Prevalence and factors of HIV associated Oral Kaposi Sarcoma at Wits Oral Health Centre, Johannesburg.

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364940

A research project submitted in partial fulfilment of the requirements for the degree of Master of Sciences (MSc) in Dentistry, University of the Witwatersrand, Johannesburg.

2024

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ABBREVIATIONS:

HIV -	**Human Immuno-deficiency Virus**
AIDS-	**Acquired Immuno- deficiency Virus**
HHV8-	**Human Herpes Virus 8**
OKS-	**Oral Kaposi Sarcoma**
KS-	**Kaposi Sarcoma**
KSHV-	**Kaposi Sarcoma Herpes Virus**
WOHC-	**Wits Oral Health Centre**
NHLS-	**National Health Laboratory Service**
AARMS -	**Academic Affairs and Research Management System**
PAAB-	**Patient Administration and Billing system**
CPS/ML-	**Copies per Milliliter**
ART/ARV -	**Anti-Retroviral Treatment**
IRIS-	**Immune Reconstitution Syndrome**

Keywords:

Oral Kaposi Sarcoma, Human Immuno-deficiency Virus, Anti-retroviral Treatment, Prevalence of OKS in South Africa .

ACKNOWLEGEMENTS

Thank you to my mother, Aroona, for her endless love, support, and strength. Thank you in memory to my late father, Sam, for his guidance and sacrifice, my education was always of the utmost importance to him. Thank you to my sisters Jelica and Nishta for their encouragement. A special thank you to my aunty Chandra and uncle Roy for their support.

I would like to acknowledge Dr. Sankeshan Padayachee, my supervisor and mentor for his guidance and commitment to this project. A sincere thank you to Dr. Tumane Mafojane for his expertise and assistance and to Prof. N Wood for his support. Thank you to Dr. Gill Hendry for her assistance with the statistics.

DECLARATION

I declare that *Prevalence and factors of HIV associated Oral Kaposi Sarcoma at Wits Oral Health Centre, Johannesburg* is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Full Name: Hasita Chetty

Date: 12/07/2024

Signature of candidate……

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ABSTRACT

Introduction:

HIV associated Oral Kaposi Sarcoma (OKS) is a neoplasm predominantly occurring in immunocompromised patients. Therefore, it is often observed in an HIV positive and AIDS population (Moore & Chang, 2003). KS is caused by the Kaposi Sarcoma Herpes Virus (KSHV) or Human Herpes Virus-8 (HHV8) (Naidoo et al, 2016). The risk of acquiring KS increases in the presence of HIV infection and immunosuppression (Kamulegeya & Kalyanyama, 2008). HIV-KS can occur at any stage of HIV infection but has been more prevalent at the stage of AIDS or severe immune impairment (Khammissa et al., 2012).

Both HHV8 infection and HIV/AIDS are highly prevalent in Africa (Kamulegaya and Kalyanyama, 2008). There is a lack of current evidence documenting the relationship between HIV/AIDS and OKS since the implementation of ART, therefore this study intends to augment the existing literature.

This study aimed to determine the prevalence and factors of HIV associated OKS, on histopathologically diagnosed patients attending the WITS Oral Health Centre, Johannesburg, between 2008 and 2018. This period predates and postdates the roll out of ART in the South African public health sector in 2012 and speaks to the relationship between ART and the occurrence of HIV associated OKS.

Materials and Method:

This is a cross-sectional study using records from the Wits Oral Health Centre (WOHC) and National Health Laboratory Service (NHLS). The study period is 10 years from January 2008 to December 2018. Prevalence of OKS was calculated based on patients with a positive histopathological diagnosis of OKS within the study period from the NHLS database.

Ethical Considerations:

Permission was requested from the Academic Affairs and Research Management System (AARMS) National Health Laboratory Service (NHLS) and WOHC to access patient files. Records of patients with a positive OKS histopathological diagnosis were noted. They were allocated a study number and patient number on a data sheet. The corresponding patients' files were accessed from WOHC to attain further information (as per the data sheet). Patient confidentiality was be maintained as no names were recorded and files were allocated a number for cross-referencing between NHLS and WOHC data.

Ethics clearance was attained from the Wits Human Research Ethics Committee to carry out this study.

Results:

The prevalence of OKS that was found in this population (137679 patients seen at WOHC) was 0.017432%(n=24). There were more females that presented with OKS than males. The mean age of presentation was 39.11 years of age (SD 12.459). There was a significant relationship between high viral loads and a low CD4 count. The palate was the site most frequently biopsied in diagnosed OKS cases.

Conclusion:

The prevalence of OKS over the study period was very low. The mean age of OKS presentation was 39.21 years of age. More females presented with HIV associated OKS than males and the palate was the site, from which most biopsy samples were taken in OKS diagnoses. There is a significant relationship between high viral loads and low CD4 cell counts. This study is suggestive that a possible reason for the low number of OKS cases could be administration of ART by state institutions in South Africa, this can be further investigated to establish the effect of ART on OKS.

CHAPTER 1

INTRODUCTION

HIV associated Oral Kaposi Sarcoma (OKS) is an HIV related condition predominantly occurring in immunocompromised patients. Therefore, it is often observed amongst an HIV positive and AIDS population (Moore & Chang, 2003). In 2018, the United Nations (UN) recorded globally, 37.9 million people living with HIV. Of that number, 20.6 million are from Eastern and Southern Africa (UNAIDS, 2019). The UN's 2018 statistics showed 13.8 million infected people in Eastern and Southern Africa were receiving antiretroviral (ART) treatment (UNAIDS, 2019).

In South Africa, circumstances around KS have evolved with changes in political decisions regarding the administration of ART (Simelela & Venter, 2014), which has, in turn, enabled a need for research into the oral manifestations of HIV/AIDS, particularly OKS in a South African setting.

CHAPTER 2

LITERATURE REVIEW

2.1 Background and Epidemiology

Kaposi sarcoma (KS) is described as four variants: Classic KS disorder, that is seen among older Mediterranean men, transplantation KS occurs in recipients of organ transplants with T-cell immunosuppression, Endemic African KS which is widespread in Sub-Saharan Africa and HIV-associated KS, seen in patients with HIV/AIDS (Khammissa et al., 2012). KS is caused by the Kaposi Sarcoma Herpes Virus (KSHV) or Human Herpes Virus-8 (HHV8) (Naidoo et al, 2016).

The risk of acquiring KS increases in the presence of HIV infection and immunosuppression (Kamulegeya & Kalyanyama, 2008). HIV-KS can occur at any stage of HIV infection but has been more prevalent at the stage of AIDS or severe immune impairment (Khammissa et al., 2012). Both HHV8 infection and HIV/AIDS are highly prevalent in Southern Africa (Kamulegaya and Kalyanyama, 2008). Globally, KS has affected more men than women but in Africa more young women are infected with HIV than young men, predisposing young women to the KS infection (Khamissa et al, 2012).

A study performed in South Africa in 2012 estimated the prevalence of HIV infection as 30% and HHV8 infection as 40% in certain regions (Khamissa et al, 2012). Zwane et al, found that KS was the most commonly occurring cancer of the head and neck in a study based in Johannesburg (Zwane et al., 2018). These statistics provide some insight into the presence of KS in a South African population (Khamissa et al, 2012). There is a lack of current evidence documenting the relationship between HIV/AIDS and OKS since the implementation of ART, therefore this study intends to augment the existing literature.

2.2 Pathogenesis

2.2.1 Viral Structure and Transmission

KSHV has a linear, double-stranded DNA genome surrounded by an icosahedral capsule, enclosed by a tegument layer consisting of host and viral proteins and an outer lipid bilayer of viral glycoproteins (Purushothaman et al., 2016).

KSHV is transmitted through sexual contact, oral secretions and cutaneous exposures (Khammissa et al., 2012). The virus infects vascular and lymphatic endothelial cells (Pantanowitz et al., 2013).

The life cycle of KSHV can exhibit an asymptomatic latent phase or an aggressive lytic phase, both with distinct viral gene-expression profiles (Purushothaman et al., 2016).

2.2.2 Kaposi Sarcoma defined as a Neoplasm

There is debate regarding KS being a true neoplasm (Kang et al., 2008). Early stage KS has not been considered a true neoplasm but rather, a hyperplastic response (Kang et al., 2008). The progression of KS is associated with neoplastic inflammatory and angiogenic processes in addition to disturbance of the innate immune system and a granulation-like tissue with abundant inflammatory cells consisting of lymphocytes, monocytes/macrophages (Kang et al., 2008). Recruitment of circulating cells into tissues is induced by inflammatory cells which promote the production of angiogenic factors that mediate angiogenesis and oedema (Kang et al., 2008). These inflammatory cells activate endothelial cells to attain the phenotypic KS spindle cells, a characteristic histological sign vital in the diagnosis of KS (Kang et al., 2008). The proliferating spindle cells form irregular microvascular channels (Moore & Chang, 2003). Due to the infiltrative nature of these lesions, uninfected tissues not containing the viral genome become entangled with tumour cells leading to further invasion of surrounding structures (Moore & Chang, 2003).

KS exhibits mechanisms which inhibit innate immune and tumour suppressor pathways facilitating viral oncogenesis (Moore & Chang, 2003). Cell cycle control is dependent on p53 tumour suppressor protein for its ability to halt the progression of the cell cycle in response to various stimuli (Ruocco et al., 2013). The primary function of p53 in oncogenesis as a tumour suppressor protein is reinforced by the fact that the TP53 gene is the most regularly mutated gene in human cancer. HHV-8 virus interferes with p53 pathway at numerous levels (Ruocco et al., 2013). The resultant effect caused by the HHV-8 genome is reduction of p53 levels, suppression of p53-mediated apoptosis and an alteration in the p53 pathway (Ruocco et al., 2013). The behaviour of the HHV-8 virus at a cellular level and its effect on p53 confirms the classification of KS as a neoplasm (Ruocco et al., 2013).

2.2.3 Role of HIV-1 in KSHV Pathology

HIV-1 is one of the most significant co-pathogens known to exacerbate KSHV induced pathologies (Thakker & Verma, 2016). Many studies have shown that individuals infected with KS who are also HIV-1 seropositive, are at significantly higher risk of developing KS compared to HIV-seronegative patients

(Thakker & Verma, 2016). In comparison of HIV-1 and HIV-2 subtypes, KS develops more frequently in those individuals that are HIV-1 seropositive (Thakker & Verma, 2016).

A major effect of uncontrolled HIV infection is severe deregulation of both adaptive and innate host immunities, a very important co-factor contributing to the progression of KS pathologies (Thakker & Verma, 2016). HIV-1 primarily establishes latency in resting memory CD4+T cells and in the cells of monocytic/macrophage CD4+ lineage (Thakker & Verma, 2016). CD4+ T cells are highly susceptible to HIV-1 induced cytopathic effects (Thakker & Verma, 2016). Progressive loss of CD4+ T cells, that stem from chronic immune activation is a major sign of KSHV co-infection with HIV (Thakker & Verma, 2016).

HIV-1 encoded Tat (transcriptional transactivator) is a small cationic peptide (Thakker & Verma, 2016). HIV-Tat is often detected in KS spindle cells, where it encourages the growth of spindle cells as well as inducing apoptosis of CD4+ T cells in KSHV (Thakker & Verma, 2016). It plays multiple roles in controlling HIV-1 replication and host immune deregulation (Thakker & Verma, 2016). Increased risk of developing KS associated with low CD4+ T cell count indicates that immune suppression associated with HIV co-infection plays essential roles in KSHV induced pathologies (Thakker & Verma, 2016). Clinical manifestations of AIDS-KS is of increasing incidence, as a result of decreasing CD4 T cell counts (Pantanowitz et al., 2013). It has been recorded that AIDS-KS shows remission in those patients receiving ART and therefore can be treated by the management of the underlying condition of AIDS (Pantanowitz et al., 2013).

A South African study found a decline in KS risk, as CD4 cell counts increased (Rohner et al., 2014). It was recommended that maintaining CD4 cell counts as ≥500/µl decreased the risk of developing KS as well as initiating ART at CD4 cell counts of >500/µl could further reduce incident KS burden (Rohner et al., 2014). This occurrence is uncharacteristic of neoplasms and leads to an understanding of the argument that KS is not a true neoplasm (Kang et al., 2008).

2.2.4 Kaposi Sarcoma (KS) and Immune Reconstitution Syndrome (IRIS)

In some cases the Immune Reconstitution Inflammatory Syndrome(IRIS) phenomenon causes development of KS or increase in severity of existing KS, following initiation of ART (Pantanowitz et al., 2013). KS-IRIS occurs shortly after commencement of ART simultaneously with a decrease in the plasma HIV load and increase in CD4+ T cell count. A clinical presence of KS as well as higher KSHV antigen load prior to the start of ART can be a predisposing factor to development of KS-IRIS (Letang et al., 2010). The onset of IRIS is eight to twelve weeks after the start of ART (Pantanowitz et al., 2013).

2. 3 Distribution of Antiretrovirals

Antiretroviral treatment (ART) has been administered within the public health sector of South Africa since 2004, but in 2016 many patients in outer lying areas were still unable to receive the medication (Naidoo et al., 2016).

Due to political and scientific debate, South Africa did not implement antiretroviral therapy (ART) until 2004 (Simelela & Venter, 2014). In 2006 the first National Strategic Plan (NSP) was released for HIV/AIDS and STI's which aimed to expand accessibility to ART to those at a significantly low CD4+ T cell count as a result of HIV and AIDS (Simelela & Venter 2014). In 2009 a new political term began and the NSP was restructured to provide ART to all HIV positive children under 1 year of age, and all pregnant women, as well as all HIV and Tuberculosis (TB) co-infected patients with a CD4+ cell count of less than 350/µl (Simelela & Venter, 2014). By 2012 the South African National Department of Health amplified the threshold to 350 cells /µl for all adults and children, in addition to administering ART to all HIV positive patients despite their CD4 count, as previously, administration of treatment was dependant on the CD4 count (Simelela & Venter 2014).

ART has reduced presentation of viral and auto-immune related lesions by 30% (Jordan et al., 2016). However, in a small percentage of patients rapid clinical progression of KS is observed shortly after initiation of ART, as a result of IRIS (Tsang & Samaranayake, 2010). AIDS-related KS is clinically the most aggressive form and requires more complex treatment compared to the other forms of KS (Thakker & Verma, 2016). The changing prevalence in HIV and AIDS-related lesions requires further investigation. It was thus expected that the administration of ART would influence the prevalence of OKS in a South African population.

Worldwide, Kaposi's sarcoma is the most common HIV-related neoplasm (De Bruin et al., 2013).

In the South African context, OKS is a lesion that is frequently observed in the public health sector; however, it's current prevalence has limited documentation (Naidoo et al., 2016). The aim of this study was to observe the presentation of OKS in a South African setting and to better define the prevalence and factors of the disease through the analysis of patient data at Wits Oral Health Centre (WOHC).

CHAPTER 3:

3. 1 STUDY DESIGN AND METHODOLOGY

3. 2 Aim:

To determine the prevalence and factors associated with HIV Associated Oral Kaposi Sarcoma (OKS) among patients attending the Wits Oral Health Centre (WOHC) from 2008 to 2018.

3. 3 Objectives:

To determine the prevalence of OKS among patients attending the WOHC from 2008 to 2018.

To determine factors associated with OKS among patients attending the WOHC from 2008 to 2018.

Methods:

Study design:

This is a cross-sectional study using records from the National Health Laboratory Service (NHLS) and WITS Oral Health Centre (WOHC). The study period is 10 years from January 2008 to December 2018.

3. 4 Study population and sample size:

All records of patients (data accessed from the National Health Laboratory Service) who attended the Wits Oral Health Centre from 1 January 2008 to 31 December 2018 were included in this study. Prevalence of OKS was calculated based on patients with a positive histopathological diagnosis of OKS within the study period from the NHLS database.

Study Sample estimation:

No sampling was conducted in this study as all records within the study period will be used for this cross-sectional study.

In accordance with a study by Zwane et al, (2018), a minimum of 46 observations are required to detect the 35.3% difference at 90% statistical power. The output with the parameters is given below;

power two proportions 0.004 0.357, power(0.9)

Estimated sample sizes for a two-sample proportions test

Pearson's chi-squared test

Ho: p2 = p1 versus Ha: p2 != p1

Study parameters:

Alpha

= 0.0500

Power = 0.9000

delta (difference in prevalence of KS between HIV+ and HIV- participants) = 0.3530

p1 (prevalence of KS among HIV- patients) = 0.0040

p2 (prevalence of KS among HIV+ patients patients) = 0.3570

Estimated sample sizes:

N = 46 (minimum number of observations required to detect the 35% difference at 90% statistical power)

3. 5 Inclusion criteria:

Records of patients seen at Wits Oral Health center with a confirmed positive histopathological diagnosis of OKS between January 2008 and December 2018

3. 6 Exclusion criteria:

Records of patients who were seen outside the years 2008 and 2018

- Records of patients with an unconfirmed histopathological diagnosis of OKS
- Records of patients with a HIV negative diagnosis

3. 7 Ethical considerations:

Permission was attained from the NHLS (Reference no. PR2119319, Appendix V) Permission was also attained from WOHC (Reference no.HRRC/July/07/2021, Appendix VI). Patients with a positive OKS histopathological diagnosis were noted, each patient allocated a corresponding patient number and study number on the data sheet. Patient confidentiality was maintained as no names were recorded.

Ethics clearance was attained to the Wits Human Research Ethics Committee to carry out this study (Clearance certificate no. M210710, Appendix IV) .

3.8 Data collection

- Data of patients with OKS was requested from the NHLS database.
- A list of patient data was received from the NHLS database containing details of the histopathological report, HIV testing, viral load and CD4 cell count.
- All records of patients found to have a positive or unknown HIV test as well as a positive histopathological diagnosis of OKS were included in the study.
- Each patient's unique file number and lab number were allocated a corresponding study number to maintain anonymity.
- This study number was then used to input the NHLS data into a data sheet.
- The PAAB system at the WOHC was then accessed and lists of patients seen at the WOHC during the study period was recorded. This was done to establish the total number of patients seen during the study period to calculate prevalence.
- Using the patient numbers from the NHLS, patient files were accessed from the WOHC Oral Medicine and Periodontology clinic to investigate and gather more information regarding clinical factors, medical history, and treatment.
- All information was recorded onto the data sheet (Appendix I, II)

3.9 Data Management Plan

Data collection

- A profile was created on the NHLS AARMS website to request patient records for patients with a positive histopathological diagnosis for OKS, seen at WOHC between the years 2008-2018.
- The NHLS mentor retrieved this data and it was sent via email in the form of an excel spreadsheet.
- Upon receiving the data, it was reviewed to exclude records of patients whose diagnosis was unconfirmed and those that were HIV negative
- The records of patients were then put into an excel data sheet, in which each patient was given a unique patient and a study number to protect their identity.
- The patient files that were available at WOHC were located and investigated for further data pertaining to patient history and management.
- The PAAB system was used to access patients logs from 2008 to 2018. A population number for patients seen over the study period was needed. These numbers were necessary for calculation of prevalence.
- A large volume of data was recovered from PAAB as patients had repeatedly returned over the study period. The data had to be sifted through to delete repeat patients and determine the correct number of patients seen over the study period.
- All the above-mentioned data was saved to a laptop and backed up to a hard drive.

Documentation and Metadata :

- Once each patient recorded had a unique patient and study number, the NHLS data was transferred to an excel spreadsheet.
- This spreadsheet captured records of patients' age, sex, viral load, CD4 count and site of biopsy.
- Both data sheets were sent to the biostatistician for analysis.
- Subcategories for viral load and CD4 counts were specified to the biostatistician for analysis of data and for use in the graphs.
- The biostatistician then did analysis of all the data received and sent a report including tests used and accompanying graphs.

Storage and back up:

- All excel sheets and documents containing data was saved to a laptop and backed up on a hard drive.

Resources:

- The cost of the biostatistician and printing cost was privately funded by me.

Data destruction:

- All data related to this study will be deleted on completion of this MSC in accordance with the POPIA.
- The biostatistician will also be instructed to destroy data
- This is necessary to protect the patient records.

3. 10 Data Analysis:

Descriptive statistics were conducted to show the prevalence of OKS among patients attending the WOHC from 2008 to 2018. A two-sided p values <0.05 was considered significant throughout. Interactions between covariates were checked. The Chi-squared goodness of fit test, a univariate test, was used to compare observed results with expected results to report on significant relationships between clinical factors. Fisher's exact test was used to determine a relationship between the viral load and the CD4 count. Statistical Software Package for Social Sciences (SPSS) was used.

Limitations:

Difficulties were experienced regarding inconsistency and lack of clinical records of the WOHC during the study period. Absence of records pertaining to HIV testing, ART administration, patient history and details of clinical findings in the patient files, prevented more in-depth investigation of factors associated with HIV associated OKS.

CHAPTER 4 :

4. 1 RESULTS

A total of 137679 patients were seen at the WOHC during the study period extending from 2008 to 2018. Of the total patients seen, 24 patients were found to have a confirmed histopathological diagnosis of Oral Kaposi Sarcoma (OKS).

Therefore, the prevalence of OKS that was found in this population was 0.017432%, n=24 (Figure 1).

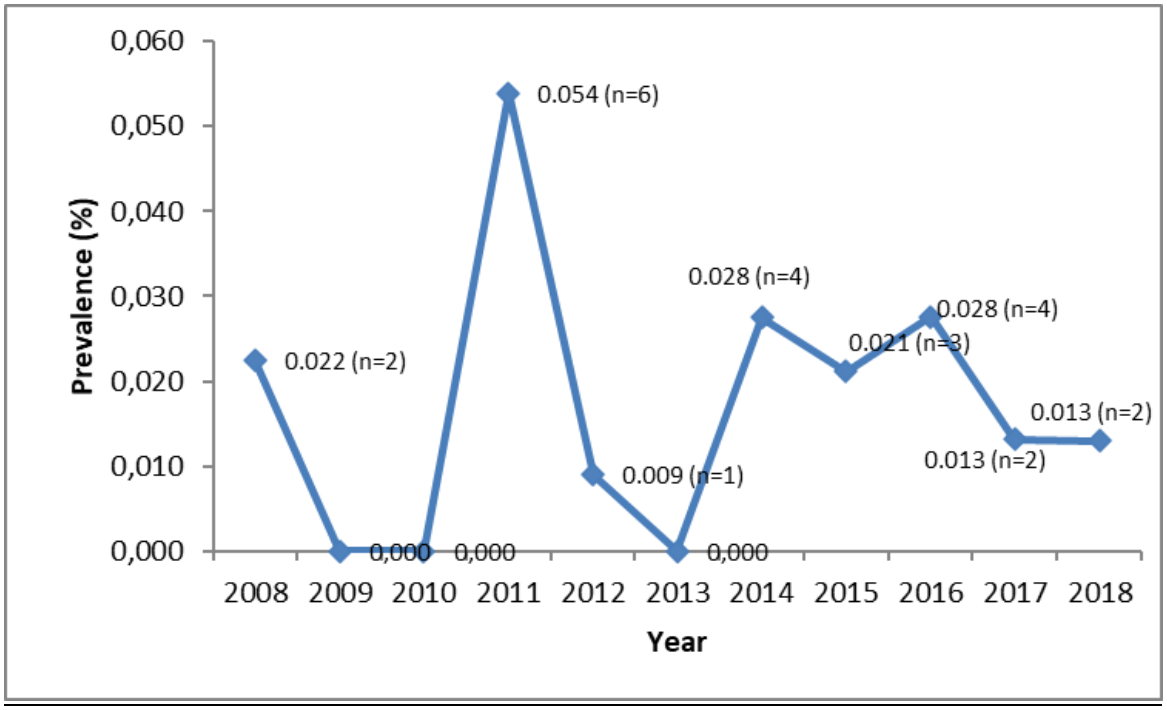

Figure 1 : Prevalence of OKS from 2008-2018, n=24

The table (table 1) below demonstrates the prevalence of diagnosed OKS patients' year-by year, from 2008 to 2018.

Table 1: Prevalence of patients seen at WOHC and number of patients diagnosed with OKS from 2008-2018

Year	Number of patients at WITS oral health clinic	Number of patients with diagnosed OKS	Prevalence (%)
2008	8900	2	0.022472
2009	8975	0	0
2010	8984	0	0
2011	11137	6	0.053874
2012	11085	1	0.009021
2013	14683	0	0
2014	14538	4	0.027514
2015	14168	3	0.021174
2016	14522	4	0.027544
2017	15242	2	0.013122
2018	15445	2	0.012949
TOTAL/ OVERALL	137679	24	0.017432

Of the 24 patients, 19 patients were confirmed as HIV positive whilst 5 patients were of unknown HIV status (Figure 2). Regarding the impact of ARV's, the sample was too small to do an inferential analysis. However, of the 9 patients for which this data is known, 3 were on an ARV treatment regimen (Figure 3). The results show that with regards to viral load, 10 of the patients had an undetectable viral load (<20 cps/ml), 4 patients had a viral load that fell within the normal range (20-1000 cps/ml) and 4 patients reported a high viral load (>1000 cps/ml). Six of the patients' viral load was unknown (Figure 4).

Of the 24 patients with a confirmed OKS diagnosis, 21 had a recorded CD4 count and 3 had no record of a CD4 count, accounting for the 12,5% of unknown (Figure 5). Of the 21 patients who had a reported CD4 count, 12 patients were found to have a CD4 count of <200 cells/mm³, this decreased level is termed AIDS. Comparatively only 2 patients were found to have a low CD4 count of between 200-500 cells/mm³, and 7 patients had a normal CD4 count of >500 cells/mm³. Of the patients who presented with a known CD4 count, a significant 46.2% had a CD4 count under 200 (AIDS), $\chi 2$ (2) = 7.143, p=.028).

There is a significant relationship between the viral load and CD4 count, Fisher's exact = 9.122, p=.017. A significant proportion of those with undetectable (<20 cells/mm³) viral load have a normal (>500 cells/mm³) CD4 count; while a significant proportion of those with high (>1000 cells/mm³) viral load have a low (200-500 cells/mm³) CD4 cell count (Table 2).

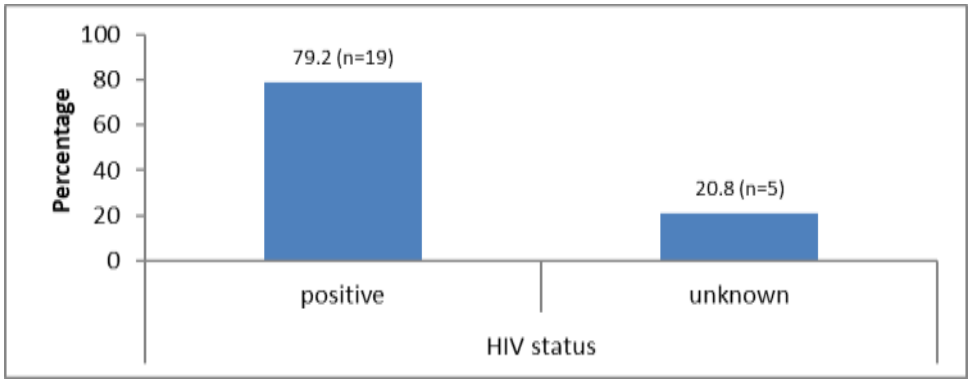

Figure 2: HIV Status

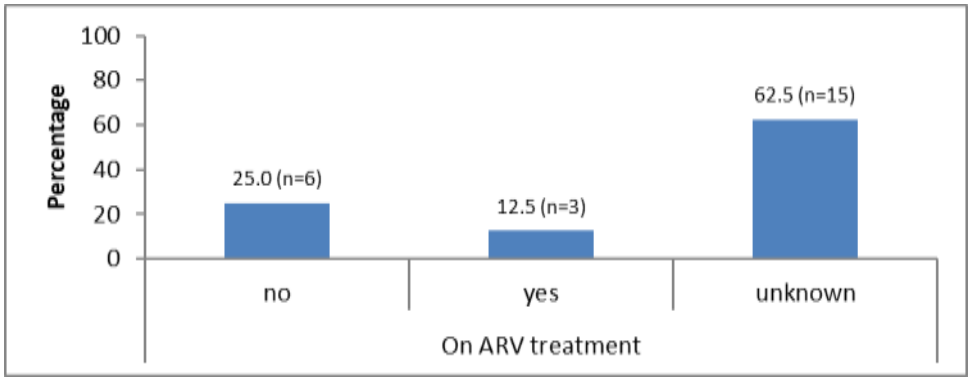

Figure 3: Patients on Anti-retroviral Treatment (ARV)

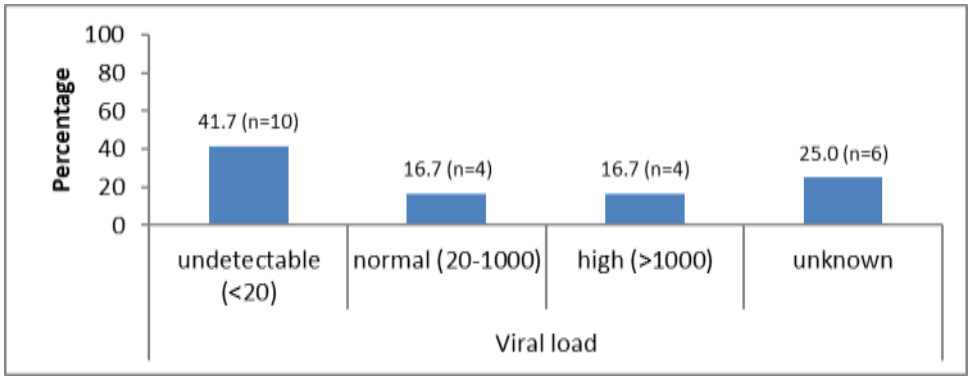

Figure 4: Percentage of patients' Viral Load

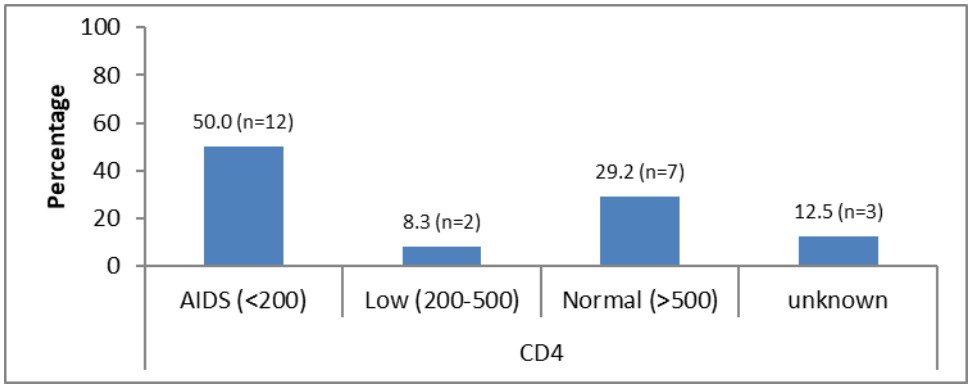

Figure 5: Percentage of patients CD4 cell count levels

Table 2: HIV Status Category and Viral Load

Viral load * CD4 count	Responses as Frequency (%)			Fisher's	p-value
	AIDS (<200)	**Low (200-500)**	**Normal (>500)**		
Undetectable (<20)	3 (30.0)	0 (0)	7 7(0.0)	9.122	0.017
Normal (20-1000)	3 (75.0)	1 (25.0)	0 (0)		
High (>1000)	2 (66.7)	1 (33.3)	0 (0)		
TOTAL	8 (47.1)	2 (11.8)	7 (41.2)		

The age of the patients in this study ranged from 23 to 70 years of age, the mean was 39.21 years (SD = 12.459) . There were 10 male and 14 female patients in the study (Table 3). More female patients were diagnosed with HIV associated OKS than males, in this study.

The sites at which the lesions were biopsied for OKS, were recorded. The site of occurrence of majority of lesions was the palate at 37.5%, n=24. Approximately 17% of lesions occurred in the mandibular region, 12.5% of lesions occurred in the maxillary region, 4.2% of lesions were found on the tongue and 20.8% occurred as multiples lesions at varied sites in the oral cavity (Figure 6). There was no significant relationship observed between site of lesion and the CD4 count and viral loads of the patients.

Table 3 : HIV Status and Sex

Variable	Categories	N (%)
Sex	*Female*	14 (58.3)
	Male	10 (41.7)
HIV status	*positive*	19 (79.2)
	unknown	5 (20.8)
	Mean/SD	Range
Age (years)	*39.21 / 12.459*	23 - 70

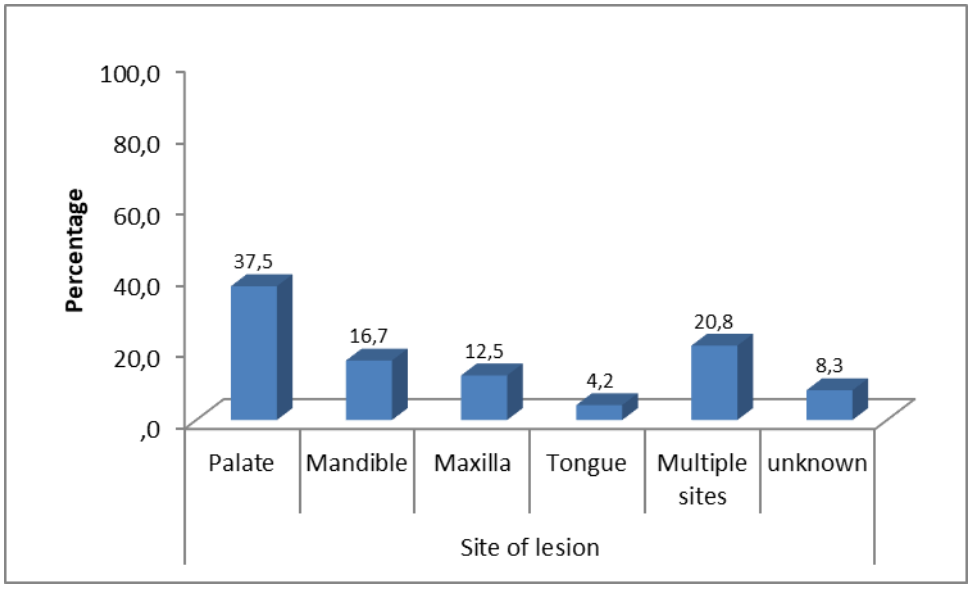

Figure 6: Site of OKS Lesions

CHAPTER 5:

5. 1 DISCUSSION

The aim of the study was to determine the prevalence of HIV associated OKS at WOHC. Records of patients seen at WOHC from 2008 to 2018 as well as patient files and data collected from the NHLS were analyzed. In this study, a total of 137679 patients were seen at WOHC over a 10 year period and of that only 0.017% received a confirmed histopathological diagnosis of OKS. One reason for the lack of cases in this study can be afforded to the inclusion criteria. Although the NHLS did provide more data, not all the cases provided had confirmed HIV pathology as well as confirmed OKS histopathology. These criteria did not allow inclusion of some cases in the study. Similar prevalence studies conducted globally have yielded varying results. A study in Brazil reported a prevalence of 4.4% of HIV associated OKS over a 12year period (Dongo et al., 2013).A study conducted in northern Tanzania reported a prevalence of 4.6% of HIV associated OKS, over a 4-year study period (Semango et al., 2018). Comparatively, a South African study reported a prevalence of 35.7% of HIV associated OKS over a 5-year period (Zwane et al., 2018). A stark contrast in the results of the study by Zwane et al, in the same setting, could be that the study was based on head and neck cancers and resulted in more cases of Kaposi Sarcoma, whereas this study focused on Oral Kaposi Sarcoma, limiting the results to those found intra-orally. Furthermore, widespread administration of ART in 2012 by the South African government, that would improve CD4 cell counts, could be a contributing factor in the lack of OKS cases. The lower the CD4 cell count, the higher the risk of developing OKS (Zwane et al., 2018).In this study the results found a significant relationship between viral load and CD4 cell counts. Patients with an undetectable viral load (<20 cps/ml) were observed to have a high CD4 cell count(>500 cells.mm^3).

The second aim of the study looked at factors relevant to HIV associated OKS. Sex of the patients in this study was found to be female dominant with 10 male patients and 14 female patients presenting with confirmed OKS. In a gender-based study in Brazil, it was found that males showed a higher frequency of HIV associated oral manifestations of KS compared to females (Dongo et al., 2013).In South Africa, a study of head and neck cancers among HIV positive patients found that more females than males presented with a confirmed HIV positive status(Zwane et al., 2018). An HIV associated OKS study, based in South Africa, noted an increase in HIV-KS disease among females (Khammissa et al., 2012).In corroboration with South African studies, the higher prevalence of HIV associated OKS among females was seen in this study. The reason for this could vary from lack of education around sexual protection or could be that females, especially of childbearing age, are more frequently visiting hospitals compared to their male counterparts (Zwane et al., 2018) . A Brazilian study postulated that

gender inequalities in certain cultures could position females in a more submissive role and therefore accept a male partners refusal of sexual protection (Dongo et al., 2013). Gender based violence could also be a cause of higher female prevalence, especially in a South African cohort (Dunkle et al., 2004). The mean age of patients in this study was 39.21 years. The mean ages of 38±11.0 years and of 33.4 years were found in studies of HIV associated OKS in South Africa (Zwane et al., 2018)(Khammissa et al., 2012).Malignancies of the head and neck present at an earlier age in patients with HIV/AIDS (Bunn and van Heerden, 2012). OKS develops at advanced stages of HIV/AIDS and if a number of patients in this study contracted HIV soon after puberty and upon initiation of sexual activity, this could explain why there is alignment between other studies in a South African cohort and the mean age found in this study.

Of the patients included in this study, a majority of the patients were confirmed as HIV positive whilst a minority of patients were of unknown HIV status. The reason for the unknown status of certain patients could be misplacement of records or poor record keeping, as well as patients having done rapid tests and not the ELISA test that is recorded by the NHLS. None of the patients who presented with a confirmed OKS diagnosis were found to be HIV negative. As this study centered around HIV associated OKS, patients who were HIV negative were excluded from the study. The cases in which the HIV status was unknown could be attributed to patients having the test done elsewhere, not presenting to the clinic for the testing or administration errors and loss of patient data (Zwane et al., 2018). In this study 46.2% of patients had a CD4 count of ≤200 cells/mm^3. Lower CD4 counts have been an indicator for developing OKS (Zwane et al., 2018)with OKS most often presenting at advanced stages of HIV/AIDS (Pantanowitz et al., 2013). Due to a lack of information in patient files and records, there cannot be confirmation, but there is a high likelihood that the patients who presented with CD4 counts ≥500 cells.mm^3 were being treated for HIV with antiretroviral therapy (ART).

ART has shown to significantly improve the prognosis of patients with OKS. Treating the patients' HIV infection results in the overall improvement of the immune response (Fatahzadeh, 2012).In this study, there was limited data available regarding the treatment of OKS in these patients and only 3 patients were known to be on ART. This lack of data could be due to lack of record keeping, patients collecting their medication from satellite clinics rather than the hospital or patients choosing not to take ART. There are instances in which ART has initiated Immune Reconstitution Inflammatory Syndrome(IRIS) and caused deterioration of the patients' health after initiation of treatment (Fatahzadeh, 2012). The data on viral load of patients in this study varied. Results indicated undetectable (lower than

detectable levels)(<20cps/ml), normal levels(20-1000 cps/ml) and high viral loads(>1000 cps/ml). Some patients presented with an unknown level of viral load. According to the literature higher viral loads are associated with HIV associated malignancy and tumour progression (Bunn and van Heerden, 2012). In this study a significant relationship was found between the viral load of patients and the CD4 cell count. Patients with a higher viral load had a low CD4 cell count. Increased viral loads and low CD4 cell counts signify immune deterioration and tumour advancement (Bunn and van Heerden, 2012).

In 22% of KS cases, the oral cavity is the first clinical site of disease (Fatahzadeh, 2012). Saliva has been shown to harbour a significantly higher concentrations of human herpes virus 8 (HHV8) compared to other bodily secretions and sero-epidemiological studies have shown that saliva is a prominent vehicle for transmission of HHV8 (Pantanowitz et al., 2013). This may explain the high frequency of KS lesions in the oral cavity. The most commonly affected oral sites in OKS are reported in the literature to be the hard palate and dorsal tongue (Fatahzadeh, 2012). In this study the most common site of presentation of OKS was the hard palate, in 37.5%; whilst the mandibular region (16.7%), maxillary region (12.5%) and tongue (4.2%) were in decreasing order of occurrence, 20.8% of cases OKS occurred as multiple lesions. Other studies in a South African cohort corroborate that the palate in the most frequently affected by OKS lesions and the tongue is rarely affected (Pantanowitz et al., 2013). Thus, the findings of this study are in accordance with findings in broader literature.

5. 2 LIMITATIONS

The key limitation in this study was the inconsistency and lack of data and patient records. The lack of patient records provided limitation on the number of factors and details of cases that could be reported on. The absence of records of patient medication and administration of ART prevented sound analysis of the effect of ART on OKS.

CHAPTER 6:

CONCLUSION

The prevalence of OKS over the study period was 0.017%. The mean age of OKS presentation was 39.21 years of age. More females presented with HIV associated OKS than males and the palate was the most common site of presentation of OKS. There is a significant relationship between high viral loads and low CD4 cell counts.

A decrease in cases of HIV and better management of HIV and CD4 counts could impact the prevalence of OKS. Although data relating to ART was unattainable, the study is suggestive that a possible reason for the low number of OKS cases could be administration of ART by state institutions in South Africa, this can be further investigated to establish the effect of ART on OKS.

FUTURE RESEARCH

This study highlights that patient information such as pathology tests, blood tests, clinical observations, medical history and treatments carried out could be recorded by WOHC in a database. The accessibility of this information to students could aid in conducting studies of this nature in the future.

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APPENDIX I

PATIENT NUMBER AND STUDY NUMBER DATA SHEET

Patient Number	Lab number	**Study Number**
JAK0010/1	TCH0512414	OKS1
TSH0667/1	HN00061360	OKS2
NDA0158/1	HN00058853	OKS3
NKO0647/1	HN00026834	OKS4
NDL1061/1	PD00172466	OKS5
ADA0196/1	HN02337478	OKS6
2661/0211	2661/0211	OKS7
MOK0970/1	HN00801524	OKS8
NOR0078/1	HN01194102	OKS9
MBA0472/1	MBA0472/1	OKS10
3614/2011	TJA1117898	OKS11
NCU0358/1	HN00050354	OKS12
MBO0143/1	HN00465984	OKS13
NDA0101/11	TJA1142596	OKS14
NKU0033/1	HN01542262	OKS15
1727/2008	TJA0834010	OKS16
SEN0119/1	HN01126845	OKS17
MTH0679/1	HN02056302	OKS18
1585/2008	TJA0834009	OKS19
SLL0001/1	HN00873396	OKS20
DHL0090	TJA1216179	OKS21
51/2011	TJA1100695	OKS22
SHA0292/11	TJA1142595	OKS23
PHA0295/1	HN01425053	OKS24

1585/2008	TJA0834009	OKS25
51/2011	TJA1100695	OKS26
MAL0693/1	HN00043426	OKS27
SEL0142/11	TJA1142597	OKS28

APPENDIX II

DATA SHEET OKS INFORMATION

Study Number	OKS1	OKS2	OKS3	OKS4	OKS5
Demographics:					
Age	33	32	70		23
Gender	FEMALE	FEMALE	MALE	MALE	FEMALE
Country of Residence	S.A JHB	SA JHB	S.A KWATHEMA	S.A JHB	S.A VOSLOORUS
HIV Status and ART:					
HIV status	POSITIVE	POSITIVE	POSITIVE	POSITIVE	POSITIVE
HIV subtype	HIV-1	UNKNOWN	HIV-1	UNKNOWN	UNKNOWN
On ARV treatment	NO	NO	YES	NO	NO
Viral load	290158 RNA cps/ml	UNKNOWN	<20 cps/ml	UNKNOWN	UNKNOWN
CD4+ T cell count	397 x 10 6/l	UNKNOWN	154 L	UNKNOWN	UNKNOWN
Diagnosis of OKS during ART	NO	NO	YES	NO	NO
Diagnosis of OKS before ART	YES	YES	NO	YES	YES
YEAR OF DIAGNOSIS	2015	2014	2017	2014	2014
SITE OF BIOPSY	ORAL + CUTANEOUS LESIONS.	ORAL + CUTANEOUS LESIONS.	ORAL EXOPHYTIC LESION ON EDENTULOOS ALVEOLAR RIDGE	MASS LESIONS HARD PALATE	VASCULAR LESION PALATE AND BUCCAL MUCOSA

OKS6
26
FEMALE
SA
UNKNOWN
UNKNOWN
UNKNOWN
UNKNOWN
33
UNKNOWN
UNKNOWN
2018
INTRA-ORAL LESIONS ON BUCCAL AND LINGUAL REGION

OKS7
38
MALE
SA
POSITIVE
UNKNOWN
UNKNOWN
<20 cps/ml
735
UNKNOWN
UNKNOWN
2011
PALATAL LESION

OKS8
35
MALE
SA
UNKNOWN
UNKNOWN
UNKNOWN
<20 cps/ml
661,7
UNKNOWN
UNKNOWN
2016
MAXILLARY GINGIVA AND HARD PALATE LES

OKS10
44
FEMALE
POSITIVE
UNKNOWN
UNKNOWN
<20 cps/ml
674
UNKNOWN
UNKNOWN
2017
LESION ON LABIAL MUCOSA

OKS11
44
MALE
POSITIVE
UNKNOWN
UNKNOWN
<20 cps/ml
547,9
UNKNOWN
UNKNOWN
2011
UNKNOWN

OKS9
46
FEMALE
UNKNOWN
UNKNOWN
UNKNOWN
21 cps/ml
41
UNKNOWN
UNKNOWN
2016
PALATAL LESION

OKS12
27
MALE
POSITIVE
UNKNOWN
UNKNOWN
<20 cps/ml
501
UNKNOWN
UNKNOWN
2014
PALATAL LESION

OKS13
21
MALE
NEGATIVE
UNKNOWN
UNKNOWN
<20 cps/ml
UNKNOWN
UNKNOWN
UNKNOWN
2015
MANDIBULAR GINGIVAL LESION

OKS14
24
FEMALE
POSITIVE
UNKNOWN
NO
UNKNOWN
92,6
UNKNOWN
UNKNOWN
2011
MANDIBULAR BUCCAL GINGIVAL LESION

OKS15
29
MALE
NEGATIVE
UNKNOWN
UNKNOWN
<20 cps/ml
UNKNOWN
UNKNOWN
UNKNOWN
2017
PALATAL LESION

OKS16
35
FEMALE
POSITIVE
HIV-1
YES
<20 cps/ml
580
UNKNOWN
UNKNOWN
2008
MANDIBULAR BUCCAL GINGIVAL LESION(3

OKS17
34
FEMALE
POSITIVE
UNKNOWN
UNKNOWN
922000 cps/ml
9
UNKNOWN
UNKNOWN
2016
LESION ON THE TONGUE

OKS18
38
MALE
POSITIVE
UNKNOWN
UNKNOWN
501000 cps/ml
UNKNOWN
UNKNOWN
UNKNOWN
2018
PALATAL LESION

OKS19
49
MALE
POSITIVE
UNKNOWN
UNKNOWN
<20 cps/ml
96
UNKNOWN
UNKNOWN
2008
MAXILLARY BUCCAL GINGIVAL LESION

OKS20
62
FEMALE
POSITIVE
UNKNOWN
UNKNOWN
<20 cps/ml
8,63
UNKNOWN
UNKNOWN
2016
PALATAL LESION

OKS21
50
MALE
UNKNOWN
UNKNOWN
UNKNOWN
399 cps/ml
89
UNKNOWN
UNKNOWN
2012
LESIONS ON HARD PALATE AND DORSUM C

OKS22
30
FEMALE
POSITIVE
UNKNOWN
YES
665 cps/ml
420
YES
NO
2011
MULTIPLE ORAL LESIONS

OKS23
35
FEMALE
UNKNOWN
UNKNOWN
UNKNOWN
<20 cps/ml
612
UNKNOWN
UNKNOWN
2011
SITE OF LESION UNKNOWN

OKS24
31
MALE
POSITIVE
UNKNOWN
NO
179 cps/ml
78
NO
YES
2017
LESIONS ON BUCCAL AND LINGUAL GINGIV

OKS25
49
MALE
UNKNOWN
UNKNOWN
UNKNOWN
<20 cps/ml
96
UNKNOWN
UNKNOWN
2008
SITE OF LESION UNKNOWN

OKS26
30
FEMALE
UNKNOWN
UNKNOWN
UNKNOWN
665 cps/ml
420
UNKNOWN
UNKNOWN
2011
UNKNOWN SITE OF LESION

OKS27
52
FEMALE
POSITIVE
UNKNOWN
UNKNOWN
UNKNOWN
106,1
UNKNOWN
UNKNOWN
2014
LESIONS ON PALATE AND MAXILLARY

OKS28
57
FEMALE
POSITIVE
UNKNOWN
UNKNOWN
UNKNOWN
91,7
UNKNOWN
UNKNOWN
2011
PALATAL LESION

APPENDIX III

HUMAN RESEARCH ETHICS COMMITTEE CERTIFICATE

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research and Innovation)

TO: Dr H Chetty
School of Oral Health Sciences
Department of Oral Medicine and Periodontology
Dental School
University

E-mail: hasitachetty@gmail.com

CC: Supervisor: Dr S Padayachee
<paddyperio@gmail.com>
and <HREC-Medical Research Office@wits.ac.za>

FROM: Mr Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain Burns@wits.ac.za

DATE: 2021/10/11

REF: R14/49

PROTOCOL NO: **M210710** (This is your ethics application reference number. Please quote it in all enquiries, oral or written, relating to this study.)

PROJECT TITLE: *Prevalence and factors of HIV-associated Oral Kaposi Sarcoma at Wits Oral Health Centre, Johannesburg*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to Government funding of the University.

MSWorks2000\Iain0007\Clearscan.wps

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R49 Dr H Chetty

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M210710

NAME: (Principal Investigator)	Dr H Chetty
DEPARTMENT:	School of Oral Health Sciences Department of Oral Medicine and Periodontology Dental School University
PROJECT TITLE:	*Prevalence and factors of HIV-associated Oral Kaposi Sarcoma at Wits Oral Health Centre, Johannesburg*
DATE CONSIDERED:	2021/07/30
DECISION:	Approved unconditionally
CONDITIONS:	
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Dr S Padayachee
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2021/10/11

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

DECLARATION OF INVESTIGATORS

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

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

Signature of Principal Investigator _____ Date _____

APPENDIX IV

WOHC APPROVAL LETTER

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

School of Oral Health Science

Department of Oral Biological Sciences, 7 York Road, Parktown, 2193. Tel: 011 717 2045 Fax: 086 553 3890 Email: Julitha.Molepo@wits.ac.za

12 July 2021

Dr H. Chetty
Oral Medicine and Periodontology Department
Faculty of Health Sciences
University of the Witwatersrand
Johannesburg

RE: PERMISSION TO CONDUCT RESEARCH

REFERENCE: HRRC/July/07/2021

It is my pleasure to grant final approval to conduct your research at Wits Oral Health Centre titled "Prevalence and factors of HIV associated Oral Kaposi Sarcoma at Wits Oral Health Centre, Johannesburg".

The Hospital Research and Risk Committee allocated a unique reference number to this application – Kindly quote this reference number in all future correspondence regarding this research.

Please note that the Hospital Research and Risk Committee should be informed of the estimated date the research will commence, as well as regular status reports until the research has been concluded. Within a month after conclusion of the research project, a written report must be submitted to the Head of School/CEO, summarizing the final result/outcome as well as the recommendations made based on the research concluded.

Regards,

Prof M. S Nemutandani

CEO/Head of School

Date: 15 July 2021

APPENDIX V

NHLS APPROVAL LETTER

NATIONAL HEALTH LABORATORY SERVICE

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

31 January 2022

Applicant: Hasita Chetty
Institution: University of the Witwatersrand
Department: Oral Medicine and Periodontology (Dental)
Email: hasitachetty@gmail.com
Tel: 031 563 4363 **Cell:** 082 891 7036

CC: Sankeshan Padayachee

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

Your application to undertake a research project **"Prevalence and Factors of HIV associated Oral Kaposi Sarcoma at WITS Oral Health Centre, Johannesburg" Ref No: PR2119319** using data from the NHLS database has been reviewed. This letter serves to advise that the application has been approved and the required data will be made available to you ***without patient names*** to conduct the proposed study as outlined in the submitted application. Submissions should be made annually on the AARMS system – https://aarms.nhls.ac.za.

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

- Ethics approval is obtained from a recognised SA Health Research Ethics Committee.
- Processes are discussed with the relevant NHLS departments (i.e. Information Management Unit and Operations Office) and are agreed upon.
- Confidentiality is maintained at participant and institutional level and there is no disclosure of personal information or confidential information as described by the NHLS policy.
- NHLS Data cannot be used to track patients as no pre-approval/consent is obtained from Patients.
- All data requested should be in accordance with the research protocol submitted and approved by the relevant Ethics Committee.
- Request for the inclusion of the NHLS as a source of data in the original protocol to be approved by Ethics as NHLS does not have a Human Research Ethics Committee.
- A final report of the research study and any published paper resulting from this study are submitted and addressed to the NHLS Academic Affairs and Research office and the NHLS has been acknowledged appropriately.

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research Office. Any data related queries may be directed to NHLS Corporate Data Warehouse, contact number: 011 386 6074 email: zarina.sabat@nhls.ac.za

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

APPENDIX VI

WITS APPROVAL OF TITLE

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

Private Bag 3 Wits, 2050
Fax: 027117172119
Tel: 02711 7172076

Reference: Mrs Sandra Benn
E-mail: sandra.benn@wits.ac.za

30 January 2021
Person No: 364940
PAG

Miss H Chetty
81 Adelaide Tambo Drive
Durban North
Durban North
4051
South Africa

Dear Miss Hasita Chetty

Master of Science in Dentistry: Approval of Title

We have pleasure in advising that your proposal entitled *Prevalence and factors of HIV-Associated Oral Kaposi Sarcoma at Wits Oral Health Centre, Johannesburg* 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