

Lymphoid Proliferation of the Parotid Gland in Paediatric Patients with HIV Infection

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A research report submitted to the Faculty of Health Science, University of the Witwatersrand,
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DECLARATION

I declare that this research report is my own work. It is being submitted in partial fulfilment for the degree of Masters of Science in Dentistry at the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted for any other degree or examination at this university or any other university.

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TSHOLOFELO KUNGOANE

This research is dedicated to my late brother

David Mbolawa Kungoane

ABSTRACT

Introduction: HIV associated parotid lymphoepithelial lesions in children are not well documented. Most studies have concentrated on the adult population.

Objectives: The present study aimed to document the disease, its risk factors and anti-retroviral treatment outcome in children.

Materials and methods: The study was conducted at 2 HIV and AIDS facilities weekly over a 6 month period. “Parotid swellings” in children below 13 years were analysed. A retrospective medical chart review was conducted.

Results: Seventy-one children were included; 47 with swelling (Group 1) and 24 without swelling (Group 2). Thirty-nine had parotid swelling of 1 or both glands, 6 had submandibular and parotid swelling and 2 with only submandibular swelling. Twenty-six children in Group 1 were receiving HAART, 19 reported a reduction in size of lesion, 6 reported no effect and only 1 had the lesion after 11 months of HAART.

Conclusion: Parotid lymphoid proliferation in children is more common than previously reported. The prevalence of this lesion could not be determined as not all children with parotid swelling presenting at the clinics were included in the study. Children with lower viral loads showed an increased risk of developing parotid lymphoid proliferation. The parotid lesions responded well to HAART but did not completely resolve.

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GLOSSARY

1. 3TC-lamivudine
2. AIDS-acquired immune deficiency syndrome
3. ARV-anti-retroviral drug
4. CDC- centre for disease control
5. CT scan- computer tomography scan
6. d4T stavudine
7. EFV- efavirenz
8. HAART- highly active anti-retroviral drugs
9. HIV –human immune deficiency virus
10. HLA-human leukocyte antigen
11. MHC-major histocompatibility complex
12. NHL-non- Hodgkin's lymphoma
13. TB- tuberculosis
14. UNAIDS- united nations-AIDS
15. WHO-world health organisation

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CHAPTER 1

1.0. INTRODUCTION

HIV infected patients frequently present with oral lesions, which are often the earliest indication of HIV infection. Amongst others, oral lesions can be used in staging of the disease, as an indication for initiating therapy and to predict disease progression to AIDS (Coogan et al. 2005). Children may have rapid HIV disease progression due to their immature immune system, resulting in the early manifestations of oral lesions (Okunseri et al. 2003).

HIV associated lesions often involve the parotid glands, presenting as slow growing unilateral or bilateral swellings associated with cervical lymphadenopathy (Cleary et al. 1990).

Numerous terms are used to describe parotid lymphoproliferation, including cystic lymphoid hyperplasia (CLHP) (Cleary et al. 1990), benign lymphoepithelial cyst (BLEC) and benign lymphoepithelial lesion (BLEL) (Smith et al. 1990) and diffuse infiltrative CD8 lymphocytosis syndrome (DILS) (Itescu et al. 1990). The terminology used is often an indication of the whole pathological spectrum of this disease.

The pathogenesis of parotid lymphoid proliferation is still not clear. In a study by Som et al (1995) the lesion was thought to originate from AIDS related proliferation of periparotid and intraparotid lymph nodes, resulting in obstruction of the salivary ducts by proliferating lymphocytes. A more popular theory is that the cysts originate from a reactive epithelial

proliferation of salivary gland acini and ducts trapped within the parotid lymph nodes (Soberman et al. 1991; Mandel et al. 1998).

HIV related parotid lymphoproliferation is sometimes associated with a slower disease progression to AIDS in the adult population (Itescu et al. 1990; Mandel et al. 1998). Some patients who develop parotid lymphoepithelial lesions are said to have diffuse infiltrative CD8 lymphocytosis syndrome (Itescu et al. 1990; Mandel et al. 1998). This syndrome is characterised by a CD8 lymphocytic infiltrate associated with the major histocompatibility complex antigen (MHC), HLA-DR5. The expression of the histocompatibility antigen HLA-DR5 is reported to play a role in mediating the immune response to the HIV, resulting in slower progression of patients to AIDS (Mandel et al. 1998).

An extensive review of the literature, including a Medline search, hand searches, a search of abstracts and conference proceedings was done. It was evident from the literature that HIV associated lymphoproliferative disease of the parotid gland has been very well studied and documented in adults (Cleary et al. 1990; Itescu et al. 1990; Mandel et al. 1998), whereas in children, this disease has been largely neglected. Anecdotal reports from HIV clinics indicate that the frequency of the lesion in children is increasing and that the clinical manifestations are being recognised and regarded by the community as indicative of HIV infection, resulting in victimisation of the sufferer. In addition, reports of malignant transformation in adults are increasingly reported in the literature (Chetty, 1996; Del Bono et al. 2000), while not much is known of this disease process in children. The present study documents the disease phenomenon in children thereby aiding in defining the entity, expounding its recognition and possible treatment strategies.

CHAPTER 2

2.0. AIMS AND OBJECTIVE OF THE STUDY

The aims of this study are to:

- determine the prevalence of parotid lymphoid proliferation in HIV infected children
- determine the risk factors for developing parotid lymphoid proliferation
- determine the treatment outcome of this disease

The overall objective of the study is to define the clinical characteristics of HIV lymphoid proliferation of the parotid glands in children.

CHAPTER 3

3.0. LITERATURE REVIEW

3.1. HIV statistics

The number of people living with HIV/AIDS has risen globally to 33.4 million in 2007 from 29 million in 2001 (UNAIDS, 2009). In 2007, 2.7 million people were newly infected with HIV and 2.1 million died from AIDS related illnesses. The majority (68%) of these infections are in Sub-Saharan Africa with an estimate of 22.4 million infected people. Southern Africa, including countries such as Botswana, Lesotho, Mozambique, South Africa, Namibia, Zimbabwe and Zambia, is the worst affected region at 32% of total HIV infections. Women make up 61% of people living with HIV in the region. An estimated 50% to 80% of the HIV infected population in Southern Africa are co-infected with tuberculosis (TB) (UNAIDS, 2009).

South Africa has the highest number of HIV infections in the world, estimated at 5.7 million in 2007 from 4.7 million in 2001. The majority of people infected are women, with a reported estimate at 3.2 million. Children (0-14 years) are estimated to have prevalence of 280 000 (UNAIDS, 2009). The majority of the adult HIV infections are through heterosexual contact. Paediatric HIV infection is reported through three major routes, namely, maternal transmission, transfusion of contaminated blood products and sexual contact, often through sexual abuse. The majority of the children acquire the infection through mother-to-child vertical transmission, however, this number has decreased through the introduction of the

prevention of mother to child transmission (PMTCT) intervention programme. In an African study by Gisselquist et al (2002) a significant proportion (20%-40%) of HIV infections occurred in individuals with HIV negative partners and children born to HIV negative parents. HIV transmission was thought to be through improper health care practices often associated with lack of infection control and the use of unsafe donor tissues.

3.2. Oral lesions in HIV and AIDS

HIV infected individuals often present with opportunistic infections caused by the progressive loss of CD4 lymphocytes. These infections frequently affect the oral cavity. HIV oral lesions have been classified into three groups based on the recommendation by EC Clearinghouse (*Classification and diagnostic criteria for oral lesions in HIV infection*, 1993) namely, those that are strongly associated with HIV infection, those less commonly associated with HIV infection and those seen in, but not indicative of HIV infection. Furthermore, oral lesions are used in the World Health Organisation (WHO) disease staging system (*Proposed 'World Health Organization staging system for HIV infection and disease'*, 1993) which predicts disease progression to AIDS. The classification system for paediatric HIV is different from that of adults and is based on an age, immune status and clinical category. A summary of HIV and AIDS associated oral lesions documented in the Centre for Disease Control and Prevention (CDC) staging 1994 is listed in Table 3.1.

An oro-facial manifestation of HIV and AIDS that has shown a marked increase in frequency is lymphoid proliferation of the parotid gland (Ortega et al. 2008). The occurrence of this phenomenon in children was reported Sculerati as early as 1990, as non-tender bilateral

enlargements of the parotid gland that often persist for months and years. As mentioned, research of HIV parotid gland lymphoid proliferation in the paediatric population is deficient.

Table 3.1. CDC (1994) revised classification system for HIV infection in children less than 13 years: based on clinical manifestations

<i>Immune category</i>		<i>Clinical oral symptoms</i>
Category N	Not symptomatic	• Persistent generalised lymphadenopathy
Category A	Mildly symptomatic	• Lymphadenopathy • Parotitis
Category B	Moderately Symptomatic	• Oral candidiasis • Oral hairy leukoplakia • Lymphoid interstitial pneumonia • Herpes simplex virus stomatitis • Herpes zoster • Cytomegalovirus infection
Category C	Severely Symptomatic	• Kaposi sarcoma • Lymphoma

3.3. Parotid lymphoid proliferation

3.3.1. Terminology

Since its discovery in the late 1980s (Shugar et al. 1988; Itescu et al. 1989) numerous terms have been used to describe lymphoproliferation of the parotid salivary glands in HIV infected patients. These have included HIV benign parotid hypertrophy (Beitler et al. 1999), cystic parotid lesions in HIV (Smith, 1990), cystic lymphoepithelial lesions in HIV (Smith, 1990),

benign lymphoepithelial cysts of the salivary glands (BLEC) (Smith, 1990), cystic lymphoid hyperplasia (CLHP) (Aguirre et al. 2000) and diffuse infiltrative lymphocytosis syndrome (DILS) (Itescu et al. 1990).

Dave et al. (2007) have proposed a three tier system to the classification of HIV associated lymphocytic parotid gland enlargement in the paediatric population,

1. persistent generalised lymphadenopathy (PGL)
2. benign lymphoepithelial lesion (BLEL)
3. benign lymphoepithelial cyst (BLEC)

These authors nonetheless emphasise that it is unclear if the lesion is a continuous spectrum of disease or distinct entities with different pathogeneses. In the present study, emphasis is made of the fact that until special confirmatory investigations are done in the HIV patient, the lesions should be referred to as HIV related parotid lymphoid proliferation of the salivary gland, based on the fact that all similar lesions previously investigated demonstrated a lymphoid component.

3.3.2. *Clinical presentation*

Lymphoid proliferation of the parotid gland presents as a unilateral and ultimately bilateral enlargement often associated with cervical lymphadenopathy (Mandel et al. 1992; Mandel et al. 1998). Lymphocytic involvement of the submandibular salivary glands has also been reported in both paediatric and adult patients (Terry et al. 1991; Chetty, 1996; Rosso et al. 2006). The liver, spleen and kidneys may have the lymphocytic infiltrate but the most common extraglandular infiltrate occurs in the lungs, often presenting as interstitial lymphocytic pneumonitis (LIP) (Som et al. 1995).

The lesions can present at any age, the youngest patient reported at 6 months by Soberman et al (1991) often with a reported male predominance both in adults and children (Schiødt et al. 1992; Morales-Aguirre et al. 2005; Rosso et al. 2006). The prevalence of the parotid lesion in the paediatric population is reported at rate of 2-10% (Fonesca et al. 2000; Gaitan-Cepeda et al. 2002; Okunseri et al. 2003) however in a South African study by Naidoo and Chikte (2004) a prevalence of 50% was reported. Children presenting with HIV parotitis with lymphadenopathy, hepatomegaly, splenomegaly and upper respiratory infections are categorised as mildly asymptomatic according to the CDC (1994) and have a better clinical course. Although bilateral parotid swelling was previously reported in HIV negative individuals (Mandel and Surattanont, 2002) the presence of these lesions today is a strong indication for HIV testing.

3.3.3. Pathogenesis

Due to the differential clinical presentation of the HIV parotid lymphoproliferation (cystic versus solid lesion), the pathogenesis of these lesions is still the subject of controversy. During embryologic development, 5 to 10 lymph nodes are trapped within the parotid gland. The HIV virus is said to induce lymphocytic infiltrate of the intraparotid lymph nodes resulting in swelling of the gland (Del Bono et al. 2000). This is thought to occur during the initial stages of HIV infection, thus the term persistent generalised lymphadenopathy (PGL). Migration of HIV infected cells from the peripheral blood circulation into the parotid glands is postulated to be the initial trigger of lymphoid proliferation and inducing metaplastic changes of the ductal epithelium. A theory supported by the presence of HIV-1 p24 protein within the follicular dendritic cells in the germinal centres (Uccini et al. 2000).

Lymphocytic infiltrate is mediated by specific major histocompatibility complex (MHC) class alleles (HLA-DR5), which recognise the viral protein expressed by epithelial cells (Mandel et al. 1998). The MHC molecule induces the proliferation of CD8 T lymphocytes, which is thought to suppress viral replication resulting in slower progression of HIV infection to AIDS. Expression of HLA-DR5 with proliferation of CD8 T cell was also reported in three of the four children with parotid enlargement in a study by Soberman et al (1991).

Cyst formation is seen as a later development in HIV parotid gland disease (Mandel, 1998). The cysts are observed to have developed within intraparotid lymph nodes, resulting in replacement of nodal tissue. While cyst formation was originally thought to be due to duct obstruction by lymphoid proliferation (Mandel, 1998; Ioachin, 1988) a more favoured hypothesis is that they arise from a lymphocytic trigger of glandular epithelium trapped within the intraparotid lymph nodes, resulting in cyst formation (Elliot, 1990).

3.3.4. *Microscopic Features*

The histology shows parotid tissue with lymphocytic infiltrate organised into follicle and germinal centres surrounding islands of epithelial tissue and myoepithelial cells. Acinar atrophy occurs as the lymphoid proliferation often leads to replacement by lymphoid tissue. Histological features are similar to that of Sjögren's syndrome. The cystic lesions consist of spaces lined by metaplastic squamous or cuboidal epithelium infiltrated by mature lymphocytes. Follicular lymphoid hyperplasia with prominent plasma cells and some multinucleated giant cells are often associated with the cyst. The lumina of the cysts may

contain keratinaceous and proteinaceous debris, which in a study by Uccini et al (2000) was found to contain HIV-1 RNA copies.

3.3.5. Radiographic Features

A number of imaging techniques are used in the diagnosis of parotid salivary lymphoepithelial lesions. Ultrasound, computed tomography (CT) and magnetic resonance imaging (MRI) are effective in the diagnosis of BLEL and BLEC, even in clinically asymptomatic patients. CT scans are the primary methods used in the diagnosis of these lesions for evaluation of the size and number of cysts within the lesion.

With regard to children, sonography may be preferred over CT scan because it is less invasive and does not require sedation. In a study by Soberman et al (1991) two main sonographic patterns were observed. The first pattern was that of diffuse small and medium hypoechoic or anechoic areas with preservation of normal gland architecture whilst the second pattern was that of large anechoic areas almost replacing the parotid gland. Calcification was noted within the large anechoic areas of one of the patients. The study by Martinoli et al (1995) showed sonographic patterns ranging from cystic to mixed masses with a predominant solid component. HIV lymphoepithelial lesions are often associated with interstitial pneumonitis. Brudnicki et al (2001) showed chest radiographs with pulmonary lymphoid hyperplasia and cyst formation.

3.3.6. Treatment

Treatment options for reactive parotid lymphoproliferative lesions include observation, repeated aspiration, antiretroviral medication, sclerosing therapy, radiotherapy and surgery.

The traditional treatments of repeated aspiration, sclerosing therapy (Lustig et al. 1998) and the least favourable surgical intervention were often used to address the cosmetic concern and social stigma associated with the swelling.

3.3.6.1. Radiotherapy

Radiotherapy was extensively used before the introduction of highly active antiretroviral therapy (HAART). External radiation of 24 gray, i.e. high dose radiation was reported to yield better results compared with low dose radiotherapy, with total regression of the lesion (Beitler et al. 1991; Kooper et al. 1998). The use of radiotherapy for a benign lesion is controversial, especially considering the possible long term consequence of malignancy associated with radiotherapy.

3.3.6.2. Parotidectomy

Parotidectomy has also been used as an alternative treatment in patients who are stigmatised by the presence of the bilateral salivary gland swelling. Due to the location of the facial nerve in the parotid gland, nerve palsy is to be anticipated during parotidectomy. Surgery, in the form of superficial parotidectomy in conjunction with fine needle aspiration (FNA), may be used to rule out malignancy (Chetty, 1996; Del Bono et al. 2000).

3.3.6.3. Antiretroviral (ARV) treatment

The use of ARVs, especially zidovudine, in the management of parotid lymphoid proliferation has been documented (Schjødt et al. 1992; Shaha et al. 1993; Hamza et al. 2006). The size of the lesions is reported to decrease or disappear as seen in follow-up images of children on HAART regimen containing zidovudine (Morales-Aguirre et al. 2005). At the same time,

contradicting reports of an increased frequency of salivary gland disease in patients on HAART have been documented (Schmidt-Westhausen et al. 2000; Ortega et al. 2008). The lesions may present as a form of immune reconstitution syndrome (IRIS), which is a reaction to the rising CD4 count and falling HIV-1 RNA level after the initiation of HAART. The first phase of immune reconstitution occurs in the first three months of therapy and is characterised by an increase in circulating naïve and memory CD4 and CD8 cells as well as B lymphocytes. The second phase is characterised by an increase in circulating naïve CD4 and CD8 cells and a decrease in circulating memory CD8 cells (Powderly et al. 1998).

The pathogenesis of the immune reconstitution syndrome is not fully understood. It is thought to result from a rapid rise in memory CD4 cells which express inflammatory cytokines resulting in an exaggerated immune response to previously known pathogens (Ortega et al. 2008). IRIS has been described for tuberculosis, cytomegalovirus retinitis, mycobacterium avium complexes (MAI), cryptococcus, hepatitis B, hepatitis C and herpes zoster. The most common oral cases reported are an increase in the frequency of human papillomavirus (HPV) related oral lesions and salivary gland diseases, especially DILS (Ortega et al. 2008).

3.3.7. *Malignant Transformation*

HIV infection predisposes individuals to the risk of cancer (Frisch, 2001). The most common cancers are Kaposi sarcoma and non-Hodgkin lymphoma (NHL). Risk for cancer is due to severe immunosuppression associated with the disease. Malignant transformation of parotid benign lymphoepithelial lesion to non-Hodgkin's lymphoma (NHL) of B cell type amongst HIV infected adult population has been reported (Del Bono et al. 2000). The use of HAART in the management of HIV and AIDS has resulted in most children surviving beyond puberty

thereby possibly increasing the risk for malignant transformation of the parotid lymphoepithelial lesion.

Due to paucity of research in children with parotid enlargement, very few cases of malignant transformation have been reported. In a study by Rosso et al (2006) only 1 child from a sample of 51 children developed NHL during a 6 months follow-up period. However, the primary site of the lymphoma occurrence was the arm and groin area, which according to the authors could mean that the occurrence of BLEL and NHL might have been coincidental. With the anticipated increasing risk of malignancy, it may be mandatory to do periodic fine needle aspiration cytology (FNA) in patients with parotid lymphoepithelial lesions and cysts. Unfortunately, FNA is less well tolerated by children making diagnosis challenging for countries with a lack of resources.

3.4. Reports of lymphoid proliferation of the parotid glands in children

As already mentioned, reports of lymphoid proliferation of the parotid gland in children are scarce. Only 6 published reports referring to paediatric HIV parotid lymphoid proliferation were found (Table 3.2.). Since children presenting with the lesion are expected to have a slow progression to AIDS, it may be that those who have the lesion are less likely to present for medical treatment. On the other hand, the lesions may have been misdiagnosed by clinicians. The South African indication for ARV treatment requires a CD4 count of $<350\text{cells/mm}^3$ or a WHO stage III or IV, which includes patients with Kaposi's sarcoma and lymphoma (*The South African Antiretroviral Treatment Guidelines*, 2010). Parotid gland swelling is categorised as Stage I according to the WHO (1993) and thus patients with parotid gland swelling only do not meet the requirements for ARV treatment.

Table 3.2. Published research on HIV paediatric parotid lymphoid proliferation

Author	Date	Country	Number of subjects	Gender	Age distribution
Goddart et al	1990	Belgium	4	4F	2-3 years
Soberman et al	1991	USA	10	7M/3F	6 months-11 years
Mayer and Haddad	1996	USA	1	1F	6 years
Pinto and Rossi	2004		Review article		
Morales-Aguirre et al	2005	Mexico	4	3M/1F	3-12 years
Rosso et al	2006	Italy	51	29M/22F	10 months-21 years
Dave et al	2007	USA	4	4F	7-17 years

Analysis of these publications (Goddart et al. 1990; Soberman et al. 1991; Mayer and Haddad, 1996; Pinto and Rossi, 2004; Morales-Aguirre et al. 2005; Rosso et al. 2006; Dave et al. 2007) shows that since the appearance of the first report in 1990 (Goddart et al. 1990) only 74 cases in children have been documented. Most of these reports consist of between 1 to 5 cases while the largest series from Italy by Rosso et al (2006) consisted of 51 patients. The gender distribution in these published reports included 39 males and 35 females, while the age range was between 6 months to 21 years. Three of the seven documented reports are imaging studies and one by Pinto and Rossi (2004), a literature review.

Only 1 child from 73 of the reported cases showed evidence of malignant transformation into non Hodgkin's lymphoma (NHL) (Rosso et al. 2006). As stated previously, the primary

lymphoma site was not in the parotid glands even though the patient had parotid enlargement. Although the presentation of parotitis is an indication for HIV testing, other causes of parotid swelling in children should be investigated (Table 3.3.).

Table 3.3. Causes of bilateral parotid swelling in children (Mandel, 2002)

Infective	Viral parotitis (mumps)
	Bacterial acute suppurative parotitis
Autoimmune	Recurrent parotitis
Congenital	Haemangioma
	Lymphangioma
	Dermoid cyst
Neoplastic	Pleomorphic adenoma
	MALT lymphoma

The present study thus constitutes one of the largest series documenting HIV parotid gland enlargement in children. In view of the high prevalence of HIV in South Africa, characterisation of this entity is important for its recognition and treatment.

CHAPTER 4

4.0. MATERIALS AND METHODS

4.1. STUDY DESIGN

This is a cross sectional study of parotid gland swelling among HIV infected children compared with children without parotid swelling.

4.2. STUDY SITE

The study took place at the HIV and AIDS wellness and treatment centres of the Jubilee and Odi District hospitals. The centres are a joint initiative of the Department of Health and the Foundation of Professional Development in partnership with UNAIDS. Both clinics are situated in semi-urban areas, 40km north; outside the Pretoria central business district (CBD). The centres offer HIV counselling, testing, wellness and treatment for both adults and children. A room with an examination light was allocated at each centre to conduct the study.

4.3. SAMPLING METHOD AND STUDY POPULATION

The study included a review of clinical records as well as examination of paediatric patients (O-13 years), who attended the clinics during the period of study (December 2008 -May 2009) which took place once weekly for 6 months. The estimated average monthly attendance of paediatric patients at the Jubilee and Odi District hospitals is 80 and 200 patients respectively. Most of the children are required to attend the clinic monthly in

order to receive treatment and counselling and as a result most of the children seen over the 6 month period were not new to the clinic and not all children with parotid swelling agreed to participate in the study. Furthermore, new patients below 13 years were constantly seeking treatment at the clinics during the 6 month period of the study but outside the allocated time for examination of patients for the study.

4.4. STUDY SAMPLE

All paediatric patients who showed any clinical physical modification of the parotid glands and a sample of patients with no physical glandular changes were recruited. All patients in this study were confirmed as being infected with HIV by enzyme-linked immunosorbent assay (ELISA) and Western Blot assays on first attending the clinic, were informed of their HIV status and had received counselling. The study sample was divided into 2 groups based on inclusion and exclusion criteria.

The inclusion criteria for children in Group 1 included only those children below 13 years with parotid swelling/enlargement that had consented to participate in the study. For the children in Group 2, the inclusion criteria included children below 13 years without parotid swelling/enlargement and who had consented to participate in the study. All children over 13 years of age were excluded from the study.

4.5. ETHICS

Ethical clearance for the study (M080926) [Appendix 1] was granted by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand to examine the patients clinically as well as to view patient hospital files in order to confirm the HIV status of the

patient, the CD4 count and the viral load. Furthermore, permission for the study was granted by the Department of Health, Tshwane Metsweding region in order to conduct the study, which included the examination of patient records, at the Jubilee and Odi District hospitals [Appendix 2].

Parents and guardians of the children to be examined were provided with a participation information leaflet and consent forms [Appendices 3(A-C) and 4], which were available in English and Setswana. The staff at the clinics was briefed on the study so that they could assist illiterate parents or guardians to fill out the consent form, and to grant permission for the clinical examination and access to patient records. Children, who were able to read and write, were also required to give assent. Patients were allocated numbers for identification purposes, to assist with follow-up, to avoid duplication and to guarantee anonymity. Furthermore, informed consent from patients and guardians was obtained for the taking and use thereafter of clinical photographs for research and publication purposes.

4.6. DATA COLLECTION

Patients were referred for evaluation by the attending staff after routine outpatient appointments based on a swelling on the side of the face or in the submandibular area. A clinical examination chart [Appendix 5] was completed on each patient detailing general patient demographics, clinical presentation, diagnostic evaluation, mode of HIV transmission, the presence and nature of the swelling, glands involved, associated symptoms, antiretroviral history and its effect on the lesion.

Oral examination of the children was conducted by the principal investigator following calibration by a certified calibrator for oral manifestations of HIV disease (certified by Guy's, Kings and St Thomas Dental Institute and the University of Witwatersrand) following repeated inter- and intra- calibration examinations until standardisation was achieved. The examination was based on the recommendation of the WHO Collaborating Centre on Oral manifestation of Human Immunodeficiency Virus (*Proposed 'World Health Organization staging system for HIV infection and disease'*, 1993) using the standard methodology to examine extra-oral head and neck, peri-oral and intra-oral soft tissue. The diagnosis of oral and dental lesions was based on clinical presentation and recorded based on the ECC-Clearinghouse criteria (*Classification and diagnostic criteria for oral lesions in HIV infection*, 1993).

The diagnosis of immune suppression is based on the CDC (1994) revised classification on HIV infection in children less than 13 years. All included patients had a confirmed HIV status and CD4 counts. No further investigations, for example ELISA, CD4 counts, viral loads, FNA or biopsy, were ordered unless prescribed for medical reasons. Information regarding HIV diagnosis, CD4 counts, viral loads, medical pathology and the type of antiretroviral therapy was obtained from the patient files.

The presence of parotid swelling was determined by visual inspection as well as manual palpation. Intra-oral examination was done by the principal investigator using a wooden spatula, a mouth mirror and dental probe under artificial and natural lighting. Patients who on examination, were found to require further oral and dental management, were referred to the nearest state dental facilities.

4.7. STATISTICAL ANALYSIS

Statistics used are largely descriptive in nature. Comparison between the 2 groups was carried out using the SAS System. Significant differences between the experimental and control groups were analysed using the Student *t* test and the Chi square test. A statistical significance level of $p = 0.05$ was used.

CHAPTER 5

5.0. RESULTS

5.1. Study sample

A total of 71 HIV infected paediatric patients, 38 males and 33 females participated in this study (Table 5.1). There were 47 children with parotid swelling (Group 1) and 24 without the swelling (Group 2).

5.2. Frequency

As mentioned in Chapter 4 (4.1.) many of the children seen over the 6 month period of the study were not new patients but were there to receive treatment and counselling. Even though new HIV infected paediatric patients were continually attending the clinic, only those patients with parotid swelling attending the clinic during the time of the investigation were included. It was therefore not possible to determine the frequency or incidence of parotid swelling in this population.

Anecdotal estimations by the clinic staff indicate that approximately 1 in 5 of the children attending the clinic for the first time showed parotid swellings.

5.3. Gender

The patients in Group 1 comprised 26 (55%) males and 21 (45%) females and those in Group 2 consisted of 12 (50%) males and 12 (50%) females (Table 5.1), [Chi square test; $p = 0.80$].

Table 5.1. Epidemiologic and clinical characteristics of HIV infected paediatric patients

	Group 1	Group 2
	Children with parotid enlargement	Children without parotid enlargement
Total	47	24
Gender		
○ Male	26	12
○ Female	21	12
Age (years)		
○ Range	2-12.8	1-12.7
○ Mean	7.00	6.28
Immunological stage (CDC category)		
1	9 (19.47%)	3 (12.50%)
2	14 (30.43%)	9 (37.50%)
3	23 (50.0%)	12 (50.0%)
CD4 cell count range (%)		
at diagnosis	19 (1.24%)-2325 (30.91%)	12 (0.87%)-1198 (22.10%)
at present	83 (9.46%)-1830 (43.80%)	16 (13.50%)-1646 (23.80%)
Viral load range		
at diagnosis	2100-340 000	2500-2 400 000
at present	Lower than detectable-920	Lower than detectable-6100
Children on HAART	26 (53.32%)	19 (79.17%)
Not on treatment	21 (44.68%)	5 (20.43%)
ARV regimen	26 (d4T, 3TC, EFV)	18 (d4T, 3TC, EFV) 1 (d4T, 3TC, Kaletra)

5.4. Age distribution

The age of the children in Group 1 ranged from 2 to 12.8 years and those in Group 2 ranged from 1 to 12.7 years (Table 5.2; Figure 5.1). The difference in ages between the 2 groups was not significant ($p = 0.35$).

Table 5.2. Statistical comparison of age difference between children in Group 1 and Group 2

P = 0.35	N	Mean	SD	SEM
Group 1	47	7.00	3.05	0.44
Group 2	24	6.28	3.43	0.70

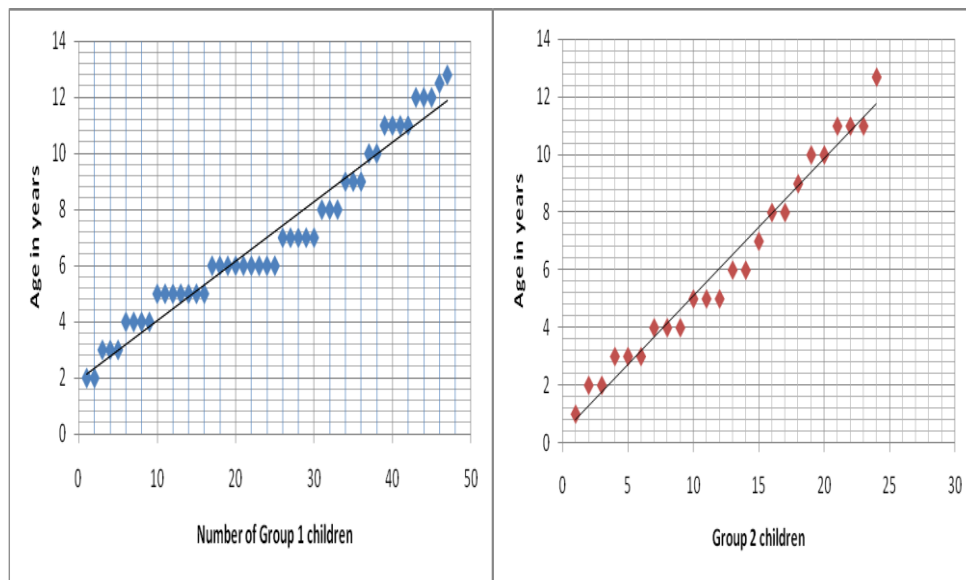


Figure 5.1. Age distribution of children in Group 1 (blue) compared to those in Group 2 (red)

5.5. HIV transmission

The route of HIV infection was predominantly vertical. Majority of the children had HIV infected mothers or one or both parents who had died from HIV related illnesses, with the exception of 4 children whose mothers were not tested.

5.6. CD 4 cell count

First visit (day of HIV diagnosis) and current value CD4 cell counts were available. The median CD4 cell count at diagnosis for Group 1 was 382.02 (14.85%) with first values ranging from 19 cells/mm³ (1.24%) to 2325 cells/mm³ (30.90%) and current median values at 552.95 cells/mm³ with ranges from 83 cells/mm³ (9.46%) to 1830 cells/mm³ (43.80%). Group 2 median CD4 count was 433.54 cells/mm³ (11.31%), ranging from 12 cells/mm³ (0.87%) to 1198 cells/mm³ (22.10%) for first values and median of 680 cells/mm³ with ranges of 16 cells/mm³ (13.50%) to 1646 cells/mm³ (23.80%) for current values. The CD4 cell count at diagnosis was not available for only 1 of the 72 children. This difference was not statistically significant (Tables 5.1. and 5.3.).

Table 5.3. Statistical comparison of CD4 cell count between children in Groups 1 and 2 at diagnosis (A) and during study period (B)

P = 0.34	N	Mean	SD
Group 1 A	46	379.68	381.65
B	22	536.13	389.33
Group 2 A	24	433.58	336.29
B	13	663.79	467.79

5.7. Viral loads

For children in Group 1, the viral loads at diagnosis ranged from 83 to 340 000 while the current values ranged from lower than detectable values to 920. Group 2 children had diagnosis values ranging from 2500 to 2400 000 and current values ranging from lower than detectable to 6100 (Tables 5.1. and 5.4.). The difference in values at diagnosis was significant ($p = 0.02$). With regard to children in Group 1, the viral loads appeared to be higher in the females as compared to the males, however this difference was not statistically significant ($p = 0.17$) (Table 5.5.).

Table 5.4. Statistical comparison of viral loads in children in Group 1 and Group 2

P = 0.02	N	Mean	SD
Group 1	31	71394.29	113184.08
Group 2	18	361805.56	708648.61

Table 5.5. Statistical comparison of viral loads in children in Group 1

P = 0.17	N	Mean	SD
Male	18	60 484.61	15 871.44
Female	13	368 884.62	255 630.08

5.8. CDC classification

Most of the children in Group 1 belonged in CDC immunological category 3 [23 (50.0%)], 14 (30.43%) in category 2 and 9 (19.57%) in category 1. CD4+ lymphocyte data was not available for 1 child. The majority of the children in Group 2 were in the

immune category 3 [12 (50%)], 9 (37.50%) in category 2 and 3 (12.50%) in category 1 (Table 5.1.).

5.9. ARV treatment

26 (55.32%) of Group 1 children and 19 (79.17%) of Group 2 children were receiving HAART consisting of stavudine (d4T), lamivudine (3TC) and efavirenz (EFV). Only 1 child in Group 2 was on regimen 2 (second line therapy) consisting of stavudine (d4T), lamivudine (3TC) and lopinavir / ritinovir (Kaletra). 21 (44.68%) of the children in Group 1 and 5 (20.83%) of those in Group 2 were not on ARV treatment.

Of the children with parotid enlargement (Group 1), 20 (73.08%) showed a reduction in the size of the parotid enlargement after the initiation of HAART, however the swelling did not completely resolve. The mean duration of HAART amongst this group was 9.89 months. HAART had no effect on 6 children (23.08%) and only 1 child (3.84%) reported that parotid swelling appeared after starting HAART. The mean duration of HAART for children with no effect on parotid enlargement was 12 months.

5.10. Clinical presentation of facial swellings

Of the children in Group 1 with facial swellings, 39 (82.98%) had swelling of 1 or both parotid glands (Figures 5.2.A-D and 5.3.A-C), while 6 (12.77%) had both parotid and submandibular gland involvement. Submandibular gland enlargement only was present in 2 (4.25%) patients (Table 5.5.). The parotid swelling had a hard texture in 36 patients (76.59%) and presented as a soft swelling in 11 children (23.40%). Both

the children and the accompanying adults indicated that the swelling started initially unilaterally before becoming bilateral, usually within 6 months. The size of the parotid swelling ranged from about 2 -3mm (Figure 5.2.B) to over 5cm in diameter in which cases the ear lobes were elevated (Figure 5.2.A,C,D). All the children in both groups (100%) had cervical lymphadenopathy (Figure 5.3.).



Figure 5.2. Children in Group 1 showing bilateral parotid swelling (A and D), with enlargement of the left parotid gland being more marked than the swelling on the right side; unilateral swelling of the right parotid gland (C and D).

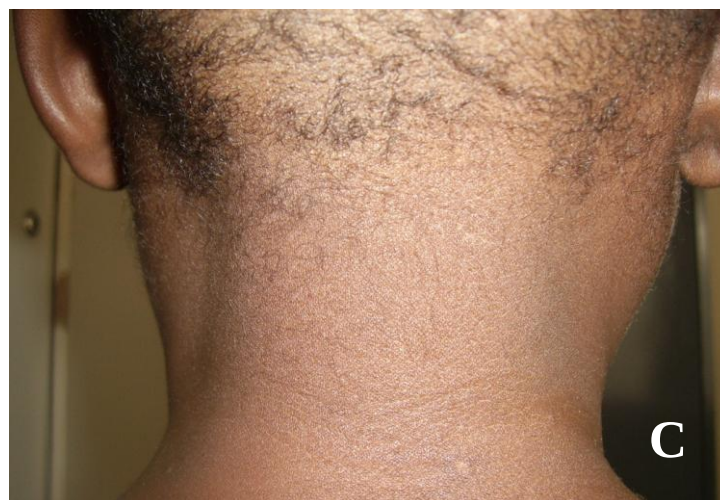
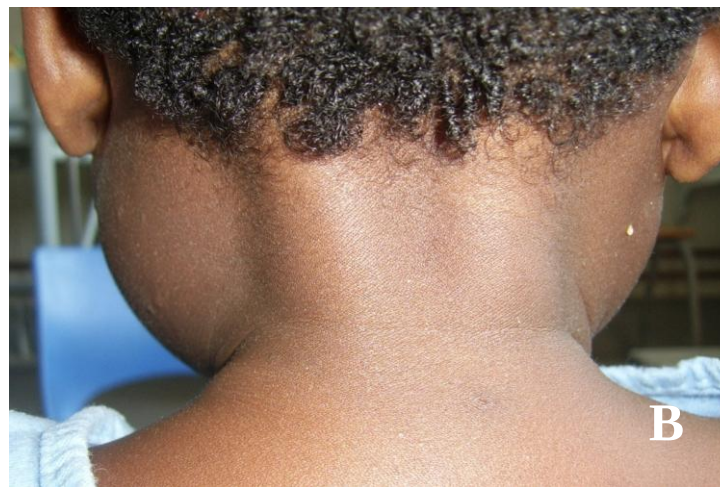


Figure 5.3. Posterior view of the unilateral (A and B) and bilateral parotid enlargement with cervical lymphadenopathy in children in Group 1 (C)

Table 5.6. Parotid lesions and associated oro-facial manifestations and treatment outcome

	Group 1	Group 2
Total	47	24
Parotid enlargement only	39 (82.98%)	0
Parotid and submandibular	6 (12.77%)	0
Submandibular enlargement only	2 (4.25%)	0
Texture		
Hard	36 (76.59%)	0
Soft	11 (23.40%)	0
Associated symptoms		
Parotid pain	12 (25.53%)	0
Dry mouth	2 (4.25%)	0
Dental caries	28 (59.57%)	12 (50%)
Oral candidiasis	20 (42.55%)	9 (37.5%)
Mouth ulcers	5 (10.63%)	3 (12.5%)
Gingivitis	5 (10.63%)	3 (12.5%)
Total on HAART	26 (55.32%)	19 (79.17%)
Effects of HAART on lesion		
No effect	5 (19.23%)	
Subsided	20 (76.92%)	
Had lesion after HAART	1 (3.85%)	
Cervical lymphadenopathy	47 (100%)	5 (20.83%)

5.11. Other oral lesions

The children in Group 1 had a higher prevalence of oral lesions as compared to the children in Group 2 (Table 5.6.; Figure 5.4.). The prevalence of dental caries (59.57%) and oral candidiasis (42.55%) (Figure 5.5.) was higher in Group 1 as compared with those in Group 2, which showed a prevalence for dental caries (50.0%) and oral candidiasis (37.0%). For children in Group 1 (Figure 5.5.) dental caries was the most prevalent oral disease, present in 28 (59.57%) children followed by oral candidiasis in 20 (42.44%) patients (Figures 5.6. and 5.7.) and pain overlying the parotid area, reported in 12 (25.53%) children. Only 5 (10.63%) children in this group presented with oral ulcers and gingivitis. One child in Group 2 presented with focal epithelial hyperplasia (Heck's disease) (Figure 5.8.).

Table 5.7. Oral lesions in HIV positive children with (Group 1) and without (Group 2) parotid swelling/enlargement

	Group 1 (47)	Group 2 (24)
Oral candidiasis	20 (42.55%)	9 (37.5%)
Caries	28 (59.57%)	12 (50%)
Gingivitis	5 (10.63%)	3 (12.5%)
Mouth ulcers	5 (10.63%)	3 (12.5%)
Viral warts	0	1 (4.17%)

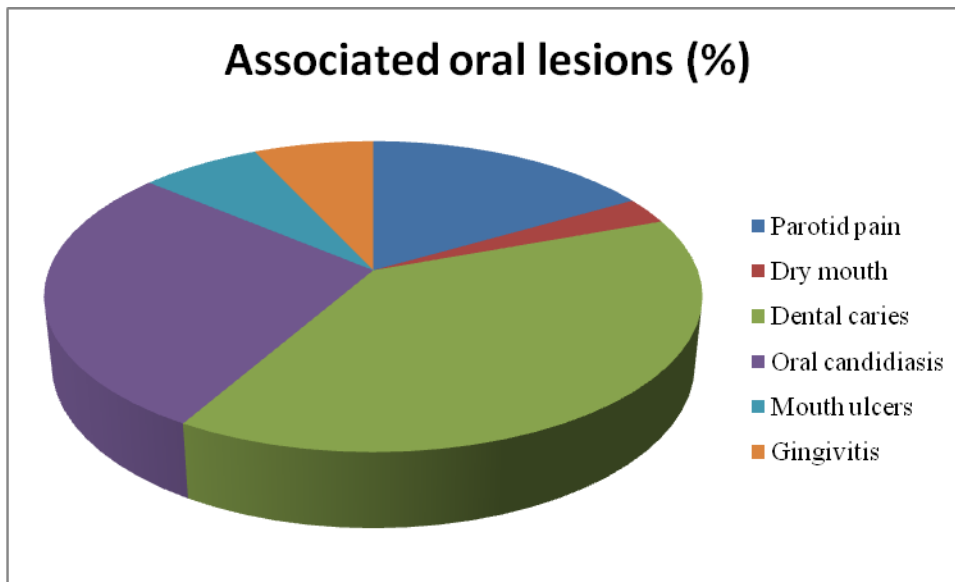


Figure 5.4. Distribution of associated oral lesions of children in Group 1

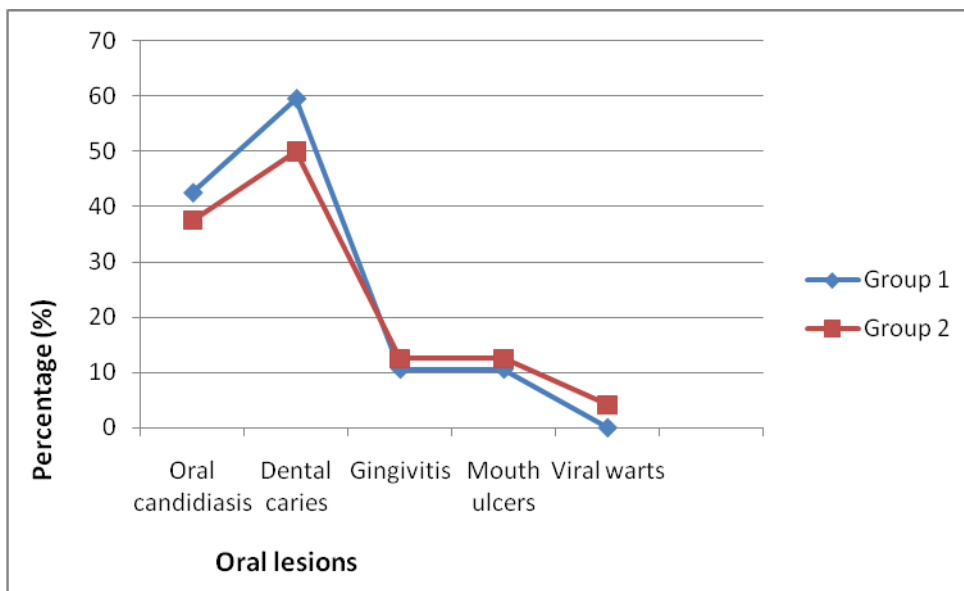


Figure 5.5. A comparison of oral lesions in children with and without parotid lymphoid proliferation



Figure 5.6. Erythematous candidiasis of the tongue with extension to the tonsillar fauces and soft palate



Figure 5.7. Bilateral angular cheilitis (oral candidiasis) and dental caries of the mandibular central incisors



Figure 5.8. Focal epithelial hyperplasia (Heck's disease) of the lower labial mucosa and gingiva

5.12 Concomitant systemic pathology

The concomitant medical conditions suffered by these children included TB and varicella-zoster. The frequency of these and other conditions are listed in Table 5.4. It can be seen that more patients in Group 2 suffered from TB whilst the frequency of the other lesions did not seem to be significantly different (Figure 5.9.).

Table 5.8. Concomitant medical pathology in HIV positive children with (Group 1) and without (Group 2) parotid lymphoid proliferation

	Group 1 (47)	Group 2 (24)
Tuberculosis	16 (34%)	13 (54.17%)
Varicella-zoster	5 (10.63%)	3 (12.5%)
Otitis media	2 (4.25%)	0
Hepato/splenomegaly	4 (8.51%)	0
Skin rash	3 (6.38%)	1 (4.17%)
Cough/respiratory infection	2 (4.25%)	1 (4.17%)

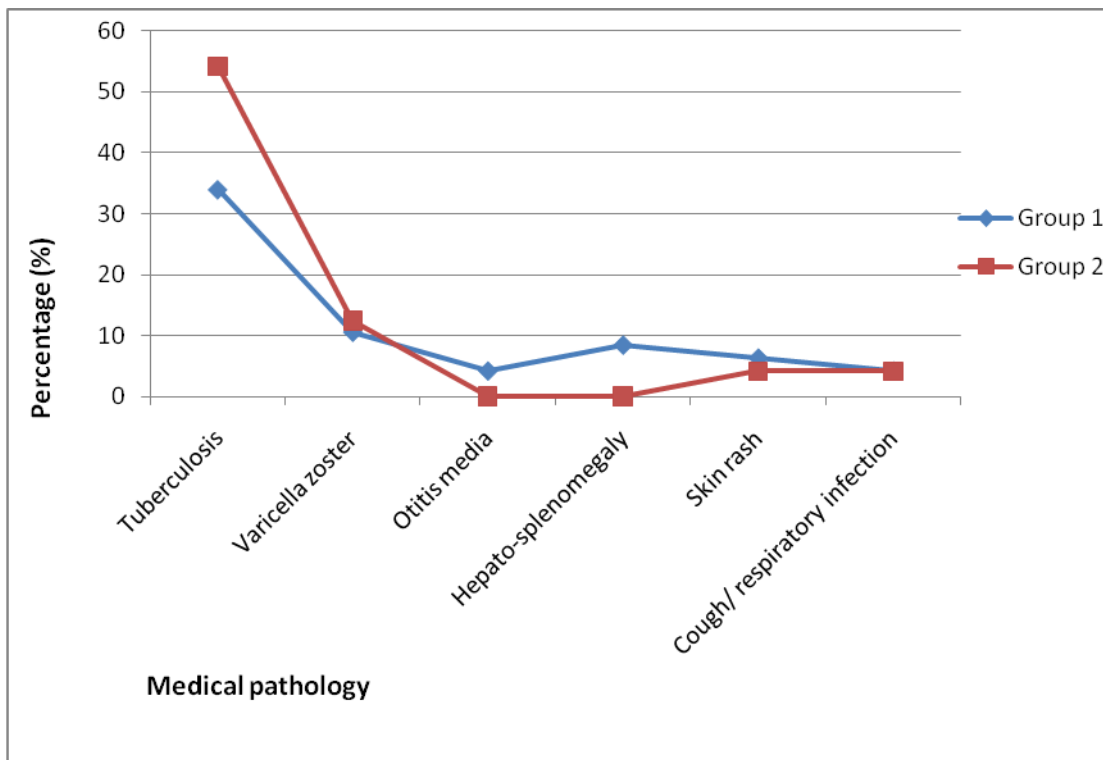


Figure 5.9. Medical pathology in HIV positive children with (Group 1) and without (Group 2) parotid lymphoid proliferation

CHAPTER 6

6.0. Discussion

Parotid lymphoid proliferation in children is not as well documented as in the adult population with relatively few cases having been reported (Table 3.2.). This study is the largest series in which the factors for the development of lymphoid proliferation in the parotid gland in children are documented. As previously mentioned, the prevalence of this condition in the sample we studied was unknown, but we estimated involvement of up to 20% or 1 in 5 of the children attending the clinics to show evidence of parotid swelling. The frequency in adults is estimated at 2-10% (Mandel et al. 1992; Mandel et al. 1998; Terry et al. 1991; Schiødt et al. 1992; Kreisel et al. 2010) while a frequency of 50% was reported by Chikte and Naidoo (2004) in institutionalised children in South Africa.

The previous largest series was a report by Rosso et al (2006) consisting of 51 patients in which ultrasonography (USG) was used to correlate the USG pattern and clinical and immunological condition of the patients. Their study is similar to the present study in that it was also conducted in vertically infected HIV patients with parotid gland and neck lymph node enlargement and included CDC staging, CD4 counts, viral loads and patients on HAART. However, whereas the present study included children ranging from 2 to 12 years with a male predominance of (1.2:1), the series reported by Rosso et al (2006), included children from 10 to 256 months (21.3 years), and thus their study was not restricted entirely to

children. Similarly, the series reported by Dave et al (2007) included a 17 year old patient. It is interesting to observe that no children younger than 2 years were found in this study. This is in contrast to other reported paediatric studies (Table 3.1.) where infants as young as 6 months were included in their series. Since the mode of HIV transmission is vertical in the children, it can only be speculated that the parotid lesion appears once the CD4 count has dropped significantly or the immune system has matured somewhat. This provides opportunity for future research.

Published reports of HIV related parotid lymphoid proliferation in adults showed a distinct male predominance with reports by Soberman et al (1991) [2.3:1], Schiødt et al (1992) [21:1], Morales-Aguirre et al (2005) [3:1], Rosso et al (2006) [1.3:1] and Martinoli et al (1995) [3.5:1] all showing a similar distinct male predominance. The gender distribution in this study was almost equal (1.2:1). This is not surprising since the mode of HIV transmission differs between adults and children. Children generally acquire HIV through parental vertical transmission while most adults in Africa are infected by sexual transmission.

All of the children included in the present study were black, however in other studies from around the world the parotid lymphoproliferation has been reported in blacks, Hispanics and whites. The association of HIV lymphoid proliferation of the parotid glands and MHC class II allele DR5 amongst black (African) patients has been reported (Itescu et al. 1990). According to these authors, the presence of HLA-DR5 confers a protection against HIV infection. As in the adult population, the expression of HLA DR5 in children with CD8 lymphocytosis has

been documented (Soberman et al. 1991). Currently there are 4 known subtypes of HLA-DR5 (Itescu et al. 1989), and in particular the allelic subtype JMV has been isolated in black patients with DILS (Itescu et al. 1990). According to these authors, the JMV subtype is rare in Caucasians. Other mechanisms to explain the pathogenesis of the parotid lymphoid hyperplasia should therefore be considered because all the children in this study, including those without the parotid enlargement were black Africans. Furthermore, there may be other HLA-DR5 subtypes in black Africans which may explain why some black patients present with parotid lymphoid hyperplasia and others do not.

When the data of the viral load on presentation in the study group (Group1) was compared with similar data from the control group (Group 2), the difference was significant; with the children in the study group showing lower viral load values, often below 100 000 RNA copies/ml. Analysis of the data of the viral loads related to possible gender differences within each group failed to show any significant differences. Our results seem to indicate that low viral load is related to a higher risk of developing parotid lymphoid hyperplasia. The possible nature of this relationship is not understood but seems to be consistent with the observation that children with parotid lesions have a better prognosis than those without the lesion. It is not the parotid lesion which confers the better prognosis but the low viral loads, with the parotid lesions merely being an indicator of such viral load. It should be noted that comparison of the values before and after treatment within the same group and in between the 2 groups was not done as there was no standardisation in the period of treatment the children had undergone.

Morales-Aguirre et al (2005) reported viral loads of below 100 000 copies/ml (8591, 58 671 and 8817), with only one male child having a viral load of 138 886 copies/ml. Similarly, Dave et al (2007) also reported viral loads below 100 000 copies/ml, however both these authors (Morales-Aguirre et al. 2005; Dave et al. 2007) did not document any correlation between low viral load and the risk of developing parotid lymphoid hyperplasia. In our study and like previous reports (Morales-Aguirre et al. 2005; Dave et al. 2007) HIV infection was acquired by vertical transmission. Since the majority of the children acquired HIV through vertical transmission, it is possible that the maternal viral load may have influenced the difference in viral load in the children and hence the development of the parotid lesion (Ioannidis et al. 2004). Children with low viral load could have mounted an effective immune response resulting in an enhanced proliferation of lymphoid tissue in both nodal and extranodal sites.

The presence of parotid gland lymphoepithelial lesions is often associated with a slower HIV disease progression in both adults and children (Katz et al.1992; Rosso et al. 2006). No correlation was found between the presence of these lesions with the CDC classification, as the parotid lesions were noted in immune categories A to C (Table 5.1.). In this study, the majority of the children in Group 1 (50.0%) were in immune category 3 as were majority of children in Group 2, (50.0%). This demonstrates that the lesion can be present at any stage of the HIV infection, including severely immune compromised children. Schiødt et al. (1992) showed 5 HIV infected adult patients (22.73%) with AIDS at the time when parotid swelling was diagnosed. In a study by Rosso et al (2006), only 2 patients with parotid lymphoepithelial lesions were asymptomatic. These findings, however, are in contrast to those reported by Morales-Aguirre et al (2005), where none of the children with parotid lymphoproliferation was

in category C. However, their series consisted of only 4 cases as compared with 47 children with lesions in this study and 22 in the study by Schiødt et al (1992).

Lymphoid proliferation usually presents as a painless, slow growing often bilateral swelling often associated with cervical lymphadenopathy. The parotid glands are commonly affected (Itescu et al. 1989; Sculerati et al. 1990; Mayer and Haddad, 1990; Dave et al. 2007) however involvement of other salivary glands has also been reported (Smith, 1990; Elliot and Oertel, 1990; Chetty, 1996; Terry et al. 1991). Only two (4.25%) children with submandibular swelling were seen in the present study. All children (100%) in Group 1 had cervical lymphadenopathy, while only 5 of the children in Group 2 (20.83%) had cervical lymphadenopathy. This shows that some children may present only with cervical lymphadenopathy, without the presence of salivary gland swelling. Most patients claimed to have experienced phases of parotid and submandibular swelling lasting from 2 to 6 weeks with alternating bouts of spontaneous remission and recurrences occurring within weeks or months. This however could not be assessed or confirmed via clinical examination of the patients in the clinics. As documented in the clinical history, the texture also correlated with the phases of the swellings, with the firmer, more solid lesions associated with the active phases of the disease, and the softer, more fluctuant and perhaps cystic lesions occurring during the remission phases. Lesions often involved one gland initially and progressed to bilateral involvement, as reported previously (Elliot and Oertel, 1990; Morales-Aguirre et al. 2005; Dave et al. 2007).

The presence of oral candidiasis and parotid enlargement are said to have prognostic significance in disease progression to AIDS (Katz et al. 1993; Magalhaes et al. 2001).

According to these authors, oral candidiasis is associated with a poor prognosis whereas the presence of parotid enlargement is associated with a better prognosis. A report by Gaitan-Cepada et al (2002) found no correlation between HIV associated oral lesions in children and the CD4 count and viral load.

The most common oral infection in HIV infected children is oral candidiasis (Magalhaes et al. 2001; Gaitan-Cepada et al. 2002). Only 6 of the 47 children (12.77%) in the present study were free of associated oral symptoms. The majority of the children presented with dental caries (59.57%), oral candidiasis (42.55%) and pain overlying the parotid area (25.45%). Pinto et al (2004) reported a link between the presence of xerostomia and the occurrence of *Candida*, only 2 children (4.25%) in the study complained of a dry mouth. They further suggest that oral candidiasis in children is mainly due to an immature immune system or immune suppression. Schiødt et al (1992) reported that salivary flow rates were more likely to be related to the degree of inflammatory infiltrate in the gland, rather than the degree of immunosuppression. The presence of dental caries in children may be due to poor oral hygiene and/or to the prolonged use of sugar containing medication as noted in the clinical history (Pomarico et al. 2008).

Reports of parotid lymphoid involvement in children often show features that are similar to those reported in the adult population (Itescu et al. 1990; Schiødt et al. 1992). However, based

on the literature review, it appears that the association between parotid lymphoproliferation and lymphoid interstitial pneumonitis (LIP) is primarily reported in the paediatric HIV population (Sculerati and Borkowsky, 1990; Soberman et al. 1991; Lepage et al. 1991; Mayer and Haddad, 1996). None of the children in this study had LIP; however 16 (34.04%) children in Group 1 presented with pulmonary TB and 13 (54.17%) children in Group 2. In South Africa, 50% to 80% of HIV infected individuals are co-infected with TB (*HIV and AIDS and STI strategic plan for South Africa 2007-2011*, 2009).

Lymphocytic infiltrates of other organs especially the liver have been reported in the literature (Itescu et al. 1990; Mayer and Haddad, 1996). The presence of hepatosplenomegaly was noted in 4 (8.51%) children in Group 1. None of the children in Group 2 presented with hepatomegaly or splenomegaly. Patients presenting with parotid swelling, cervical lymphadenopathy and hepato-splenomegaly often present with DILS (Itescu et al. 1990; Mandel et al. 1998). Without histological confirmation, it is uncertain whether the 4 patients in our study had DILS; however they did present with 3 clinical symptoms and signs: namely parotid gland enlargement, cervical lymphadenopathy and hepato-splenomegaly, which are consistent with DILS. The 4 children comprised 3 males and 1 female with an initial viral load ranging from 12 000 to 31 000. All the male children were not on ARV treatment. The lower viral loads observed in these children may be an indication of the role of the lymphocytosis in viral suppression.

The use of antiretroviral drugs especially AZT (zidovudine) has been shown to reduce and even eliminate the parotid lymphoproliferative enlargement (Terry et al. 1991; Uccini et al. 2000). In the present study the parotid enlargement subsided but did not completely resolve in 20 (76.92%) children and showed no effect in 5 children (19.23%). Children whose lesions persisted had been on HAART for 3 to 36 months. One (3.85%) child developed parotid enlargement following the initiation of HAART. None of the children in this study were on AZT. The parotid lymphoproliferative enlargement may be part of an immune reconstitution inflammatory syndrome (IRIS) (Powderly et al. 1998) as the parotid lesion appeared 8 months after ARV treatment. Studies conducted have mainly concentrated on oral lesions after the initiation of HAART (Aquino-Garcia et al. 2008; Hamza et al. 2006) and no studies have focused on the development of parotid lymphoepithelial lesions following the institution of ARV treatment as part of the oro-facial manifestations of HIV and AIDS.

Although fewer studies of the parotid lymphoepithelial lesions have been documented in children than adults, it is important to recognise parotid swelling in children as it is important in the diagnosis of HIV in this population. Unlike reports in the adult population (Shugar et al. 1988; Itescu et al. 1989; Cleary and Batsakis, 1990; Itescu et al. 1990; Mandel et al. 1998) parotid lymphoproliferation in children may be present at any CD4 count and CDC classification (Rosso et al. 2006; Dave et al. 2007). Also, the parotid lesion in children goes through phases of proliferation and remission, in contrast with the adult population where slow growing parotid lesions of up to 7 years have been reported (Mandel et al. 1998).

The role HIV viral load plays in the development of the parotid lymphoepithelial lesions has never been explored, with most studies reporting on CD4 and CD8 counts and correlating these to the development (Itescu et al. 1990; Mandel et al. 1998), radiological presentation (Shugar et al. 1988; Soberman et al. 1991; Som et al. 1995; Morales-Aguirre et al. 2005; Rosso et al. 2006; Dave et al. 2007) and histological classification (Smith, 1990; Mayer and Haddad, 1996; Uccini et al. 2000; Dave et al. 2007) of these lesions. Cytology and imaging studies are not carried out routinely, however, the recording of CD4 count and HIV RNA viral loads are standard in HIV clinics in South Africa. Records of CD4 cell count and viral loads may be used to predict patient progression from HIV infection to AIDS, especially in resource poor settings.

A weakness of this study is the lack of cytology or imaging correlation, and more importantly the lack of histological confirmation of the parotid lymphoepithelial lesions. As stated previously in the literature review, other causes of parotid swelling in children need to be considered. Even though the lymphoid proliferations were not confirmed cytologically or histologically, given the strong association between parotid gland swelling and HIV infection, based on evidence of clinically similar lesions in HIV positive children described in the literature, these swellings are probably due to HIV induced lymphoepithelial proliferation.

Vertically HIV infected children are surviving beyond 15 years of age mainly due to the use of ARVs and are therefore at a prolonged risk for cancer development (Frisch et al. 2001). A longitudinal follow-up study will be useful.

CHAPTER 7

7.0. CONCLUSION

This study has documented the largest global series of parotid lymphoproliferation in vertically infected HIV children. It was not possible to determine the prevalence of the disease in the population studied. There was no difference in demographic data, nor was there any correlation with regard to CD4 cell count and CDC classification in children presenting with HIV lymphoid proliferation of the parotid glands and children who do not have the parotid lymphoid proliferation. There was however a correlation between viral load and the risk of developing parotid lymphoid proliferation with children with lower viral loads being at an increased risk.

Although the exact nature of the relationship between parotid lymphoid proliferation and viral load is not fully understood, it might explain why children with parotid lymphoid proliferation seem to have a better prognosis to HIV infection than children without parotid lesions; i.e. it is not the parotid lesions which confer a better prognosis but the lower viral load, with the parotid lesions merely being a visible manifestation of the lower viral loads. The parotid swellings responded well to ARV treatment, however, they did not completely resolve. Only one child developed parotid enlargement after ARV initiation.

CHAPTER 8

8.0. REFERENCES

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APPENDICES

Appendix 1: Ethics clearance letter

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

R14/49 Kungoane

CLEARANCE CERTIFICATE

PROTOCOL NUMBER M080926

PROJECT

Lymphoid Proliferation of the Pseudo
Gland in Paediatric Patients with HIV
Infection

INVESTIGATORS

Dr T Kungoane

DEPARTMENT

Division of Oral Pathology

DATE CONSIDERED

08.09.26

DECISION OF THE COMMITTEE*

Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE

09.08.28

CHAIRPERSON


(Professor P E Cleaton Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Prof M Altini

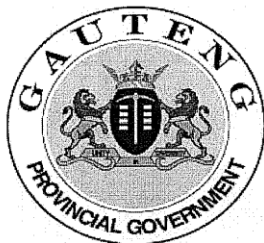
DECLARATION OF INVESTIGATOR(S)

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

I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

Appendix 2: Letter of approval from the Gauteng Department of Health



Department of Health
Lefapha la Maphelo
Departement van Gesondheid
Umnyango wezeMpilo
TSHWANE-METSWEDING REGION
 Office of the Director: Mrs L Volkwyn
 Tel: (012) 303-9012/9217
 Fax: (012) 324 2538

10 November 2008

TO WHOM IT MAY CONCERN:

PERMISSION TO DO A RESEARCH

TOPIC: **Lymphoid Proliferation of the Parafid Gland in Paediatric Patients with HIV Infection**

This letter serves as a confirmation that permission has been granted for Dr Tsholofelo Kungoane to do a research in all Tshwane District Clinics.

Best regards,

DR JV NDUMANDE
PRINCIPAL FAMILY PHYSICIAN
TSHWANE METSWEDING DISTRICT HEALTH SERVICES

Cc: Ms ON Matjebe – Deputy Director: HAST

Appendix 3A: Participation information form (English)

Study number: M080926

Study title: Lymphoid Proliferation of the parotid gland in paediatric patients with HIV infection.

Investigator: Dr Tsholofelo Kungoane

Institution: University of the Witwatersrand, Johannesburg

Contact number: 0827289269/ 0127032732

Ethics chairman contact details: 011 7171234

Dear parent/ guardian.

Hello. My name is Tsholofelo Kungoane. I am a student doing a master's degree at the University of the Witwatersrand, WITS. I am currently undertaking a study as part of my degree and request your help by allowing your child to participate.

In this study, I want to examine occurrence of swelling on the side of the face in children who are infected with the HIV, in order to identify the effects of the swelling in predicting the improvement of the disease. The intention is create awareness of parotid swellings and to encourage early diagnosis and treatment.

The **examination** will be carried out during the routine check-up of your child. It involves physical palpation for facial and neck swellings. I will also check your child's mouth for rotten teeth, dryness, swollen gums and oral infection. I also need your permission to access your child's medical record for information regarding CD4 counts and viral loads and to use the information collected in the study. I will also take photos which will be used for the study. After examination, should we find that your child needs any further dental treatment, he/she will be referred to the nearest state dental facility for treatment. If you wish, you may take him/her to a private dentist at own cost. Please note that this is an anonymous study, and you and your child's name will remain anonymous.

The examination is **voluntary** with no financial compensation. If you do not want your child to participate in the study you may opt do so; you may also withdraw your child from the study at any point. This will not be held against you or your child.

Please read the document carefully before agreeing to participate. If you do not understand the contents, do not hesitate to call me on the above numbers.

Appendix 3B: Participation form for children**Study number: M080926****Study title:** Lymphoid Proliferation of the parotid gland in paediatric patients with HIV infection.**Investigator:** Dr Tsholofelo Kungoane**Institution:** University of the Witwatersrand, Johannesburg**Contact number:** 0827289269/ 0127032732**Ethics chairman contact details: 011 717 1234**

Hello.

My name is Tsholofelo; I am a dentist and a student from WITS. I am doing a study/ assignment for my studies at WITS and I need your help in completing the study.

You are invited to take part by giving me permission to examine you for any swelling of the face and neck. The examination is painless procedure using only gloved hands. I also ask for permission to look at your file and take photos. The information found will be used for the study. If after the examination you have rotten teeth, I will, with your permission refer you to a local dental clinic.

This is a nameless study. No other person except for me will know who you are. You are free to refuse the examination. Nobody can force you to participate, even the nursing sister, your parent or guardian

Appendix 3C: Participation information form (Setswana)

Study number: M080926

Study title: Lymphoid Proliferation of the parotid gland in paediatric patients with HIV infection.

Investigator: Dr Tsholofelo Kungoane

Institution: University of the Witwatersrand, Johannesburg

Contact number: 0827289269/ 0127032732

Ethics chairman contact details: 0117171234

Go motswadi le motlhokomedi wa ngwana

Madume. Ke nna Tsholofelo Kungoane, moithuti wa masters ko WITS. Ke kopa tetla ya gago

go tlhatlhoba le go lebelela faele ya ngwana ya sepetlela. Mo tlhatlhobong e, ke tlile go

lebelela bolwetsi ba maue bo bo tlhaselang bana ba ba na leng kokwana-tlhoko ya HIV.

Tlhatlhobo e, e tlile go dirwa ka letsatsi la ngwana la go tla bookelong. Ke tlile go tlhatlhoba

le legano la ngwana go lebelela meno a go bola, marinini a go ruruga le malwetse a mangwe a

legano. Ke kopa o ntetlelle go okomela faele ya ngwana mo ke tlo go lebelela dilo ditshwana

le di CD4 le viral load le tetla ya go tsaya ditshwantsho. Fa go ka fitlhelwa gore ngwana o

tlhoka tlhokomelo ya legano, o tlile go romelwa ko bookelong kana tliliniking ya meno.

O itsisewe gore tlhatlhobo e ke mahala, ka boithapo (voluntary). Fa o sa dumele tlhatlhobo e, o

kana wa dira jalo ntle le kotlhao. Fa o na le dipotso, ntetsetse mogala mo dinomorong tse di

filweng.

Appendix 4: Consent form

Study number: M080926

Study title: Lymphoid Proliferation of the parotid gland in paediatric patients with HIV infection.

Investigator: Dr Tsholofelo Kungoane

Institution: University of the Witwatersrand, Johannesburg

Contact number: 0827289269/ 0127032732

Ethics chairman contact details: 011 7171234

Please complete and return.

I, the undersign parent/guardian ofagree to have my child participate in the study. I have read and understand the purpose of the study and the procedures involved. I also understand that I am free to withdraw my child from the study at any time without any consequences to me or my child. I also give permission for photos to be taken and their use for the purpose of the study.

.....

Parent/Guardian Name	Signature/thumbprint	Date
----------------------	----------------------	------

My name isMy parent/guardian has explained to me about the study. I understand what will be done in my mouth and face. I am aware that even if my parent/ guardian agree to enrol me in the study, I have a right to refuse or to stop my involvement anytime during the study.

.....

Child Name	Signature/thumbprint	Date
------------	----------------------	------

Appendix 5: Data collection form

The clinical examination

1. Age
2. Gender
3. Race
4. Date of diagnosis
5. Mode of HIV transmission
6. CD4 count: at diagnosis..... at present.....
 CD8 count: at diagnosis.....at present.....
 Viral load: at diagnosis.....at present.....

Facial examination

Swelling present Yes..... No.....

Glands involved: Parotid..... Submandibular.....
 Unilateral..... Bilateral.....

Size: Left..... Right.....

Texture of swelling: Hard..... Soft.....

Associated symptoms: Pain Y/N
 Dry mouth Y/N
 Caries Y/N

Other symptoms Oral candidiasis Y/N
 Gingivitis/bleeding gums Y/N
 Mouth ulcers Y/N

Associated cervical lymphadenopathy: Yes/No

Other medical pathology

Antiretroviral therapy history

Effect of ARV: On parotid swellings
 On CD4 count
 On viral load

Appendix 6 Raw data Group 1

[illegible]

Group 2 children data

patient number	AGE	Gender	Race	Date of diagnosis	mode of HIV transmission	CD4 at diagnosis	CD4 % diagnosis	Present CD4	CD4 % present	VL AT DIAGNOSIS	PRESENT VL	Oral lesions	Cervical Lymphadenopathy	ARV treatment	Treatment duration(months)	Medical Pathology
1	1	male	African	2007	vertical	889	14.6	1261	28.7	2 100 000		caries	no	d4T, 3TC, EFV	1	
2	2	female	African	2008 Nov	vertical	983	18.8						no	not on treatment		
3	2	male	African	2008 May	unknown	690							no	d4T,3TC, EFV	11	coughing
4	3	female	African	2006	vertical	759	9.3			160 000			no	d4T, 3TC, EFV	5	TB
5	3	female	African	2007 Oct	vertical	429	10.1			2 400 000			yes	d4T,3TC, EFV	36	TB, Varicella zoster
6	3	male	African	2008 July	unknown	262	10.9					caries	no	d4T, 3TC, Kaletra	3	
7	4	female	African	2006 Dec	vertical	861		1646		33 000	<25	caries	no	d4T,3TC, EFV	24	TB
8	4	male	African	2006 Nov	vertical	399		57		55 000	6100		no	d4T,3TC, EFV	24	
9	4	male	African	2005 May	vertical	1198	22.1	558	22.2	150 000	lower than detectable	oral candidiasis, erythematous	no	d4T,3TC, EFV	36	
10	5	female	African	2003	vertical	197	7.62			5 200		oral candidiasis	no	not on treatment	13	TB
11	5	female	African	2003	vertical	198				5 300	1900	oral candidiasis	no	d4T,3TC, EFV	14	TB
12	5	male	African	2008 Jun	vertical	224				58 000		caries, oral candidiasis, oral ulcers, gingivitis	yes	d4T, 3TC,EFV	3	varicella zoster, wasting syndrome
13	6	female	African	2005 Jun	vertical	306	12	952	34.9	350 000	0		no	d4T,3TC, EFV	36	TB
14	6	male	African		vertical	700	8.11	929		27 000	lower than detectable	caries, oral candidiasis, gingivitis	no	d4T, 3TC, EFV	8	TB
15	7	female	African	2007	vertical	16	1.4	16	13.5	26 000	37	caries	no	d4T, 3TC,EFV	17	TB
16	8	female	African	2005 Jul	vertical	110	5.66	631	22.7	160 000	<25	caries, oral candidiasis	no	d4T,3TC, EFV	36	TB
17	8	female	African	2007 Oct	vertical	650	25	316	16.1			caries	yes	not on treatment		TB
18	9	female	African	2005 Aug	vertical	55	4.7	871	39.5	140 000	lower than detectable	caries	no	d4T, 3TC, EFV	48	TB
19	10	male	African	2005	vertical	391	8.36	649	35.1	98 000	lower than detectable	caries	no	d4T,3TC, EFV	36	TB, Lipodystrophy
20	10	male	African	2005	vertical	489	17.8					gingivitis, mouth ulcers	no	not on treatment		
21	11	female	African	2009 mar	vertical	381	14.7			720 000		caries, oral candidiasis, erythematous	no	not on treatment		facial and head fungal infection
22	11	male	African	2008 Aug	vertical	164	19.3			2 500		oral candidiasis	yes	d4T,3TC, EFV	3	varicella zoster
23	11	male	African	2008 Oct	vertical	43	3.64					oral candidiasis, mouth ulcers	no	d4T,3TC, EFV	1	
24	12.7	male	African	2006 Dec	vertical	12	0.87	282	13.7	50 000	lower than detectable	caries	yes	d4T, 3TC, EFV	18	TB