The effect of antiretroviral therapy on fine needle aspiration of salivary gland masses in HIV-infected patients

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A research report in submissible format of a paper, in submission to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine in the branch of Anatomical Pathology

Johannesburg 2020

Dedication

To Dr Albert William Coleman

Letter stating role of candidate in writing the article

I declare that this research report is my own work, aided by my supervisor. It is being submitted for the Degree Master of Medicine (MMED) at the University of the Witswatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

My contribution towards this article: 80%

My supervisor's contribution towards this article: 20%

----------Date---06/08/2019

Signature of Candidate

Dr.Amanda Ama Coleman Eghan

----------Date---6/8/2019

Signature of Supervisor

Professor Pamela Michelow

Presentations and publications arising from this study

Presentations: None

Publication: This manuscript has been submitted to Diagnostic Cytopathology where peer review is currently underway.

Abstract

Background

South Africa has one of the highest prevalence rates of HIV/ AIDS. Salivary gland lesions are common in HIV- infected patients. The aim of this study was to determine the pathologic entities diagnosed on FNA of salivary gland masses in an HIV-infected study population that now has free access to ARVs and how this differs from the pathologic entities before the advent of widespread ARV availability.

Methods

A retrospective review was performed on confirmed HIV-infected patients who underwent FNA of salivary gland masses over a two year period. A computer generated search was conducted using the Cytopathology archives of the Cytopathology unit, Department of Anatomical Pathology, University of Witwatersrand and National Health Laboratory Service (NHLS), Johannesburg, South Africa from September 2014 to August 2016. The lesions were classified according to the Milan system for Reporting Salivary Gland Cytopathology.

Results

A total of 360 patients underwent FNA of salivary gland masses within the designated time frame, 58.3% females and 41.7% males. The parotid gland was the most biopsied salivary gland at 55.3%. The most common diagnosis made in patients on antiviral therapy was lymphoepithelial cyst and the most common diagnosis in patients not on antiviral therapy was infective (including abscess and mycobacterial infection). Common neoplasms sampled included non-Hodgkin lymphoma, pleomorphic adenoma and squamous cell carcinoma.

Conclusion

Patients on antiretroviral therapy had higher CD4 counts, fewer infective lesions, and more reactive and benign lesions. Patients not on treatment had significantly lower CD4 counts and were frequently diagnosed with infective processes.

Acknowledgements

I would like to express my sincere gratitude to my supervisor Professor Pamela Michelow for her continuous support of my MMED research, for her patience, motivation, enthusiasm and immense knowledge. Her guidance, experience and expertise in this field have been invaluable during this journey.

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Section 1: Author Guidelines

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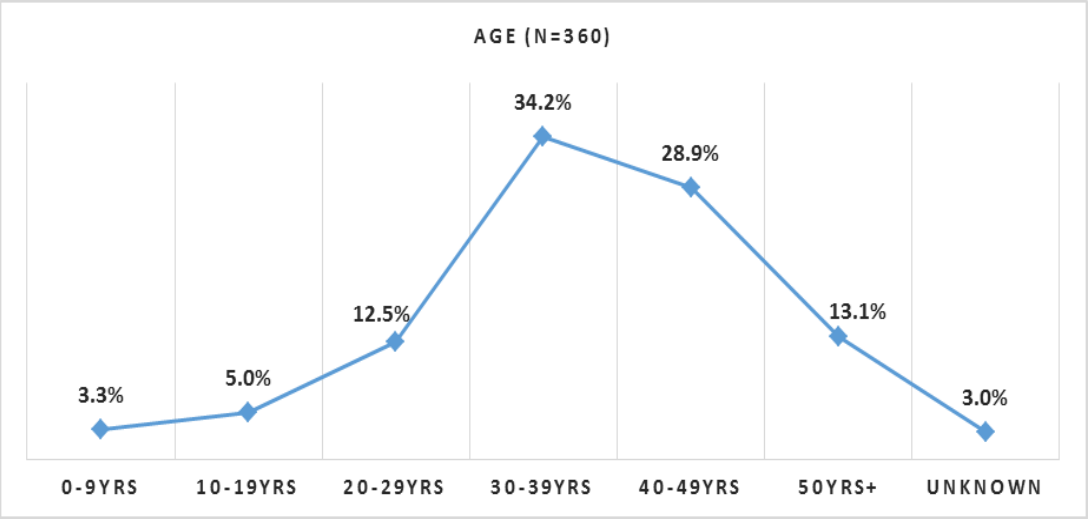

Figure 1: Age distribution

B. List of tables

Table 1: FNA results classified according to the Milan System for reporting salivary gland cytopathology.

Milan System for Reporting salivary gland cytopathology (16)	**Number of cases**	**Percentage (%)**
I - Non-diagnostic	**61**	**16.94**
• Salivary gland tissue only	• **40**	• **11.1**
• Non-mucinous cyst	• **6**	• **1.6**
• Other causes e.g. blood only	• **15**	• **4.1**
II - Non-neoplastic	**253**	**70.2**
• Lymphoepithelial lesion	• **98**	• **27.2**
• Infective (abscess, mycobacterial infection)	• **81**	• **22.5**
• Reactive lymphoid hyperplasia	• **68**	• **18.8**
• Epidermal inclusion cyst	• **6**	• **1.6**
III - Atypia of undetermined significance	**4**	**1.1**
• Atypical cells	• **3**	• **0.8**
• Mucus cyst	• **1**	• **0.2**
IVA - Benign neoplasm	**8**	**2.2**
• Pleomorphic adenoma	• **7**	• **1.9**
• Oncocytoma	• **1**	• **0.2**
IVB - Salivary gland neoplasm of uncertain malignant potential (SUMP)	**5**	**1.3**
• Spindle cell lesion	• **3**	• **0.8**

• SUMP, NOS	• **2**	• **0.5**
V - Suspicious for malignancy	**0**	**0**
VI - Malignant	**25**	**6.94**
• Non-Hodgkin lymphoma	• **18**	• **5**
• Squamous cell carcinoma	• **2**	• **0.5**
• Low grade mucoepidermoid carcinoma	• **1**	• **0.2**
• Poorly differentiated carcinoma	• **3**	• **0.8**
• Undifferentiated malignancy	• **1**	• **0.2**
Total	***356**	***99**

***There were 4 cases where no description/comment was supplied.**

<u>Histopathological follow-up</u>

- NO Histology results were traced on the system for all cases in the *non-diagnostic* category.
- Histology confirmed 1 case of epidermal inclusion cyst and 1 case of lymphoepithelial cyst in the *non-neoplastic* category.
- No histology found on the system for any of the cases in the category *atypia of undetermined* significance
- Histology confirmed a PA in one case in the category *benign neoplasm*
- No histology was traced on the system for any of the cases in the category *Salivary gland neoplasm of uncertain malignant potential (SUMP)*
- No cases were recorded under the category *Suspicious for malignancy*
- *In the Malignant category h*istology confirmed the presence of Non Hodgkin lymphoma in 8 cases; this includes 1 case of plasmablastic lymphoma, 1 case of follicular lymphoma, 4 cases of diffuse large B cell lymphoma of which 2 had a differential diagnosis of Burkitt

lymphoma, and 2 cases classified as Non Hodgkin lymphoma. The squamous cell carcinoma was confirmed on histology. Low grade mucoepidermoid carcinoma was diagnosed as acinic cell carcinoma on histology. Poorly differentiated carcinoma was diagnosed as a squamous cell carcinoma on histology.

Table 2: Cytologic diagnosis by FNA site

Pathology	Parotid Mass Aspiration (n=199)	Submandibular Mass Aspiration (n=148)	Submental (n=13)	All FNAs (n=360)
Benign lymphoepithelial cyst	36.2%	17.6%	0.0%	27.2%
Infective (Abscess; mycobacteria)	9.5%	36.5%	61.5%	22.5%
Reactive lymphoid hyperplasia	21.6%	14.2%	30.8%	18.9%
Normal salivary gland tissue	15.1%	6.8%	0.0%	11.1%
Other	8.5%	10.1%	0.0%	8.9%
Non Hodgkin Lymphoma	3.0%	7.4%	0.0%	4.7%
Inadequate	3.0%	5.4%	7.7%	4.2%
Pleomorphic adenoma	2.0%	2.0%	0.0%	1.9%
Squamous cell carcinoma	1.0%	0.0%	0.0%	0.6%

Table 3: Cytologic diagnosis by ARV treatment

Pathology	On ARV treatment (n=45)	Not on ARV treatment (n=93)
Infective (Abscess; mycobacteria)	8.9%	28.0%
Normal salivary gland tissue	22.2%	21.5%
Benign lymphoepithelial cyst	22.2%	20.4%
Reactive lymphoid hyperplasia	17.8%	15.1%
Other	13.3%	6.5%
Non Hodgkin Lymphoma	6.7%	3.2%
Inadequate	2.2%	3.2%
Pleomorphic adenoma	6.7%	0.0%
Squamous cell carcinoma	0.0%	2.2%

Table 4: Cytologic diagnosis by sex

	Sex		
Pathologies	**Male (n=150)**	**Female (n=210)**	**All patients (n=360)**
Benign lymphoepithelial cyst	27.3%	27.1%	27.2%
Infective (Abscess; mycobacteria)	28.0%	18.6%	22.5%
Reactive lymphoid hyperplasia	18.7%	19.0%	18.9%
Normal salivary gland tissue	8.0%	13.3%	11.1%
Other	10.7%	7.6%	8.9%
Non Hodgkin Lymphoma	2.7%	6.2%	4.7%
Inadequate	3.3%	4.8%	4.2%
Pleomorphic adenoma	0.0%	3.3%	1.9%
Squamous cell carcinoma	0.0%	0.0%	0.6%

Table 5: Cytologic diagnosis by age

Pathologies	**0-9yrs**	**10-19yrs**	**20-29yrs**	**30-39yrs**	**40-49yrs**	**50yrs+**
	n=12	*n=18*	*n=45*	*n=123*	*n=104*	*n=47*
Benign lymphoepithelial cyst (BLEC)	16.7%	44.4%	20.0%	22.8%	30.8%	34.0%
Infective (Abscess; mycobacteria)	16.7%	33.3%	28.9%	29.3%	18.3%	8.5%
Reactive lymphoid hyperplasia	41.7%	16.7%	13.3%	25.2%	16.3%	10.6%
Normal salivary gland tissue	16.7%	5.6%	8.9%	7.3%	14.4%	14.9%
Other	0.0%	0.0%	11.1%	8.9%	7.7%	14.9%
Non Hodgkin Lymphoma	0.0%	0.0%	6.7%	3.3%	5.8%	8.5%
Inadequate	8.3%	0.0%	8.9%	2.4%	1.9%	4.3%
Pleomorphic adenoma	0.0%	0.0%	2.2%	0.0%	3.8%	4.3%
Squamous cell carcinoma	0.0%	0.0%	0.0%	0.8%	1.0%	0.0%

Table 6: Pathologies by CD4 count

Pathologies	Number of Patients	Mean Count	Std. Dev	Std. Error
Pleomorphic adenoma	3	538.7	362.839	209.485
Other	**16**	**535.0**	419.422	104.855
Normal salivary gland tissue	4	528.8	311.450	155.725
Reactive lymphoid hyperplasia	**41**	**392.7**	273.941	42.782
Squamous cell carcinoma	2	387.5	306.177	216.500
Benign lymphoepithelial cyst	**54**	**363.4**	248.240	33.781
Non Hodgkin Lymphoma	12	284.0	247.967	71.582
Inadequate	9	144.3	127.301	42.434
Infective (Abscess; mycobacteria)	**43**	**126.5**	157.175	23.969
Total	**184**	**320.3**	**283.294**	**20.885**

C. Nomenclature

ARVs – antiretrovirals

HIV – Human immunodeficiency virus

AIDS – Acquired immunodeficiency virus

FNA – Fine needle aspiration

NHLS – National Health Laboratory Service

MSRSGC – The Milan System for Reporting Salivary Gland Cytopathology

CD4 – Cluster of differentiation

BLEC – Benign lymphoepithelial cysts

TB – Tuberculosis

SUMP – Salivary gland neoplasm of uncertain malignant potential

ROM – Risk of malignancy

PA – Pleomorphic adenoma

HAART – Highly active antiretroviral therapy

2.1 Title Page

Title: The effect of antiretroviral therapy on fine needle aspiration of salivary gland masses in HIV-infected patients

Running title: FNA of salivary glands in HIV-infected patients on ARVs.

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ETHICAL APPROVAL

The study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg, South Africa.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

FUNDING

No funding was obtained.

2.2 ABSTRACT

ABSTRACT

Background

South Africa has one of the highest prevalence rates of HIV/ AIDS. Salivary gland lesions are common in HIV- infected patients. The aim of this study was to determine the pathologic entities diagnosed on FNA of salivary gland masses in an HIV-infected study population that now has free access to ARVs and how this differs from the pathologic entities before the advent of widespread ARV availability.

Methods

A retrospective review was performed on confirmed HIV-infected patients who underwent FNA of salivary gland masses over a two year period. A computer generated search was conducted using the Cytopathology archives of the Cytopathology unit, Department of Anatomical Pathology, University of Witwatersrand and National Health Laboratory Service (NHLS), Johannesburg, South Africa from September 2014 to August 2016. The lesions were classified according to the Milan system for Reporting Salivary Gland Cytopathology.

Results

A total of 360 patients underwent FNA of salivary gland masses within the designated time frame, 58.3% females and 41.7% males. The parotid gland was the most biopsied salivary gland at 55.3%. The most common diagnosis made in patients on antiviral therapy was lymphoepithelial cyst and the most common diagnosis in patients not on antiviral therapy was infective (including abscess and mycobacterial infection). Common neoplasms sampled included non-Hodgkin lymphoma, pleomorphic adenoma and squamous cell carcinoma.

Conclusion

Patients on antiretroviral therapy had higher CD4 counts, fewer infective lesions, and more reactive and benign lesions. Patients not on treatment had significantly lower CD4 counts and were frequently diagnosed with infective processes.

KEY WORDS: FNA Salivary glands, HIV, Milan system for Reporting Salivary Gland Cytopathology

2.3 Manuscript

2.3.1 INTRODUCTION

South Africa has one of the highest prevalence rates of HIV (human immunodeficiency virus)/ AIDS (acquired immunodeficiency virus). HIV is a T lymphotropic retrovirus that infects cells with CD4 receptors. This causes a gradual decline in CD4 lymphocytes with resultant decrease in host immunity. This places the individual patient at risk for developing a multitude of opportunistic diseases ranging from infectious processes to neoplasms, benign and malignant. [1]

Salivary gland lesions are common in HIV-infected patients, including children. The majority of salivary gland lesions occur in the parotid gland and include inflammatory processes, infections, benign lesions such as benign lymphoepithelial cysts (BLEC) and neoplasms which include pleomorphic adenoma, lymphoma, Kaposi sarcoma and squamous cell carcinoma. [2]

Clinically, patients with salivary gland lesions usually present with painless palpable masses in the parotid region or in the floor of the mouth for submandibular masses. Other signs include pain, facial nerve palsy and enlarged lymph nodes which could suggest malignancy .[3] FNA is frequently used in the diagnosis of lesions of the salivary glands. It is most often the first tissue-based procedure applied to establish a diagnosis before any surgical intervention. FNA of salivary gland is a good diagnostic test and has a sensitivity and specificity, ranging from 60% to 100% and 90% to 100%, respectively [4-5]. There is a degree of false negatives and false positives in salivary gland FNA. False negatives are mainly due to underdiagnosing of low grade tumour because of bland cytological features as well as the difficulty in diagnosing hypocellular lesions. False positives can occur when reactive inflammatory lesions are called non-benign. Also challenging can be shared cytological features between benign, low grade and high grade malignant salivary tumours. [6]

The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC*)* has recently been developed. The MSRSGC was conceptualised in 2015 to address the limitations for the overall effectiveness of salivary gland FNA.[7] It presents logical, pragmatic and flexible reporting terminology that allows the pathologist and the clinician to communicate effectively with improved patient care as the desired outcome.[8] This system is designed to include the best practice guidelines pertaining to

salivary gland FNA, this includes indications for salivary gland FNA, standardised reporting of results and application of ancillary techniques including immunohistochemistry and molecular testing to complement cytomorphology. [7] Studies of this nature are important to test the robustness of the MSRSGC.

The pathologies described in FNA of salivary gland masses in HIV –infected patients, including those on ARVs, need to be studied and described to better prepare clinicians for disease burden and management of these patients. A previous study interrogating FNA of salivary gland masses in HIV infected individuals before the introduction of antiretroviral therapy in a low middle income country showed reactive lymphoid hyperplasia, benign lymphoepithelial lesion and mycobacterial infection to be the most common diagnoses. [9] The study referred to was done in an era where ARVs was not widely available. With the progressive ease with which ARVs are now available, this current study will demonstrate the spectrum of disease in HIV-infected patients receiving antiretroviral therapy (ARVs) may be somewhat different. The long term management for patients with HIV is ARVs. In the South African public health sector, ARV rollout to HIV-infected patients began in 2004. A CD4 count of < 200 cells/uL was required before the initiation of ARVs. In 2012, the CD4 count was revised to <350 cells/uL and in 2014 the CD4 count was revised to <500 cells/uL if the patient was motivated and ready to start treatment. In August 2015, the CD4 count was discarded as a guide and the guideline now states that any patient in South Africa diagnosed with HIV/AIDS is eligible for initiation of antiviral therapy.[10-11]

The aim of this study was to determine the pathologic entities diagnosed on FNA of salivary gland masses in an HIV-infected study population that now has free access to ARVs and how this differs from the pathologic entities before the advent of widespread ARV availability.

The objectives of this study is to determine the difference in fine needle aspiration of salivary gland masses prior to earlier antiviral therapy initiation and the current status with introduction at an earlier stage of the disease.

2.3.2 MATERIALS AND METHODS

To undertake this study, ethics approval was obtained from the University of the Witwatersrand, Johannesburg, Human Research Ethics Committee. A computer generated search was conducted

using the Cytopathology archives of the Cytopathology unit, Department of Anatomical Pathology, University of Witwatersrand and National Health Laboratory Service (NHLS), Johannesburg, South Africa was performed over a two year period from September 2014 to August 2016. The study population consisted of HIV-infected patients who underwent FNA of salivary gland lesions on the request of their attending clinicians from several public health sector hospitals in and around Johannesburg. Most FNAs were performed by personnel from the Cytology Unit in an FNA clinic with additional FNAs performed by clinicians and surgeons. Aspirated material was smeared onto two slides; one was fixed with alcohol and stained with a Papanicolaou stain and the other slide was allowed to air dry and fixed with a Diff-Quik stain. In cases where mycobacterial infection was suspected, material was submitted additionally for TB culture and Gene Xpert. Where relevant microscopy, culture and sensitivity was performed on purulent material and flow cytometry was requested in cases where lymphoma was suspected. Additional information elucidated from the database included patient age, gender, salivary gland site and cytologic diagnosis, CD4 count and results of ancillary investigations. Duration of ARVs was not available to us.

Inclusion criteria included all cases where HIV-infected patients with salivary gland masses underwent FNA during the study time. All HIV negative patients were excluded from this study. HIV-infected patients, who presented with a lesion other than in salivary gland, were also excluded.

Follow up in terms of microbiologic investigations and histopathological diagnosis was undertaken for all study participants however, clinical follow-up e.g. response to anti-microbials was beyond the scope of this study.

The data was subsequently analysed using IBM SPSS Statistics. Descriptive statistics were applied to present the basic features of the study sample in frequencies, percentages and means. The Pearson Chi-square test was used to examine associations between cytologic diagnosis results and the categorical variables of specimen type (FNA site) and sex of patient. In cases where the variables being tested exceeded two categories, pairwise comparisons were performed and the Bonferroni method used to adjust the p-value and lower the chance of making Type 1 errors. One-way analysis of variance (ANOVA) was used to test for associations between the cytologic diagnosis results and variables that were measured on a continuous scale, such as CD4 count. Statistical significance testing was done at a 95% confidence level.

2.3.3 RESULTS

A total of 360 patients were included in the study. There were 41.7% male patients and 58.3% female patients. Paediatric patients, defined in our setting as patients 19 years and younger, were the least number of patients represented in the HIV-infected population within our study; 3.3% of patients were between the ages 0-9 years old and 5.0% were between the ages 10-19% (Figure 1).

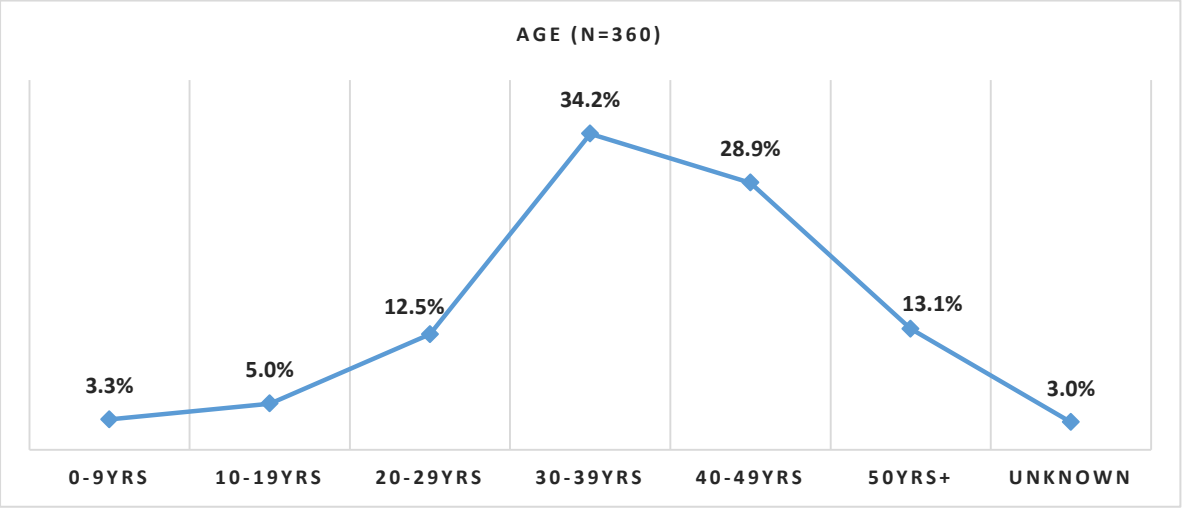

Figure 1: Age distribution

While all patients were known to be HIV-infected, the laboratory was not informed in 61.7% if patients were on ARVs. In the remainder of patients, 12.5% were on ARVs and 25.8% were not on ARVs.

The CD4 count information was provided for only 184 (51.1%) of the patients in the dataset and the mean (average) count was 320.3 as shown below. CD4 counts were included only if they were determined within 3 months of the salivary gland FNA being performed.

A total of 199 (55.3%) of the specimens in the analysis were parotid mass aspirations, 148 (41.1%) were submandibular mass aspirations and 13 (3.6%) were submental. Table 1 provides the entities that were encountered classified according to the MSRSGC [7] .

Table 1: FNA results classified according to the Milan System for reporting salivary gland cytopathology.

Milan System for **Reporting salivary gland cytopathology (16)**	**Number of cases**	**Percentage (%)**
I - Non-diagnostic • Salivary gland tissue only • Non-mucinous cyst • Other causes e.g. blood only	**61** • **40** • **6** • **15**	**16.94** • **11.1** • **1.6** • **4.1**
II - Non-neoplastic • Lymphoepithelial lesion • Infective (abscess, mycobacterial infection) • Reactive lymphoid hyperplasia • Epidermal inclusion cyst	**253** • **98** • **81** • **68** • **6**	**70.2** • **27.2** • **22.5** • **18.8** • **1.6**
III - Atypia of undetermined significance • Atypical cells • Mucus cyst	**4** • **3** • **1**	**1.1** • **0.8** • **0.2**
IVA - Benign neoplasm • Pleomorphic adenoma • Oncocytoma	**8** • **7** • **1**	**2.2** • **1.9** • **0.2**
IVB - Salivary gland neoplasm of uncertain malignant potential (SUMP) • Spindle cell lesion • SUMP, NOS	**5** • **3** • **2**	**1.3** • **0.8** • **0.5**
V - Suspicious for malignancy	**0**	**0**

VI - Malignant • Non-Hodgkin lymphoma • Squamous cell carcinoma • Low grade mucoepidermoid carcinoma • Poorly differentiated carcinoma • Undifferentiated malignancy	**25** • **18** • **2** • **1** • **3** • **1**	**6.94** • **5** • **0.5** • **0.2** • **0.8** • **0.2**
Total	***356**	***99**

***There were 4 cases where no description/comment was supplied.**

The Pearson Chi Square test showed a statistically significant association between cytologic results and FNA site/specimen type (Chi square=70.281, p=0.00). There is significantly higher diagnosis of benign lymphoepithelial cysts in parotid mass aspirations (36.2%) compared to submandibular aspirations (17.6%) and submental (0.0%). There is significantly lower diagnosis of infective (Abscess, mycobacteria) in parotid mass aspirations (9.5%) compared to submandibular aspirations (36.5%) and submental (61.5%). There is significantly higher diagnosis of normal salivary gland tissue in parotid mass aspirations (15.1%) compared to submandibular aspirations (6.8%) and submental (0.0%) (Table 2).

Table 2: Cytologic diagnosis by FNA site

Pathology	**Parotid Mass Aspiration (n=199)**	**Submandibular Mass Aspiration (n=148)**	**Submental (n=13)**	**All FNAs (n=360)**
Benign lymphoepithelial cyst	36.2%	17.6%	0.0%	27.2%
Infective (Abscess;	9.5%	36.5%	61.5%	22.5%

mycobacteria)				
Reactive lymphoid hyperplasia	21.6%	14.2%	30.8%	18.9%
Normal salivary gland tissue	15.1%	6.8%	0.0%	11.1%
Other	8.5%	10.1%	0.0%	8.9%
Non Hodgkin Lymphoma	3.0%	7.4%	0.0%	4.7%
Inadequate	3.0%	5.4%	7.7%	4.2%
Pleomorphic adenoma	2.0%	2.0%	0.0%	1.9%
Squamous cell carcinoma	1.0%	0.0%	0.0%	0.6%

The cytologic diagnosis outcomes were compared between patients on ARVs and those not on ARVs. The other cases whose ARV treatment status was not known were left out of this comparison. There was significantly less diagnosis of infective (abscess, mycobacteria) in patients on ARVs at 8.9% compared to 28.0% among those not on ARVs (Chi square 15.017, p=0.50). BLEC (22.2%), reactive lymphoid hyperplasia (17.8%), non-Hodgkin lymphoma (6.7%) and pleomorphic adenoma (6.7%) were seen more frequently in patients receiving ARVs compared to those not on ARVs. (Table 3). On the Pearson Chi-squared test, there was a significant statistical difference between patients receiving antiviral therapy and those not receiving antiviral therapy.

Table 3: Cytologic diagnosis by ARV treatment

Pathology	**On ARV treatment (n=45)**	**Not on ARV treatment (n=93)**
Infective (Abscess; mycobacteria)	8.9%	28.0%
Normal salivary gland tissue	22.2%	21.5%
Benign lymphoepithelial cyst	22.2%	20.4%
Reactive lymphoid hyperplasia	17.8%	15.1%

Other	13.3%	6.5%
Non Hodgkin Lymphoma	6.7%	3.2%
Inadequate	2.2%	3.2%
Pleomorphic adenoma	6.7%	0.0%
Squamous cell carcinoma	0.0%	2.2%

The cytologic diagnosis outcomes were compared between female and male patients and there were significant differences in the diagnosis by sex (Chi Square=17.149, p=0.03). Infective (abscess, mycobacteria) diagnosis was significantly higher in males (28.0%) compared to females (18.6%). Female patients were diagnosed more often with normal salivary gland tissue (13.3%), non-Hodgkin lymphoma (6.2%) and pleomorphic adenoma (3.3%). (Table 4).

Table 4: Cytologic diagnosis by sex

	Sex		
Pathologies	**Male (n=150)**	**Female (n=210)**	**All patients (n=360)**
Benign lymphoepithelial cyst	27.3%	27.1%	27.2%
Infective (Abscess; mycobacteria)	28.0%	18.6%	22.5%
Reactive lymphoid hyperplasia	18.7%	19.0%	18.9%
Normal salivary gland tissue	8.0%	13.3%	11.1%
Other	10.7%	7.6%	8.9%
Non Hodgkin Lymphoma	2.7%	6.2%	4.7%
Inadequate	3.3%	4.8%	4.2%
Pleomorphic adenoma	0.0%	3.3%	1.9%

Squamous cell carcinoma	0.0%	0.0%	0.6%

The cytologic diagnosis outcomes were also compared by age and the differences in the diagnosed pathologies by age shown in Table 5 were not statistically significant (Chi Square=50.02, p=0.13).

Table 5: Cytologic diagnosis by age

Pathologies	**0-9yrs**	**10-19yrs**	**20-29yrs**	**30-39yrs**	**40-49yrs**	**50yrs+**
	n=12	*n=18*	*n=45*	*n=123*	*n=104*	*n=47*
Benign lymphoepithelial cyst (BLEC)	16.7%	44.4%	20.0%	22.8%	30.8%	34.0%
Infective (Abscess; mycobacteria)	16.7%	33.3%	28.9%	29.3%	18.3%	8.5%
Reactive lymphoid hyperplasia	41.7%	16.7%	13.3%	25.2%	16.3%	10.6%
Normal salivary gland tissue	16.7%	5.6%	8.9%	7.3%	14.4%	14.9%
Other	0.0%	0.0%	11.1%	8.9%	7.7%	14.9%
Non Hodgkin Lymphoma	0.0%	0.0%	6.7%	3.3%	5.8%	8.5%
Inadequate	8.3%	0.0%	8.9%	2.4%	1.9%	4.3%
Pleomorphic adenoma	0.0%	0.0%	2.2%	0.0%	3.8%	4.3%
Squamous cell carcinoma	0.0%	0.0%	0.0%	0.8%	1.0%	0.0%

Reactive lesions are commonly diagnosed in paediatric patients; reactive lymphoid hyperplasia was seen most often in patients between 0-9 years old and benign lymphoepithelial cysts in patients aged 10-19 years. Women aged 20-39 were frequently diagnosed with infective processes. Women over the age of 40 years were most often diagnosed with BLEC. (Table 5).

The association between cytologic diagnosis results and CD4 count was tested using ANOVA which showed significant differences in the pathologies by CD4 count (F=6.311, p=0.00). The Tukey HSD

test was then performed to identify where exactly the differences existed and the results show that the mean CD4 count of 126.5 for infective (abscess, mycobacteria) cases was significantly lower compared to benign lymphoepithelial cyst cases (mean count = 363.4), reactive lymphoid hyperplasia cases (mean count = 392.7) and other cases (mean count = 535.0). (Table 6).

Table 6: Pathologies by CD4 count

Pathologies	Number of Patients	Mean Count	Std. Dev	Std. Error
Pleomorphic adenoma	3	538.7	362.839	209.485
Other	**16**	**535.0**	419.422	104.855
Normal salivary gland tissue	4	528.8	311.450	155.725
Reactive lymphoid hyperplasia	**41**	**392.7**	273.941	42.782
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Infective (Abscess; mycobacteria)	**43**	**126.5**	157.175	23.969
Total	**184**	**320.3**	**283.294**	**20.885**

There was a significant difference in CD4 count according to ARV treatment status (F=4.278, p=0.04). The results show that of the 20 patients known to be on ARVs, the mean CD4 count was 453.8; this is significantly higher than the mean of CD4 count of 276.4 in 46 patients who were not on ARVs.

Histopathological follow-up

- NO Histology results were traced on the system for all cases in the *non-diagnostic* category.
- Histology confirmed 1 case of epidermal inclusion cyst and 1 case of lymphoepithelial cyst in the *non-neoplastic* category.
- No histology found on the system for any of the cases in the category *atypia of undetermined* significance
- Histology confirmed a PA in one case in the category *benign neoplasm*
- No histology was traced on the system for any of the cases in the category *Salivary gland neoplasm of uncertain malignant potential (SUMP)*
- No cases were recorded under the category *Suspicious for malignancy*
- *In the Malignant category h*istology confirmed the presence of Non Hodgkin lymphoma in 8 cases; this includes 1 case of plasmablastic lymphoma, 1 case of follicular lymphoma, 4 cases of diffuse large B cell lymphoma of which 2 had a differential diagnosis of Burkitt lymphoma, and 2 cases classified as Non Hodgkin lymphoma. The squamous cell carcinoma was confirmed on histology. Low grade mucoepidermoid carcinoma was diagnosed as acinic cell carcinoma on histology. Poorly differentiated carcinoma was diagnosed as a squamous cell carcinoma on histology.

2.3.4 DISCUSSION

Salivary gland lesions are common in HIV-infected patients.[2] There is conflicting evidence as to whether antiviral therapy causes an increase or a decrease in the incidence of salivary gland pathology in HIV- infected patients. The theory behind the former state the initiation of antiviral therapy decreases the HIV viral load and increases the CD4 count, therefore the body is able to mount an appropriate immune response to opportunistic infections that have otherwise been clinically silent, this phenomenon is known as immune reconstitution syndrome; this suggests that an infectious agent may play a role in salivary gland enlargement in the HIV positive patient. On the other hand,

initiation of antiviral therapy has also shown a decrease in salivary gland pathology. This is due in part to cessation of viral replication, lower viral load and an increase in the CD4/CD8 count.[12]

The challenge with cytologic evaluation of salivary gland tumours is the wide range and heterogeneous nature of benign and malignant tumours; many have overlapping cytologic features and this renders a definitive diagnosis problematic in some cases[6]. The benefit of MSRSGC in everyday practice of cytopathology is that it is practical with a limited number of diagnostic categories and well defined criteria. The uniformity of reporting salivary gland FNA will improve clarity, quality and reproducibility of salivary gland FNA diagnoses among various national and international institutions, improving patient care.[7] The diagnostic criteria and risk of malignancy (ROM) in the MSRSGC are as follows: I- Non diagnostic with a 25% ROM, II – Non neoplastic with a 10% ROM, III – Atypia of undetermined significance (AUS) with a 10% ROM, IVA- Benign neoplasm with a less than 5% ROM, IVB Salivary gland neoplasm of undetermined malignant potential(SUMP) with a 35% ROM, V – Suspicious for malignancy with a 60% risk of malignancy and VI- Malignant with a 90% ROM. [7]

With the MSRSGC in mind, the following was noted in our study. In the non-diagnostic category which includes salivary gland FNA with significant limitation often as a result of scant cellularity and preservation artefact. [7] There were a total of 61 cases (16.94%) and these comprised 40 cases which showed salivary gland tissue only (11.1%), 6 cases which showed non mucinous cyst (1.6%) and 15 cases which were unsatisfactory for diagnosis e.g. blood only (4.1%).

In the non- neoplastic category which includes benign entities such as reactive, metaplastic and inflammatory processes. The risk of malignancy is low in this category. The major pitfall is the possibility of a false negative diagnosis due to inadequate sampling; this category therefore requires close clinical and radiological correlation [7]. There were a total of 253 cases (70.2%) and these included 98 cases of BLEC (27.2%), 81 cases of infective process (22.5%), 68 cases of reactive lymphoid hyperplasia (18.8%) and 6 cases of epidermal inclusion cyst (1.6%). The AUS category represents a subset of all salivary gland neoplasms with atypical features. It is expected that the majority of these lesions represent reactive atypia, cystic lesions with abundant mucin or scant epithelial component and poorly sampled lesions. This category is useful in reducing the number of

false negative diagnosis in the non-neoplastic category [7]. There were a total of 4 cases (1.1%) and included 3 cases categorised as atypical cells (0.8%) and 1 case described as a mucus cyst (0.2%). According to the MSRSGC, mucinous cysts with few epithelial cells should be designated as atypia of uncertain significance[8]. The category benign neoplasm is reserved for cases where the benign neoplasm is diagnosed based on conventional cytomorphologic criteria. These cases are managed either by follow-up or conservative surgical management.[8] The current study had a total of 8 cases (2.2%) composed of 1 case of oncocytoma (0.2%) and 7 cases of pleomorphic adenoma (1.9%). The cases in the category SUMP lack the diagnostic criteria of a specific entity and importantly, a malignant neoplasm cannot be excluded. These are usually cellular benign neoplasms, neoplasms with atypical features or low grade carcinomas [8]. There were a total of 5 cases (1.3%) and included 3 cases of spindle cell lesions (0.8%). It is possible that these represented Kaposi sarcomas but neither immunocytochemistry nor histology confirmed the diagnosis. There were 2 cases diagnostic of salivary gland neoplasms (0.5%) that could not further be classified on cytology. There were no cases in our study within in the category suspicious for malignancy. In the malignant category, there were a total of 25 cases (6.94%) composed of 18 cases of non-Hodgkin lymphoma (5.0%), 2 cases of squamous cell carcinoma (0.5%), 3 cases of poorly differentiated carcinoma (0.8%) and 1 case of low grade mucoepidermoid carcinoma (0.2%). There was 1 case of undifferentiated malignant cells (3.2%). Poor histopathological follow-up of malignancy precludes accurate assessment for ROM in this study.

A previous study conducted by Michelow *et al* [9] looked at salivary gland fine needle aspiration in the setting of HIV-infected patients in South Africa. The study was conducted at a time where antiviral therapy was initiated at a CD4 count of less than 200 cells/uL. The findings of that study concluded that most diagnosis of FNA salivary gland masses in HIV-infected patients were benign and comprised 33.9% reactive lymphadenopathy, 23.2% benign lymphoepithelial cysts, 12.5% mycobacterial infections and 10.5% abscesses. Neoplasms accounted for 6.7% and included 11 pleomorphic adenomas, 13 lymphomas, 3 Kaposi sarcoma, 1 squamous cell carcinoma, 1 metastatic carcinoma and 1 rhabdomyosarcoma .[9] In comparison to the current study, there appears to be an increase in non-Hodgkin lymphoma (non-Hodgkin lymphoma was diagnosed in 5% (18 cases) of the sample population in our current study and was reported in 2.2% (11 cases) in the previous study) [9].

This could be attributed to prolonged immunosuppression that HIV-infected patients on ARVs face; this leads to an increase in malignancies mediated by oncogenic viruses (Epstein-Barr virus and/or human herpesvirus-8) that is responsible for the increase in lymphoproliferative disorders in this clinical setting [13.]

As confirmed by the study, the majority of salivary gland lesions occur in the parotid gland at 55.5%. The most prevalent pathology diagnosed in patients on antiretroviral therapy is benign lymphoepithelial cysts (BLEC); this was also the most common pathology diagnosed in female patients. Benign lymphoepithelial cyst (BLEC) is common in salivary gland pathology associated with HIV. They occur in both adults and children. The aetiopathogenesis is one that has not clearly been defined but is thought to arise from lymphoid proliferation that leads to duct obstruction and dilation of salivary gland. The other theory is that they develop from lymphoid proliferation in intraparotid lymph nodes, which causes glandular epithelium to become trapped within the nodes and results in cystic entrapment. Inspissated secretions can lead to duct obstruction causing pain and sialadenitis. BLEC usually comprises cystic and solid components and radiologic evaluation usually demonstrates bilateral changes. [2] The cytomorphology of benign lymphoepithelial cyst comprises a heterogeneous lymphoid population, scattered foamy macrophages and anucleated squamous cells in a proteinaceous background. [2]

Infection was the most common pathology diagnosed in patients not on antiretroviral therapy and is also the most common cytopathology diagnosed in male patients. Mycobacterium tuberculosis is responsible for most of the infectious processes within the salivary gland in HIV positive patients. This is followed closely by mycobacterium avium complex. These can be diagnosed on cytology, histology, culture, genexpert etc. Acid-fast bacilli can be demonstrated on the Ziehl-Neelsen stain. Of the 81 cases in our study categorised as infective (mycobacterium infection or abscess), follow-up results demonstrated 16 cases where mycobacterium tuberculosis was confirmed either with a positive Ziehl Neelsen stain for acid-fast bacilli or positive Gene Xpert. No other organisms were cultured.

In the case of benign neoplasms, pleomorphic adenoma (PA) was diagnosed in 7 cases (1.9%). There were too few follow-ups available to calculate ROM for benign salivary gland lesions.

Pleomorphic adenoma is the most common tumour in all salivary glands, both children and adults. Two thirds of parotid tumours and 50% of all salivary gland tumours are PAs. The most commonly encountered site is the superficial parotid. On examination, the nodule is firm, reflecting abundance of chondromyxoid matrix; the aspiration is painless. In this study, the cytomorphology of PA was consistent with the classic description, namely varying quantities of epithelial and myoepithelial cells in a chondromyxoid background .[14]

There is a stigma attached to being HIV infected in South Africa. [15] HIV/AIDS management requires patients to attend designated HIV health care facilities for routine screening procedures and treatment. The stigma associated with HIV infection encourages non-compliance resulting in high viral loads, low CD4 counts and susceptibility to a host of infective processes. In our study, the majority of cases did not state whether patients were on antiviral therapy or not; we thus looked to the CD4 count as a guide in deciding the likelihood that a patient was receiving antiviral therapy. Given the average low CD4 counts, we can assume that many patients were not on antiviral therapy or non-compliant with therapy. Southern Africa has a high burden of HIV and since the release of previous guidelines; the scale-up of antiviral therapy has continued to grow. Cohort studies from the region show excellent clinical outcomes; however, antiviral therapy is still being started late in some patients resulting in relatively high morbidity and mortality rates. [11]

There is a significant difference in the CD4 count of patients on antiretroviral therapy versus patients not on treatment. The goals of therapy are achieved by completely suppressing viral replication for as long as possible using well-tolerated and sustainable treatment. With prolonged viral suppression, the CD4 lymphocyte count usually increases with which is accompanied by pathogen-specific immune function for most patients. The result is a reduction in risk of HIV associated morbidity and mortality. [10] In the current study, the mean CD4 count of patients on antiretroviral therapy is 453.8 and the mean count of patients not on treatment is 276.4. This translates to the pathologies diagnosed by CD4 count (Table 6) which highlights patients with the lowest mean CD4 count had the highest number of infective process diagnosed. In a country like South Africa with a high prevalence of HIV infection and new cases reported daily, access to antiretroviral treatment is crucial. Resources need to be

dedicated to this cause in order to improve HIV counselling, testing and quality of life of HIV infected patients and decrease morbidity and mortality associated with this disease.

The study also highlights that female patients of child- bearing age are the most represented in the HIV- infected population. This could be attributable to the fact that women of child- bearing age usually have access to additional health resources in the form of antenatal clinics where HIV testing is a part of routine work-up in an attempt to identify and prevent mother to child transmission and cervical cancer screening programmes. Therefore more HIV- infected women are identified and initiated on treatment. The most common cytologic diagnosis in female patients undergoing salivary gland FNA is benign lymphoepithelial cyst at 27.1%.Male patients have fewer reasons to attend primary health facilities hence fewer are tested and managed for HIV and many will present with AIDS defining illnesses with an associated high rate of morbidity and mortality. The most common pathology diagnosed on salivary gland FNA in males is infective at 28%.

In conclusion, this study was an assessment of the pathologies described in HIV infected patients and the impact of antiretroviral therapy. It is clear that patients on antiretroviral therapy had higher CD4 counts, fewer infective lesions, and more reactive and benign lesions were diagnosed with BLEC being the most common pathology seen. Patients not on treatment had significantly lower CD4 counts and were frequently diagnosed with infective processes. FNA is a useful method of assessing salivary gland lesions in HIV infected patients. The method is cheap, minimally invasive and results are available immediately for quick and effective patient management. The Milan System for Reporting Salivary Gland Cytopathology is well-suited to reporting out salivary gland FNAs in an HIV-infected population.

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Section 3: Research Proposal

The effect of antiretroviral therapy on the pathology of salivary gland masses in HIV-infected patients

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3.1 Introduction.

Salivary glands are found in and around the mouth and throat. The major salivary glands include the parotid, submandibular and sublingual glands. (1)

The function of the salivary glands is to secrete saliva into the mouth. The parotid glands secrete saliva through the salivary ducts, submandibular glands beneath the tongue and the sublingual glands secrete saliva through many ducts in the floor of the mouth. The saliva produced is used to moisten the mouth, aid in digestion, and prevent tooth decay. (1)

Minor salivary glands are present in the linings of the mouth and throat as well as the buccal mucosa. (1)

There is a great burden of Human immune deficiency virus (HIV) in South Africa and subsequent acquired immune deficiency syndrome (AIDS). The virus is a T lymphotropic retrovirus that infects cells with CD4 receptors. This causes a gradual decline in CD4 lymphocytes with resultant decline in host immunity. This places the individual patient at risk for developing a multitude of opportunistic diseases ranging from infectious processes to neoplasms, benign and malignant. Salivary gland lesions are common in HIV-infected patients, especially children. The majority of salivary gland lesions occur in the parotid gland and include inflammatory processes, infections; benign lesions such as benign lymphoepithelial cysts (BLEC) and neoplasms which include pleomorphic adenoma, lymphoma, Kaposi Sarcoma, Non Hodgkin Lymphoma and squamous cell carcinoma. (7) Clinically, patients with salivary gland lesions usually present with painless palpable masses in the parotid region or in the floor of the mouth for submandibular masses. Other signs include pain, facial nerve

palsy and enlarged lymph nodes which could suggest malignancy. The long term management for patients with HIV is antiviral therapy (ARVs). The guidelines for the initiation of ARVs have changed multiple times since it was introduced in the country in 2004. The initiation of ARVs is based predominately on CD4 count and clinical condition of the patient. In 2004, a CD4 count of < 200 cells/uL was required before the initiation of ARVs. In 2012, the CD4 count was revised to <350 cells/uL and in 2014 the CD4 count was revised to <500 cells/uL if the patient was motivated and ready to start treatment. Currently, in August 2015 the CD4 count was discarded as a guide and the guideline now states that any patient diagnosed with HIV/AIDS is eligible for initiation of antiviral therapy.

Imaging modalities for salivary gland lesions include sonography as the first step in children and pregnant women; especially for lesions in the superficial lobe of the parotid. Computed tomography (CT) and magnetic resonance imaging (MRI) are other modalities used in the radiologic diagnosis of salivary gland lesions. CT is the method of choice in patients suspicious for inflammatory disease or in patients with contraindication for MR imaging. MR is preferred where malignancy is suspected.

The differential diagnosis between reactive, benign and malignant lesions cannot be established by simple physical examination but requires complementary diagnostic methods. This includes imaging and FNA guided by ultrasound imaging (12).

Fine-needle aspiration (FNA) biopsy is frequently used in the diagnosis of lesions of the salivary glands. It is most often the first tissue-based procedure applied to establish a diagnosis before any surgical intervention. FNA of the salivary gland is useful in that, it is minimally invasive, easy to perform, the smear can be evaluated immediately and it can be performed multiple times to obtain adequate representation of lesional tissue. If a non-benign lesion is diagnosed, the patient and surgeon are given the opportunity to discuss possible management options. (2)

FNA of salivary gland is a good diagnostic test and has a sensitivity and specificity, ranging from 60% to 100% and 90% to 100%, respectively (5;12). However, as pointed out by Stanley and Schwarz et al (2), it is difficult to compare the accuracy of statistical data because many of the published series are small and unsatisfactory or non-diagnostics were excluded in the studies. There is a degree of false negatives and false positives in salivary gland FNA. False negatives are mainly due to underdiagnosing of low grade tumour because of their bland cytological features as well as the difficulty in diagnosing hypocellular lesions. False positives are a problem because of the over-diagnosing of reactive inflamed lesions as non-benign. There is also the problem of shared cytological features between benign and malignant tumours; this also accounts for the long lists of differential diagnosis given when these lesions are encountered (2).

The issue of post FNA–related tissue changes is important, as these changes may affect the histologic interpretation of resected specimens. Potential tissue changes include tumour infarction, haemorrhage, granulation tissue, and metaplastic changes. Granulation tissue and metaplastic cellular changes potentially can be confused with sarcoma, carcinosarcoma, or both. Seeding of the needle tract by tumour cells is a rare but serious complication of FNA. (2)

A previous study conducted by Michelow *et al (4)* looked at salivary gland fine needle aspiration in the setting of HIV-infected patients in South Africa. The study was conducted at a time where antiviral therapy was initiated at a CD4 count of less than 200 cells/uL. The findings of that study concluded that most diagnosis of FNA salivary gland masses in HIV-infected patients were benign and comprised 33.9% reactive lymphadenopathy, 23.2% benign lymphoepithelial cysts, 12.5% mycobacterial infections and 10.5% abscesses. Neoplasms accounted for 6.7% and included 11 pleomorphic adenomas, 13 lymphomas, 3 Kaposi sarcoma, 1 squamous cell carcinoma, 1 metastatic carcinoma and 1 rhabdomyosarcoma.

There is conflicting evidence as to whether HAART causes an increase in salivary gland lesions or a decrease in the incidence of salivary gland pathology in HIV infected patients. The theory behind the former state the initiation of antiviral therapy decreases the HIV viral load and increases the CD4 count, therefore the body is able to mount an appropriate immune response to opportunistic infections that have otherwise been clinically silent, this phenomenon is known as immune reconstitution syndrome; this suggests that an infectious agent may play a role in salivary gland enlargement in the HIV positive patient. On the other hand, initiation of HAART has also shown a decrease in salivary gland pathology. This is due in part to cessation of viral replication, lower viral load and an increase in the CD4/CD8 count.

As mentioned previously, the indication for initiation of antiviral therapy has changed since that study was conducted, therefore the changes, if any, in the pathologies described in FNA of salivary gland masses in HIV –infected patients needs to be studied and described to better prepare clinicians for disease burden and management of these patients.

The impact of this in the setting of disease manifestation and progression is something we would like to explore further, specifically addressing salivary gland lesions in the HIV patient.

3.2 Problem Statement

Antiviral therapy has been life saving for patients infected with HIV since it was introduced many years ago. However, initiation of antiretroviral therapy was dependent on CD4 count as set out in the antiretroviral therapy guidelines. With the implementation of the new guidelines which allows initiation of antiviral therapy to patients regardless of CD4 count, the pathologies noted in HIV-infected patients should be revised in order to assess the impact of this on these patients.

3.3 Aim and Objectives

3.3.1 Aim

The aim of this study is to identify the impact of earlier antiretroviral therapy introduction on FNA of salivary gland masses in HIV-infected patients.

3.3.2 Objectives

The objectives of this study is to determine the difference in fine needle aspiration of salivary gland masses prior to earlier antiviral therapy initiation and the current status with introduction at an earlier stage of the disease.

3.4 Demarcation of Study field

The study will be conducted from the Cytology Laboratory of the National Health Laboratory Services (NHLS) in Braamfontein, Johannesburg, which is affiliated with University of the Witwatersrand. This study will use FNAs performed at several public sector hospitals in and around Johannesburg, Gauteng and sent to the cytology lab at Braamfontein. A data search will determine exactly how many FNAs are received by the lab; and of those how many are from salivary gland masses in HIV-infected patients.

3.5 Ethical considerations

Application to the Human Research Ethics committee (Medical) and Postgraduate Office of the Faculty of Health Science, University of the Witwatersrand will be submitted for clearance of this study.

This is a retrospective study and no new procedures will be performed. These FNAs were performed on the request of the attending physician as a diagnostic procedure. The study will be conducted on archived departmental slides. The patients shall remain anonymous. Patient details (name and HIV status) will only be available to the primary investigator. The patients included in this study will not be known by the investigatory and no direct contact will be possible. The information will be discussed with the statisticians in a completely anonymous form. The patient and the institutions will not be financially liable for any part of this study and the study will not influence patient care in any way.

Permission to undertake this study in the Cytology Unit, Dept. of Anatomical Pathology, University of the Witwatersrand and National Health Lab Services has been obtained (Appendix 1).

3.6 Research methodology

3.6.1 Research design

The study will be contextual and retrospective. The study will be contextual as it will be conducted on FNAs received by the NHLS Braamfontein Cytology department from various clinics in and around Johannesburg. It will be a retrospective study as archived slides shall be examined and no new procedures will be performed.

3.6.2 Study population

The study population consists of HIV-infected patients from August 2015 to August 2016 (i.e.1 year) who underwent FNA of salivary gland lesions on the request of their attending clinicians.

3.6.3 Study sample

i)Sample size

After ethics approval is obtained, a data search will be conducted to determine how many FNAs of salivary gland masses in HIV-infected patients. A retrospective power analysis will then be performed in order to determine if this number is appropriate. A statistician may be consulted in this regard.

ii) Sampling method and data collection

The data base of the Cytology Unit, Department of Anatomical Pathology, University of the Witwatersrand and NHLS will be searched to find all HIV-infected patients who have FNAs of salivary gland masses from 01 September 2014 till 31 August 2016. These shall be recorded by an allocated number, with a separate key corresponding to the case number. This key shall be kept separately such that patients remain anonymous.

The glass slides of a randomly selected 10% of cases will be reviewed by Dr AA Coleman Eghan and Dr P Michelow for quality control purposes. The randomisation process will be determined after the protocol is approved, ethics permission obtained and a computer search performed so the number of cases will then be known. If the original and reviewed diagnoses are discrepant, all the study cases will require review by Drs Eghan and Michelow.

iii) Inclusion and exclusion criteria

All cases where HIV-infected patients with salivary gland masses underwent FNA shall be included.

All HIV negative patients will be excluded from this study.

HIV-infected patients who presented with a lesion other than in salivary gland, will be excluded.

4.6.4 Data collection

The cases reported during the study time frame will be identified by a computer-based record search. The cases are identifiable by an administrative code unique to salivary gland FNA, entered into the NHLS database at the time of receipt of the specimens.

Data points will be captured from each case into an anonymised spreadsheet, as follows (Appendix 2):

1. Each case will be allocated a study number, starting at 1 and proceeding chronologically.
2. Patient age
3. Patient sex
4. Clinical presentation (e.g. salivary gland mass)
5. Cytology laboratory reference number
6. Cytology diagnosis (in words)
   6.1 Reactive lymphoid hyperplasia
   6.2 Benign lymphoepithelial cyst
   6.3 Normal salivary gland tissue
   6.4 Inadequate
   6.5 Infective (Abscess; mycobacteria)
   6.6 Pleomorphic adenoma
   6.7 Squamous cell carcinoma
   6.8 Kaposi sarcoma
   6.9 Non Hodgkin Lymphoma
   6.10 Other

7. HAART status at time of FNA

8. Duration of HAART at time of FNA

9. CD4 count at time of FNA (If the CD4 count is not stated of the initial lab request form, the Trakcare lab system will be used to retrieve these results if available)

10. FNA site

Once pathologies have been recorded according to the data capture sheet, this information shall be returned to the primary investigator. At this point the primary investigator will use the numbers to retrieve the required patient information, if available (HAART status, duration of HAART, CD4 count at time of FNA) as was submitted with the slide from the health care provider. This information will not be available at the time of slide examination in order to limit bias.

4.6.5 Data analysis

The pathologies diagnosed on FNA slides that have been reviewed for quality assurance purposes will be noted.

Descriptive statistics including calculation of measures of centre mean and median as well as measures of variation including standard deviation and IQR.

Difference in means (if data is parametric) will be analysed using a two sampled t-test with a p-value of <0.05 indicating statistical significance.

Difference in medians (if data is non-parametric) will be analysed using a Mann-Whitney U test with a p value of <0.05 indicating statistical significance.

Categorical data will be analysed using a Chi squared test.

The Primary Investigator shall be responsible for the data search, assisting in case retrieval, numbering of cases, examination of a proportion of slides, data collection and analysis and then write up of the findings.

3.7 Significance of the study

IF this study is statistically significant, it would reliably assess whether the initiation of antiviral therapy at an earlier stage in the disease has impacted the HIV-infected population positively or negatively in terms of salivary gland masses.

It is envisaged that this knowledge will streamline HIV-infected patients with a salivary gland mass presenting to a South African health care facility, leading to a more satisfactory outcome.

3.8 Validity and reliability

This study is valid because it will illustrate the impact antiviral therapy use in the context of initiation and the impact that has on patients with salivary gland pathology.

By employing the skills of experienced individuals, looking at an appropriate number of cases, excluding inappropriate cases and analysing data obtained with accepted tools, the results should be reliable.

3.9 Potential limitations

The important limitation in this study would be that the referring clinician does not always provide information in the form of CD4 count and whether patient is on therapy and duration thereof.

3.10 Project outline

Time frame

Activity	Jan '17	Feb '17	Mar '17	Apr '17	May '17	Jun '17	Jul '17	Aug '17	Sept' 17	Oct '17	Nov '17	Dec '17	Jan '18
Proposal													
Lit. review													
Submit proposal													
Ethics													
Corrections													
Data collection													

Data analysis													
Write up													
Submission													

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3.12 APPENDICES

Appendix 1 – Data capture sheet

NAME	Study number	Cytology lab reference number	Sex	Age	Clinical presentation	FNA Site	*Diagnosis	HAART status at time of FNA	CD4 count at time of FNA	Duration of HAART	Comments

* Codes for cytologic diagnosis

1 Reactive lymphoid hyperplasia
2 Benign lymphoepithelial cyst
3 Normal salivary gland tissue
4 Inadequate
5 Infective (Abscess; mycobacteria)
6 Pleomorphic adenoma
7 Squamous cell carcinoma
8 Kaposi sarcoma
9 Non Hodgkin Lymphoma
10 Other