in the age of patients with oral HPV.

The level of significance was set at $p<0.05$

2.6. Ethical considerations

- Ethical clearance for the study was granted by the Human Research Ethics Committee (HREC) of the University of Witwatersrand (M181010).
- Permission to conduct this study was received from the Wits Oral Health Centre.

- Permission to access the information on patient records pertaining to this topic was granted by the Departments of Oral Medicine, Oral Pathology-WOHC, Maxillofacial and Oral Surgery as well as NHLS.
- A reference number was allocated to each patient record to protect their identities.

3. CHAPTER THREE: RESULTS

3.1. Test for normality

A total of 128 cases found on the archives met the inclusion criteria for this study. Test for normality was done using the Shapiro-Wilk test for continuous variables (age and size – volume and area) (Table 3.1).

Table 3:1: Test for normality

Tests of Normality			
	Shapiro-Wilk		
Variables	**Statistic**	**Df**	**Sig.**
Age	0.882	125	0.00
Size volume	0.741	125	0.00
Size area	0.894	125	0.00

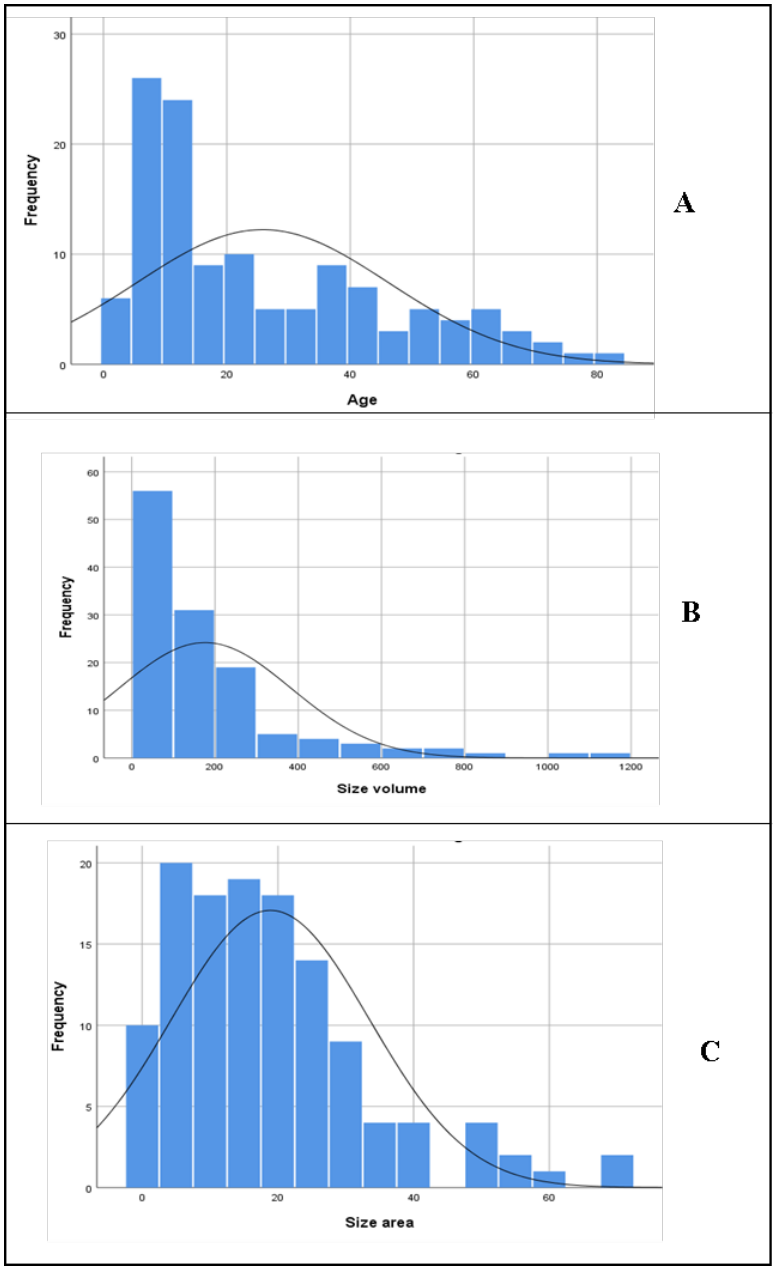

Figure 3:1: Histogram chart – test of normality

Continuous variables (age, volume and area) were not normally distributed, using the Shapiro-Wilk test; p-value was < 0.05. Figure 3.1 represents the histogram of the normality curve. Therefore, non-parametric statistical tests were used to analyse the data (Table 3.2).

Table 3:2: Data analysis matrix

Variables		
To present the demographic characteristics of the patients		
Demographics	Data normality	Statistical test
Gender	Not applicable	Frequency and percentage
Age	Skewed data	Median and interquartile range
HIV status and HAART	Not applicable	Frequency and percentage
To determine the prevalence and incidence of HPV associated oral lesions and evaluate the types at the WOHC-Oral Medicine clinic.		
HPV	Data normality	Statistical test
Diagnosis, site of lesion, colour, number, pedunculated	Not applicable	Frequency and percentage
Size and volume	Skewed data	Median and interquartile range
To determine the relationship between HPV associated oral lesions and HIV		
Association between diagnosis and HIV	Not applicable	Fischer's exact test and binary logistic regression test Level of significance set at $p<0.05$
Measure of significant difference in the size of the lesion according to diagnosis	Area and volume are skewed	Kruskal Wallis – H test Level of significance set at $p<0.05$
To determine the association relationship between HPV and patient demographics		
Association between diagnosis and gender	Not applicable	Fischer's exact test Level of significance set at $p<0.05$
Measure of significant difference in the age of the participants according to diagnosis	Age is skewed	Kruskal Wallis – H test Level of significance set at $p<0.05$

3.2. Demographic characteristics of the patients

A total of 128 cases of oral HPV were diagnosed at the WOHC-Oral Pathology between 2007 and 2017, the median age of the patients was 17 years (IQR: 9 – 39.5). Majority of the patients were aged ≤ 19 years old (Table 3.3).

Table 3:3: Age distribution (n=125)

Age in years	N	%
2-9	32	25.6
10-19	33	26.4
20-29	15	12
30-39	14	11.2
40-49	10	8
50-59	9	7.2
60-81	12	9.6

As shown in Figure 3.2 below, majority (58%, n=74) of the patients were females.

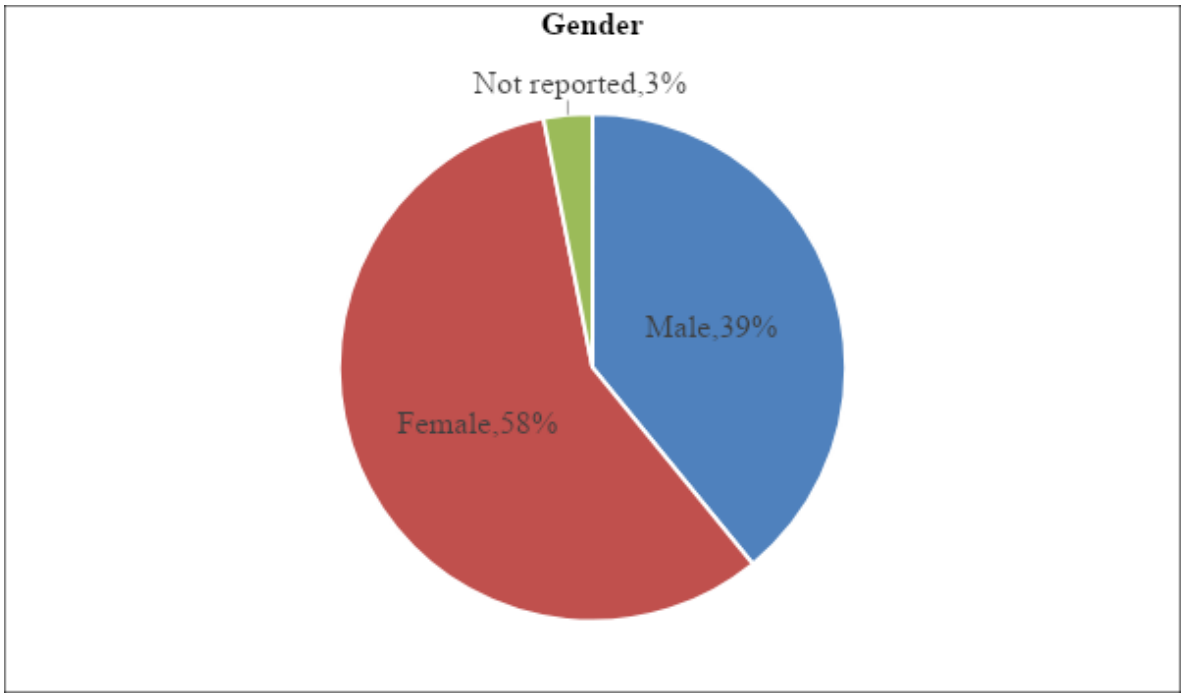

Figure 3:2: Gender distribution of the patients (n=128)

3.3. HIV status of the cases recorded

Of the 128 cases of HPV associated oral lesions diagnosed at WOHC-Oral Pathology department, 21% of them were confirmed HIV positive whilst the HIV status of the remaining cases was not reported as illustrated in Figure 3.3.

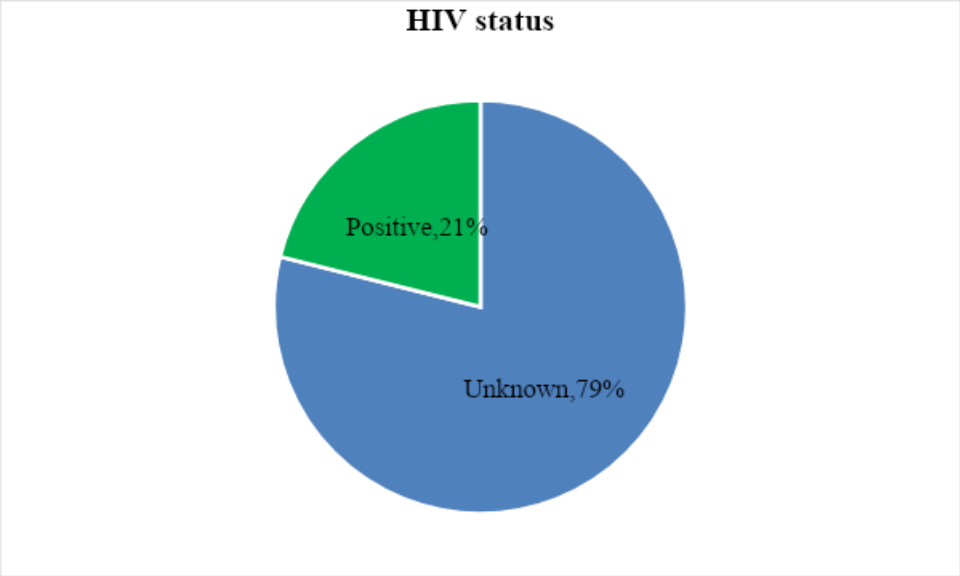

Figure 3:3: HIV status of the patients (n=128)

Of the 27 cases living with HIV, majority (66.67%) were already on antiretroviral therapy as shown in Figure 3.4. Only 3.7% reported as not receiving HAART yet. HAART status was unknown for 29.63% of the HIV - infected patients.

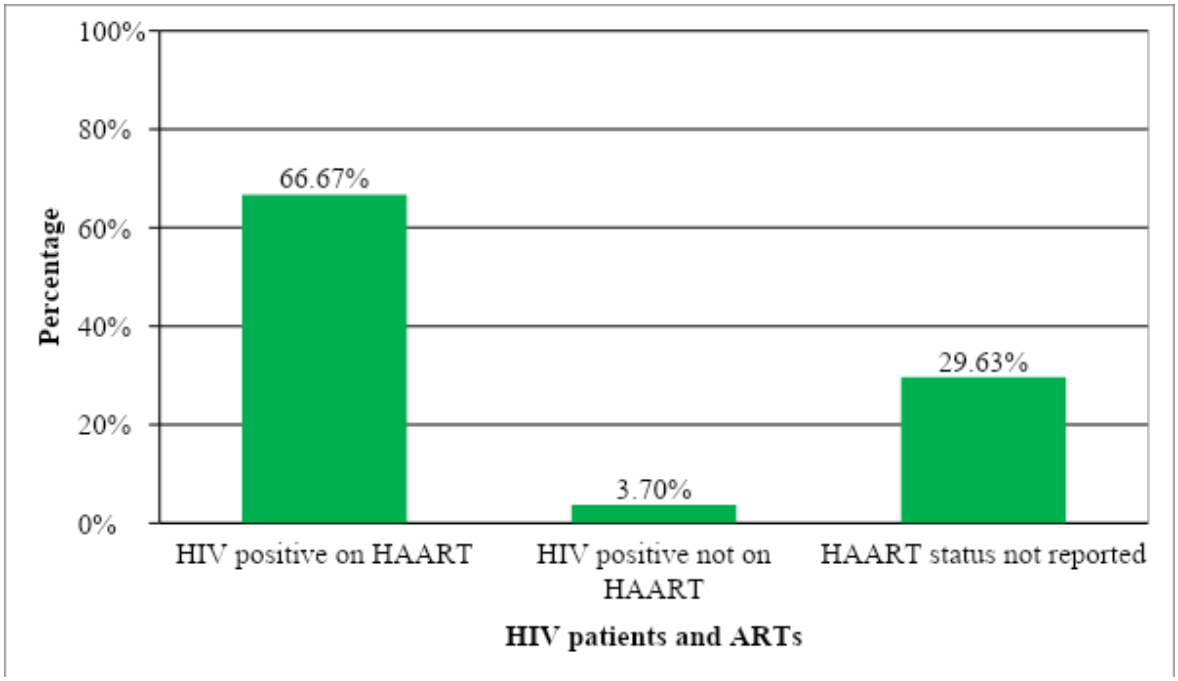

Figure 3:4: Patients with HIV on HAART (n=27)

3.4. Incidence and diagnosis of Oral HPV

A total of 128 cases of oral HPV was recorded and managed at the WOHC, South Africa with two cases documented as reoccurring. Therefore, the incidence rate of oral HPV between 2007 and 2017 was 12.8 per 1000 patients, based on the records from the Oral Pathology department. The most common diagnosis of the oral HPV was FEH: Heck's disease and squamous papilloma; diagnosed in 64.06% and 32.81% of the patients respectively (Figure 3.5).

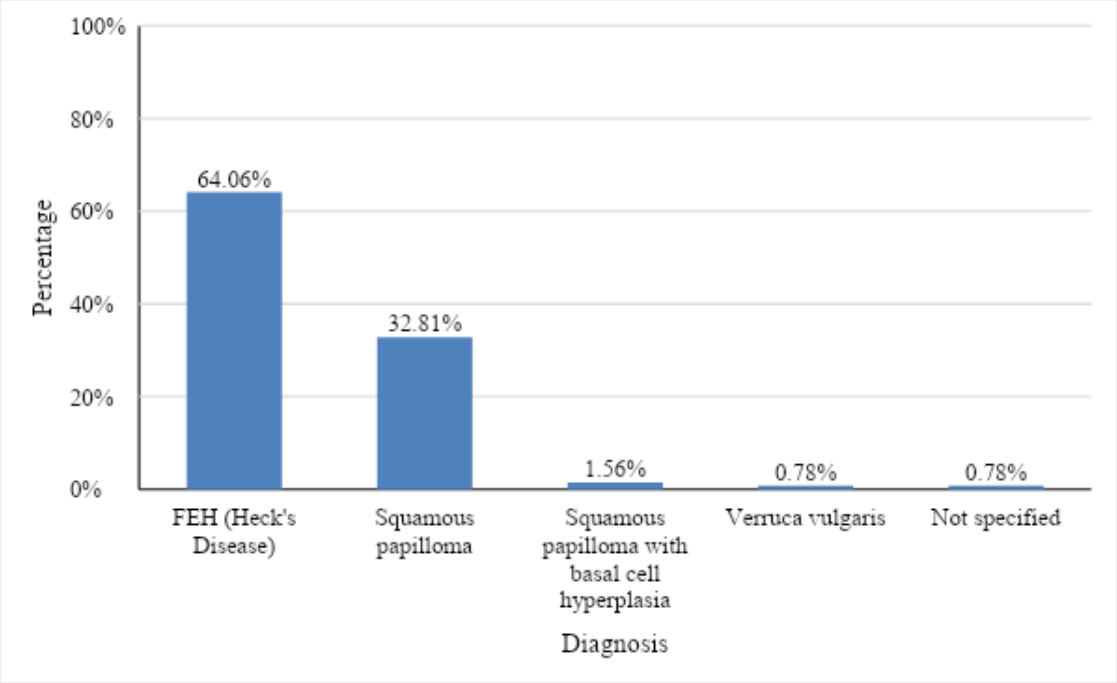

Figure 3:5: HPV diagnosis among the participants (n=128)

3.5. Site of the HPV associated lesion

The most common sites affected by HPV associated oral lesions per case were the labial mucosa (n=54), buccal mucosa (n=44) and the tongue (n=42) as outlined in **Table 3.4** below.

Table 3:4: Site of lesion

Site	**N**
Labial Mucosa	54
Buccal Mucosa	44
Tongue	42
Palate	19
Gingiva	9
Not specified	6
Floor of mouth	5
Lip Vermillion	1
Sulcus	1
Retromolar	1

3.6. Size of the lesion

The size of the lesion was presented in area and volume, the median volume and area of the lesion irrespective of diagnosis was 105 mm^3 (IQR: 42.5 – 216) and 15 mm^2 (IQR: 8-24) respectively (Table 3.5).

Table 3:5: Size of lesion (n=128)

		Interquartile range	
Size of lesion	**Median**	**25%**	**75%**
Volume (mm^3)	105	42.50	216
Area (mm^2)	15	8	24

Using Kruskal-Wallis H test, there was no significant difference between the size of the lesion and the specific diagnoses of HPV associated lesion, (p-value = 0.63) as illustrated in Table 3.6.

Table 3:6: Measure of significance between specific diagnosis and size of the lesion

Diagnosis	**Volume, Median (25 - 75% IQR) mm^3**	**Kruskal-Wallis H**	**p-value**
FEH (Heck's Disease)	105 (48 - 216)	1.75	0.63
Squamous papilloma	130 (40 - 280)		
Squamous papilloma with basal cell hyperplasia	200 (200 - 200)		
Verruca Vulgaris	57 (16 - 98)		
Not specified	320 (320 - 320)		
Diagnosis	**Area, Median (25 - 75% IQR) mm^2**	**Kruskal-Wallis H**	**p-value**
FEH (Heck's Disease)	15 (9 - 24)	2.45	0.49
Squamous papilloma	18 (8 - 30)		
Squamous papilloma with basal cell hyperplasia	20 (20 - 20)		
Verruca Vulgaris	9 (4 - 14)		
Not specified	32 (32 - 32)		

3.7. Colour of the lesion

The colour of the lesions biopsied was majorly not reported (79.69%). Only 8.59% of the lesions were reported to be the same colour as the surrounding mucosa and 3.91% as pink in colour (Table 3.7).

Table 3:7: Colour of HPV lesions

Colour	N	%
Not specified	103	80.47
Normal colour	18	14.06
Paler than surrounding mucosa	2	1.56
White	2	1.56
Erythematous	2	1.56
Non-homogenous	1	0.78

Although the colour for most of the lesions was not specified, in 18 of the lesions diagnosed as FEH (Heck's disease), the colour was documented, either as normal colour (n=14), paler than the surrounding mucosa (n=2) or white (n=2) as shown in Table 3.8.

Table 3:8: Diagnosis and colour of HPV lesions

Diagnosis	Colour	n	%
FEH (Heck's Disease) (n=82)	Not reported	64	78
	Normal colour	14	17.1
	Paler than surrounding mucosa	2	2.4
	White	2	2.4
Squamous papilloma (n=42)	Not reported	35	83.3
	Normal colour	4	9.5
	Erythematous	2	4.8
	Non-homogenous	1	2.4
Squamous papilloma with basal cell hyperplasia (n=1)	Not specified	1	100
Verruca vulgaris (n=2)	Not specified	2	100
Not specified (n=1)	Not specified	1	100

The number of lesions reported as solitary or multiple was 10.94% and 32.81% respectively and 56.25% of the lesions were not reported as represented in Figure 3.6.

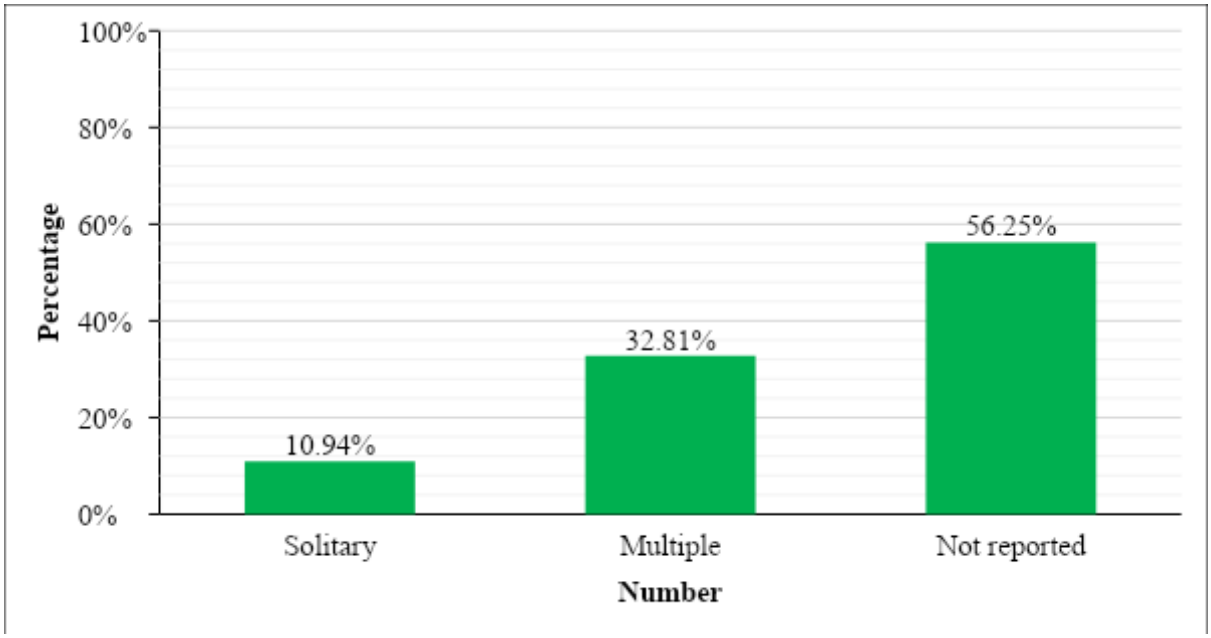

Figure 3:6: Number of solitary/multiple lesions (n=128)

The number of solitary / multiple lesions was partially reported for FEH and squamous papilloma. The majority of cases diagnosed with FEH (Heck's disease) predominantly presented with multiple lesions (50%, n=41) and only (2.4%, n=2) had solitary lesions, whereas patients diagnosed with squamous papilloma mainly presented as solitary lesions (28.6%, n=12) whilst 2.4%(n=1) presented as multiple lesions (Table 3.9).

Table 3:9: Diagnosis and the number of lesions

Diagnosis		**Number**	**%**
FEH (Heck's Disease) (n=82)	Not specified	39	47.6
	Solitary	2	2.4
	Multiple	41	50
Squamous papilloma (n=42)	Not reported	29	69
	Solitary	12	28.6
	Multiple	1	2.4
Squamous papilloma with basal cell hyperplasia (n=1)	Not specified	1	100
Verruca Vulgaris (n=2)	Not specified	2	100
Not reported (n=1)	Not specified	1	100

3.8. Characteristics of HPV associated oral lesions

As represented in Figure 3.6, the characteristics of most (80.7%) HPV associated oral lesions was not reported, with 12.5% of them reported as sessile, 6.25 % as pedunculated and 6.25% reported as both pedunculated and sessile.

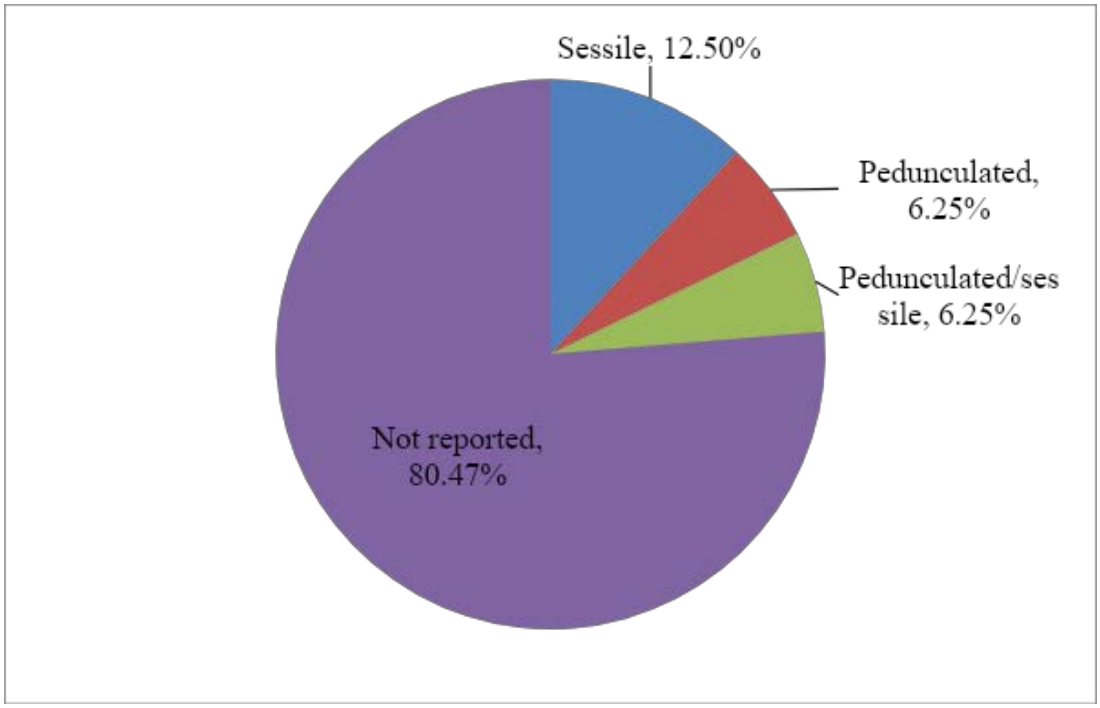

Figure 3:7: Lesion characteristics - Pedunculated/sessile (n=128)

On cases where lesions were characterized, 18.3% of lesions diagnosed as FEH (Heck's disease) were sessile, 1.2% pedunculated and 1.2% were reported as being both pedunculated and sessile. Of the lesions diagnosed as squamous papilloma, only 16.7% were recorded as pedunculated and 2.4% as sessile (**Table 3.10)**.

Table 3:10: Diagnosis and lesion characteristics

Diagnosis	Pedunculated/sessile	n	%
FEH (Heck's Disease) (n=82)	Not specified	65	79.3
	Pedunculated	1	1.2
	Sessile	15	18.3
	Pedunculated and sessile	1	1.2
Squamous papilloma (n=42)	Not specified	34	81
	Pedunculated	7	16.7
	Sessile	1	2.4
Squamous papilloma with basal cell hyperplasia (n=1)	Not specified	1	100
Verruca Vulgaris (n=2)	Not specified	2	100
Not reported (n=1	Not specified	1	100

The surface texture for most of the lesions was not documented (89.84%), and where reported 3.13% of the lesions had a papillary surface texture, 2.34% had finger-like projections, 2.34% a cobblestone appearance, and 0.78% had a smooth, rough, or lobulated surface (Figure 3.8).

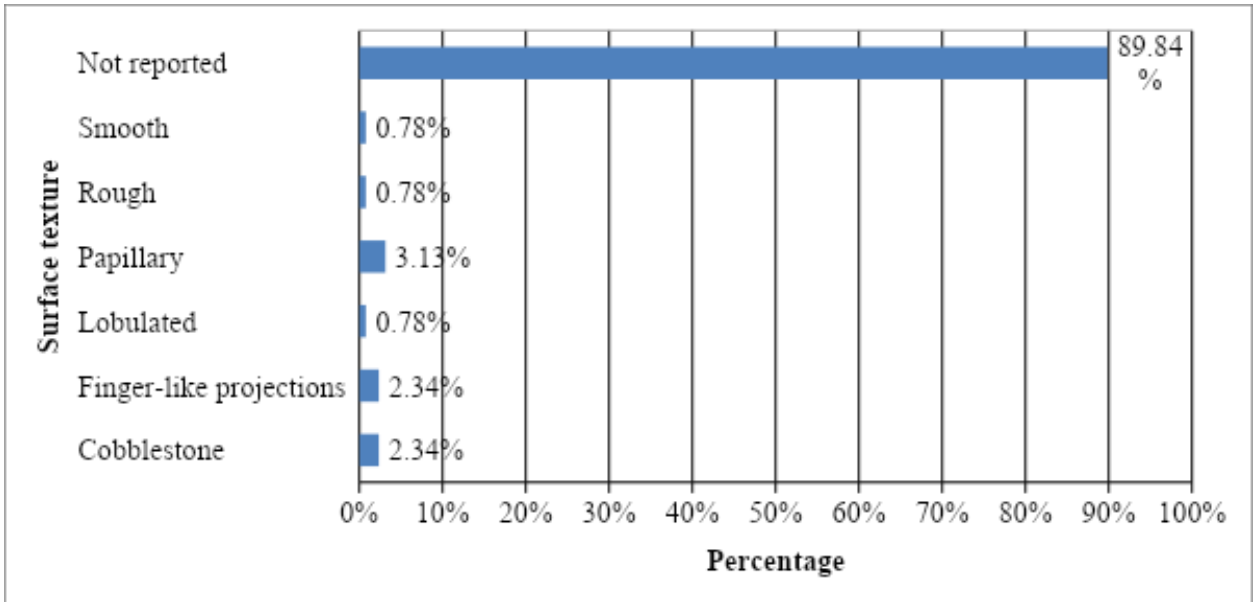

Figure 3:8: Surface texture of the lesions (n=128)

Cobblestone and finger-like projections surface texture were documented in 3.7% and 2.4% of the lesions diagnosed as FEH (Heck's disease) respectively, and 1.2% of the lesions had a smooth or papillary surface texture. Lesions diagnosed as squamous papilloma, had 4.8% reported as having a papillary surface texture, and 2.4% as lobulated, rough or finger-like projections (Table 3.11).

Table 3:11: Diagnosis and surface texture

Diagnosis	**Surface texture**	**n**	**%**
FEH (Heck's Disease) (n=82)	Not specified	75	91.5
	Cobblestone	3	3.7
	Finger-like projections	2	2.4
	Papillary	1	1.2
	Smooth	1	1.2
Squamous papilloma (n=42)	Not specified	37	88.1
	Finger-like projections	1	2.4
	Lobulated	1	2.4
	Papillary	2	4.8
	Rough	1	2.4
Squamous papilloma with basal cell hyperplasia (n=1)	Not specified	1	100
Verruca Vulgaris (n=2)	Not specified	2	100
Not reported (n=1)	Papillary	1	100

3.9. Relationship between the diagnoses of HPV associated lesion and demographics

3.9.1. Association between diagnosis and gender

Fischer's exact test was used to determine the association between diagnoses and gender. The results show that 67.6% of females and 60% of males were diagnosed with FEH (Heck's disease) respectively. However, there was no significant association between gender and the specific diagnoses of HPV associated oral lesion, (p-value = 0.711) as shown in Table 3.12.

Table 3:12: Association between diagnosis and gender (n=128)

Gender	Diagnosis	n	%	Fischer's exact test
Female (n=74)	FEH (Heck's Disease)	50	67.57	0.711
	Squamous papilloma	22	29.73	
	Squamous papilloma with basal cell hyperplasia	1	1.35	
	Verruca Vulgaris	1	1.35	
Male (n=50)	FEH (Heck's Disease)	30	60	
	Squamous papilloma	18	36	
	Verruca Vulgaris	1	2	
	Not reported	1	2	
Not reported (n=4)	FEH (Heck's Disease)	2	50	
	Squamous papilloma	2	50	

3.9.2. Association between the diagnoses and age of the patient

The median age of patients diagnosed with FEH (Heck's disease) was 16 (9-34), 29.5 (8.5-54) for squamous papilloma, 12 (12-12) for squamous papilloma with basal cell hyperplasia and 43.5 (29-58) for verruca vulgaris. Kruskal Wallis test showed that there was no significant difference between a specific diagnosis of an HPV associated oral lesion and the age of the patient, (p-value =0.16) (Table 3.13).

Table 3:13: Association between diagnoses and age (n=128)

Diagnosis	Age, Median (25-75%IQR)	Kruskal-Wallis H	p-value
FEH (Heck's Disease)	16 (9 - 34)	5.167	0.16
Squamous papilloma	29.5 (8.5 - 54)		
Squamous papilloma with basal cell hyperplasia	12 (12 - 12)		
Verruca Vulgaris	43.5 (29 -58)		
Not reported	65 (65 - 65)		

3.9.3. Association between diagnoses and HIV status of the patient

FEH (Heck's disease) and squamous papilloma were diagnosed in 59.4% and 37.6% of patients who were HIV negative and in 81.2% and 14.8% of patients who were HIV positive respectively. Fischer's exact test showed significant association between the overall diagnosis of HPV associated oral lesion and the HIV status of the patient (Table 3.14).

Table 3:14: Association between HIV and diagnosis (n=128)

HIV status	Diagnosis	n	%	Fischer's exact test
HIV unknown	FEH (Heck's Disease)	60	59.4	0.045*
	Squamous papilloma	38	37.6	
	Squamous papilloma with basal cell hyperplasia	1	1	
	Verruca Vulgaris	2	2	
HIV positive	FEH (Heck's Disease)	22	81.5	
	Squamous papilloma	4	14.8	
	Not reported	1	3.7	

Further analysis using binary logistic regression of the results, showed that the odds of being diagnosed with FEH (Heck's disease) if you are HIV positive is significantly increased by 3.48 [p=0.03, (95% CI = 1.11 – 10.89)].

4. DISCUSSION

The aim of this study was to report on HPV-associated oral lesions, histologically diagnosed in the Oral Pathology Department at WOHC over a period of 10 years (2007 - 2017).

4.1. HPV infection and patient demographics

The patient demographics that were observed in the study included gender, race and age. A large constituent of the sample was female (58%), males at (39%) and gender was not reported in 3%. The impact of HPV infection on gender was not significant, showing no direct correlation between the two variables. This was consistent with reports from other studies that reported gender as not being directly related to oral HPV infection. Neville and Damm (2009) reported that FEH affected males and females equally as did the results in this study, which showed that there was no statistical significance in gender predilection and oral HPV infection. Osazuwa-Peter *et al* (2019) conducted a study based on gender and race and the risk factors of HPV infection and found that there were no statistical differences in terms of race; however, in this study race could not be included because it was not documented.

Squamous papilloma was diagnosed in more males than females in this study. The reason for this minor difference seen is not known.

4.2. HPV associated oral lesions in relation to HIV status of the patient

HIV infection leads to a reduction in $CD4^+$ T lymphocyte which makes individuals infected with the virus more susceptible to opportunistic infections (Almeida *et al*, 2017-ref 1). Studies by Cameron *et al* have shown a strong association between HPV and HIV infection. HIV has been known to cause a plethora of oral lesions, some of which have been categorised according to how

strongly associated they are with the condition. However, HPV associated oral lesions are in Group 2, which are categorised as lesions that are less commonly associated with HIV infection (EC-Clearinghouse and WHO on oral manifestations of HIV, 1993). In this study 21% of the patients were diagnosed HIV positive, whilst 79% had an unknown status. The reasons for this high percentage of unknown cases could be due to undocumented data, the unwillingness of patients to disclose their status to the practitioner or poor history taking by the interviewing practitioner. The total percentage of HIV infected individuals who were already on HAART was 66,67%, with 3,7% of them recorded as not yet receiving HAART. The remaining 29,63% of the individuals were unconfirmed cases.

In 2008, Cameron *et al* conducted a study to determine the impact of HAART in seropositive individuals with HPV infection. There was a significant difference between the risk factors for infection in African American males and Caucasian males who were on HAART. This difference was associated with possible compliance amongst the two race groups. HAART has led to improvement in health, quality of life as well as longevity for patients living with HIV infection with a decline in a wide variety of infections of the oral cavity (Cameron *et al*, 2008). However, ART initiation is not without complications especially in the first 6 months. (Haddow *et al*, 2012). HIV associated immune reconstitution inflammatory syndrome (IRIS) is now known to cause early complications in ART initiation. IRIS can be described as a paradoxical phenomenon observed in patients recovering from immunodeficiency, a response to an overwhelming inflammatory response to either a viable, dormant or non-viable antigen in patients with rapidly recovering immune systems (DeSimone *et al*, 2000, Brenton, *et al*, 2006). Its initiation and progression seems to be linked to an increase in $CD4^+$ T-helper cells and $CD8^+$ T-suppressor cell count and a reduction in T-regulatory cells with the assistance of exaggerated cytokine release and activity (Tappuni, Fleming 2001).

Other studies reported that the use and duration of ART was associated with detection of HPV DNA in oral secretions (D'Souza *et al,* 2007) and that oral HPV DNA has been detected more frequently in HIV-infected patients who were virologically suppressed and on ART than those who were not virologically suppressed and on ART (Cameron *et al,* 2005). This was also demonstrated in this study, that the likelihood of an HIV-infected individual being diagnosed with an HPV associated oral lesion is increased. The undesirable findings in our study reflected that most variables investigated were not reported on. The high number of unreported/unknown cases is alarming and warrants further investigation to ascertain the reason for this large discrepancy.

4.3. HAART and IRIS

Since the introduction and initiation of HAART in the treatment of HIV there has been improvement in immune reconstitution of patients on this treatment, however, development of clinical outbreaks of infectious diseases has increased (Murdoch *et al,* 2007). In our study, 21% of the cases were HIV positive and of that percentage, 66.67% were already on HAART with 3.70% not on treatment. In 29.63% of the cases, HAART was not reported. This number is relatively high and in a country where HAART is freely available in government institutions, this finding cannot be reasonably explained. We could however, speculate that this might be due to practitioners taking inadequate medical and drug history from patients or that the patients themselves are not being forthright with practitioners about their treatment regimens and hence, the skewed data capturing. HAART has contributed to the reduction in clinical prevalence of numerous oral conditions commonly associated with HIV infection such as oral hairy leukoplakia and a moderate reduction in prevalence of necrotizing gingivitis, necrotizing periodontitis, necrotizing stomatitis, oral candidiasis as well as Kaposi sarcoma especially in resource-rich countries (Tappuni *et al,* 2001; Peppes *et al,* 2013).

The response to HAART by HIV-positive patients can result in IRIS. This response occurs usually within 3 months after ART initiation and it develops from a dysregulated immune response to a variety of antigenic stimuli following its initiation (Sharma *et al*, 2011). This means that some people experience a clinical deterioration that is believed to be the restored ability to mount an inflammatory response (Robertson *et al*, 2006). The relationship between HPV infection and HAART treatment is still not clear and its interaction has not been studied yet (Syrjanen, 2011). A study conducted by Anaya-Saavedra *et al* in 2013 on the impact of long-term use of HAART in HIV-positive patients, found that these patients were more likely to present with an HPV associated oral lesions (HPV-OL), and those on HAART for longer than 12 months were 5 times more likely to present with HPV-OL.

4.4. HPV associated oral lesions in relation to colour, surface texture and number

In our study the nature of the colour, number of lesions and specific histological diagnosis the patients presented with had no significant deviations from what these benign HPV-associated oral lesions characteristically present as according to Neville and Damm, 2009. FEH typically present as multiple discrete or clustered non-tender, flat or convex, sessile, soft papules 3-10mm in size with normal mucosal colour. In this study, lesions diagnosed as FEH also presented with the same known features. Squamous papilloma presents as a solitary lesion with finger-like projections and pink in colour, although when they are excessively keratinized, they may appear white (Prabhu and Wilson, 2013). However, in this study we are not able to substantiate this fact because the colour of lesions diagnosed as squamous papilloma was mostly not captured, with only a few cases that reported the colour of the lesions as being the same as that of surrounding mucosa. This again proves how proper data collection and capturing can help alleviate the skewed results that researchers end up with, making these important studies be less significant then they ought to be.

Other significant features of the suspected HPV-OL such as surface texture, and the age of the patients, were also not captured for all the lesions. Figure 5 in the results section illustrates the most frequently histologically diagnosed HPV-OL was FEH at 64.06%, with 32.81% diagnosed as squamous papilloma, 1.56% verruca vulgaris, and 0.78% squamous cell papilloma with basal cell hyperplasia and 0.78% of the lesions not reported as any specific type. The results also revealed FEH as the most common HPV-OL diagnosed in HIV-positive patients. The study conducted by Anaya-Saavedra (2013) however found squamous cell papilloma to be the most prevalent HPV-OL in HIV-positive patients at (%), followed by verruca vulgaris (%), a result that is different from our study. This shows that there might be some geographical differences seen in the prevalence of the type of lesions diagnosed between the different studies conducted.

4.5. HPV associated oral lesions in relation to site

When looking at the different HPV-OL, site predilection is not always the same for the different lesions as mentioned in the descriptors for these lesions. Some sites can be affected more commonly than others. In this study, the sites were categorized according to the most affected areas by the lesions, rather than with its association to the lesion. The most affected site was found to be the labial mucosa with 54 sites affected, followed by the buccal mucosa at 44 sites in number and lastly the tongue with 42 sites in number. Other affected areas included the palate, gingiva, floor of the mouth and a small number of unspecified sites. The least affected site was the lip vermillion, followed by the vestibular and retro-molar areas. The sites were recorded as per number and not as a percentage as we found that some had the same site involved. This resulted in a more accurate depiction of the site prevalence affected by HPV-OL, as opposed to a specific lesion having a particular site involved, which would result in a more skewed picture of HPV-OL involvement.

4.6. Focal epithelial hyperplasia, HIV infection and HAART

Focal epithelial hyperplasia or multifocal epithelial hyperplasia is a squamous epithelial proliferation primarily attributed to LR-HPV type 13 and 32, hence it being considered a benign lesion affecting most commonly the labial, buccal and lingual mucosa. However, the gingiva, floor of the mouth and tonsillar area may also be involved (Neville and Damm, 2009). There have not been enough studies conducted focusing on the prevalence of FEH (oral warts) in the general population versus people living with HIV-infection. On the 1st of April 2004 a rollout of the ART program in South Africa was initiated, where ART was delivered to pregnant women and their neonates as a second phase of prevention and treatment. a couple of years later around 2005 a comprehensive plan was set up to roll out ART to 53 of the country's districts, these were primarily tertiary hospitals; however, the number of people receiving ART remained far below the number targeted (Simelela and Venter, 2014).

4.7. Squamous Papilloma, HIV-infection and HAART

Squamous papilloma is a benign proliferation of stratified squamous epithelium that results in a papillary mass. The implicated HPV subtypes are 6 and 11 which are low risk types (Neville and Damm, 2009). Squamous papilloma was diagnosed in 32.81% of cases in this study, making it the second most common lesion found. There was no gender predilection associated with this lesion, supporting what the literature says, as it was found to affect both males and females equally. The sites mostly commonly affected include the palate, tongue, and lips although any oral site may be affected (Neville and Damm, 2009). As with FEH many of the features of the lesions were not reported on.

4.8. Verruca Vulgaris, HIV infection and HAART

Verruca vulgaris also known as the common HPV associated oral lesion. It is a benign HPV-induced hyperplasia of stratified squamous epithelium with HPV 2 strain as the most commonly present subtype. Verruca vulgaris is contagious and can spread to other parts of the body such as the skin or mucosa by autoinoculation (Neville and Damm, 2009). In this study the number of cases histologically diagnosed as Verruca vulgaris was 1.56%, which was very low compared to the other most commonly diagnosed lesions. In the study conducted by Anaya-Saavedra (2013), verruca vulgaris also had the lowest incidence as compared to the other HPV-OL. There are no current studies that can explain why verruca vulgaris is found at a lower rate than its counterparts.

The research conducted by Anaya-Saavedra *et al* found numerous reports of increased HPV-OL in patients on HAART and ART, however, even though the aforementioned report in that particular study proposed that the increase in HPV-OL were as a result of HAART and the age of the patient; there has not been enough scientific evidence to conclude that the therapy could be a risk factor for the development and progression of these lesions. (Anaya-Saavedra *et al*, 2014).

4.9. Limitations of the study

- The lack of comprehensive information in the reports compiled by the consulting practitioner, leading to inconclusive results due to incomplete/ insufficient data
- The data selection for this study could have been a limitation in that it was only focused on oral HPV associated lesion and not the oral cavity in its entirety as well as the variables that were chosen that did not include a wider spectrum such as CD4+ count.

4.10. Conclusion

This study's objectives were to ascertain how particular variables were associated with the occurrence of HPV oral lesions and how these manifested in the oral cavity, with a focus on lesions only limited to the oral cavity and not oropharynx. We were also focused on the benign group of HPV associated oral lesions that encompassed mostly the low-risk subtypes which included; Focal epithelial hyperplasia, Squamous papilloma, Condyloma acuminatum and Verruca vulgaris.

Our results showed that some variables such as; age and HIV-status were strongly linked to the frequency of HPV associated oral lesion occurrence as opposed to gender and demographics of individuals, which had a much less association with the occurrence of HPV associated oral lesions. There are numerous ways that HPV can be contracted, and depending on the mode of transmission, it can manifest as any of the above mentioned lesions. It is important to know that HPV induces cellular proliferation and LR-HPV associated oral lesions are limited to the epithelium and mucosal surfaces.

HIV infection has led to numerous manifestations of HPV-associated oral lesions. In an era where HIV infection rate is still so high, especially in Sub-Saharan Africa, documenting the incidence of HPV-associated oral lesions has become even more important.

In this study it was apparent that there was a major deficit in how data was captured regarding HPV-OL by practitioners, leading to inconclusive results. This has highlighted the importance of training practitioners on proper record keeping for future referencing and research purposes.

The recommendations from this study are that descriptive examination forms specifying what is required for a description of a lesion be designed to assist the dental/ medical practitioner working at a primary health care facility formulate a differential diagnosis that can assist in the proper

management of patients. This data collection sheet would specify details such as the site, colour, size, surface texture, base of lesion, demographics of the patients as well as specific medications they may be taking.

It is now evident how imperative proper history taking is, and the importance of using the correct and appropriate medical terms when filling-in or capturing medical reports. In the South African context patients are often referred from surrounding local clinic to tertiary facilities with referral letters that often do not contain a detailed history.

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APPENDIX ONE: APPROVAL OF TITLE

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05 April 2019
Person No: 0702967T
PAG

Miss S Golele
Po Box 54176
0156
South Africa

Dear Miss Sagwati Golele

Master of Science in Dentistry: Approval of Title

We have pleasure in advising that your proposal entitled has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

APPENDIX TWO: ETHICAL APPROVAL

R14/49 Dr S Golele

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M181010

NAME: (Principal Investigator)	Dr S Golele
DEPARTMENT:	School of Oral Health Sciences Department of Oral Medicine and Periodontology Medical School University
PROJECT TITLE:	An audit of oral lesions associated with HPV infection in the Oral Pathology Department, Wits Oral Health Centre
DATE CONSIDERED:	26/10/2018
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Professor S Shangase and Dr N Zwane
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	20/11/2018

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 Research Office Secretary on 3rd floor, Phillip V Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/We fully understand the conditions under which I am/we are authorised 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 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 of the meeting when the study was initially reviewed. In this case, the study was initially reviewed in October and will therefore reports and re-certification will be due early in the month of October each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX THREE: DATA COLLECTION TOOL

Case number:	Patient Race	Age of patient	Gender (M/F)	HIV-status	Site of lesion	Type of Oral HPV Lesion
1.						
2.						
3.						
4.						
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10.						